48th Annual Drosophila Research Conference

March 7-11, 2007
Philadelphia Marriott Downtown
Philadelphia, Pennsylvania

Program and Abstracts

- **2007 Meeting Organizers**

  Steve DiNardo, University of Pennsylvania School of Medicine
  Liz Gavis, Princeton University
  Tom Jongens, University of Pennsylvania School of Medicine
  Jessica Treisman, NYU Medical Center

- **2007 Drosophila Board of Directors Officers and Regional Representatives**

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Midwest	Pam Geyer	University of Iowa

- **International Representatives**

Australia/Oceana	Robert Saint	Australian National University
Asia	Yasushi Hiromi	National Institute of Genetics
Europe	David Ish-Horowicz	Cancer Research UK

- **Sponsored by**

The Genetics Society of America
9650 Rockville Pike, Bethesda, MD 20814-3998
Telephone: (301) 634-7300 fax: (301) 634-7079
e-mail: society@genetics-gsa.org
Web site: http://www.genetics-gsa.org/
Conference site: http://www.drosophila-conf.org/

TABLE OF CONTENTS

FUTURE CONFERENCE DATES

2008
April 2-6
San Diego, California

2009
March 4-8
Chicago, Illinois

2010
April 7-11
Washington, DC

2011
March 30-April 3
San Diego, California

2012
March 7-11
Chicago, Illinois

2013
April 3-7
Washington, DC

SCHEDULE OF EVENTS
48th Annual Drosophila Research Conference

WEDNESDAY, MARCH 7		
Time	Event	Location
12:00 noon - 6:00 pm	**Ecdysone Workshop** *Organizers:* Robert Ward, University of Kansas, Lawrence; and Craig Woodward, Mount Holyoke College, South Hadley, Massachusetts	Grand Ballroom Salons A-D Level Five
3:00 pm - 6:00 pm	**Drosophila Board of Directors Meeting**	Room 407-409 Level Four
3:30 pm - 9:00 pm	**Registration & Book Pickup**	Franklin Hall Foyer Level Four
7:00 pm - 9:00 pm	**Opening General Session** *Moderator:* Steve DiNardo, University of Pennsylvania Medical School, Philadelphia	Grand Ballroom Salons E-H Level Five
7:00 pm	**Welcome and Opening Remarks** Steve DiNardo, University of Pennsylvania Medical School, Philadelphia	
7:10 pm	**Introduction of Historical Address Speaker** Mark Fortini, National Cancer Institute, Frederick, Maryland	
7:20 pm	**Historical Address** Spyros Artavanis-Tsakonas, Harvard Medical School/MGH Cancer Center, Boston, Massachusetts	
8:10 pm	**Introduction of Larry Sandler Memorial Lecture** Helen Salz, Case Western Reserve University, Cleveland, Ohio	
9:00 pm - 12:00 am	**Mixer/Reception**	Grand Ballroom Salons A-D and Foyer Level Five
THURSDAY, MARCH 8		
7:15 am - 8:30 am	**Continental Breakfast**	Grand Ballroom Foyer Level Five
8:00 am - 5:00 pm	**Registration & Book Pickup**	Franklin Hall Foyer Level Four

Time	Event	Location
8:30 am - 12:00 noon	**Plenary Session I** *Moderator:* Liz Gavis, Princeton University, New Jersey	Grand Ballroom Salons E-H Level Five
8:30 am	**Image Award Presentation** Liz Gavis, Princeton University, New Jersey	
8:35 am	**Circadian Clocks and Sleep** Ravi Allada, Northwestern University, Evanston, Illinois	
9:00 am	**Membrane and Organelle Traffic in Neurons** Thomas Schwarz, Children's Hospital, Boston, Massachusetts	
9:30 am	**Taste Recognition in Drosophila** Kristin Scott, University of Iowa, Iowa City	
10:00 am	**Break**	
10:30 am	**Regulation of Cell Surface Mechanics Underlying Tissue Morphogenesis** Thomas Lecuit, IBDM, Marseille Cedex, France	
11:00 am	**Progressive Heterochromatin: Players and Pathways** Lori Wallrath, University of Iowa, Iowa City	
11:30 am	**Mechanisms of P Element Transposition and Alternative Pre-mRNA Splicing** Donald Rio, University of California, Berkeley	
1:00 pm - 5:00 pm	**FlyMine Drop-in Demonstrations** **Presentation at 2:15 pm**	Room 414-415 Level Four
1:00 pm - 6:00 pm	**FlyBase Demonstrations** **Demo Room Open for Tutorials and Discussions** 1:30 pm-2:00 pm Presentations: Navigating the new FlyBase website. Accessing the dozen fly genomes. Increasing your query power: TermLink & QueryBuilder. 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website. Accessing the dozen fly genomes. Increasing your query power: TermLink & QueryBuilder.	Room 401-403 Level Four
2:00 pm - 4:00 pm	**Exhibits & Poster Viewing** 2:00 pm-3:00 pm: Even-Numbered Poster Authors 3:00 pm-4:00 pm: Odd-Numbered Poster Authors	Franklin Hall Level Four
4:30 pm - 6:30 pm	**Concurrent Platform Sessions I**	
	Immune System and Cell Death	Grand Ballroom Salon E Level Five

Time	Event	Location
	Organogenesis	Grand Ballroom Salons H-J Level Five
	Neurophysiology and Behavior	Grand Ballroom Salons G, K-L Level Five
8:00 pm - 11:00 pm	**Exhibits & Poster Viewing** 8:00 pm-9:00 pm: "A" Poster Authors 9:00 pm-10:00 pm: "B" Poster Authors 10:00 pm-11:00 pm: "C" Poster Authors	Franklin Hall Level Four
FRIDAY, MARCH 9		
8:30 am - 5:00 pm	**Registration & Book Pickup**	Franklin Hall Foyer Level Four
8:30 am - 10:15 am	**Concurrent Platform Sessions II**	
	Evolution and Quantitative Genetics	Grand Ballroom Salon E Level Five
	Regulation of Gene Expression	Grand Ballroom Salons H-J Level Five
	Cytoskeleton and Cell Biology	Grand Ballroom Salons G, K-L Level Five
9:00 am - 5:00 pm	**FlyMine Drop-in Demonstrations** **Presentations at 10:30 am and 2:15 pm**	Room 414-415 Level Four
9:00 am - 6:00 pm	**FlyBase Demonstrations** **Demo Room Open for Tutorials and Discussions** 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website. Accessing the dozen fly genomes. Increasing your query power: TermLink & QueryBuilder.	Room 401-403 Level Four
10:15 am - 10:45 am	**Break**	Grand Ballroom Foyer Level Five
10:45 am - 12:30 pm	**Concurrent Platform Sessions III**	
	Evolution and Quantitative Genetics	Grand Ballroom Salon E Level Five

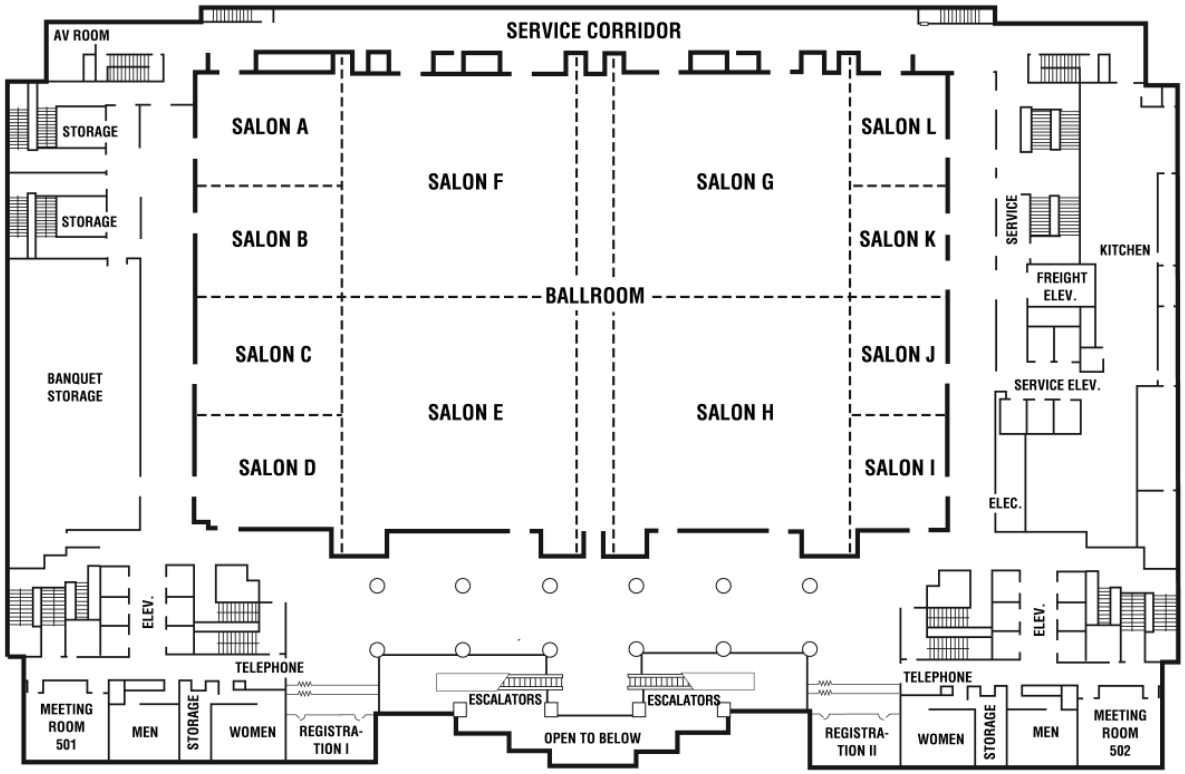
Time	Event	Location
	Regulation of Gene Expression	Grand Ballroom Salons H-J Level Five
	Cytoskeleton and Cell Biology	Grand Ballroom Salons G, K-L Level Five
12:45 pm - 1:45 pm	**GSA Mentor Roundtable** Limited attendance. Sign up by 5:00 pm on March 8 at DROS registration desk, Level 4.	Room 413 Level Four
1:45 pm - 3:45 pm	**Concurrent Workshops**	
	Cell Death *Organizers:* Rebecca Hays, University of Kansas, Lawrence; Andreas Bergmann, University of Texas, Houston; and Jamie Rusconi, SUNY Albany, NY	Grand Ballroom Salon E Level Five
	A Dozen Fly Genomes: What Have We Learned and What's Next? *Organizers:* William M. Gelbart, Harvard University, Cambridge Massachusetts; and Thomas C. Kaufman, Indiana University, Bloomington	Grand Ballroom Salons H-J Level Five
	Drosophila Research and Pedagogy at Primarily Undergraduate Institutions *Organizer:* Don Paetkau, Saint Mary's College, Notre Dame, Indiana	Grand Ballroom Salons G, K-L Level Five
2:00 pm - 4:00 pm	**Visit the Exhibits**	Franklin Hall Level Four
4:30 pm - 6:30 pm	**Concurrent Platform Sessions IV**	
	Gametogenesis and Sex Determination	Grand Ballroom Salon E Level Five
	Drosophila Models of Human Diseases	Grand Ballroom Salons H-J Level Five
	Signal Transduction I	Grand Ballroom Salons G, K-L Level Five
8:00 pm - 11:00 pm	**Exhibits & Poster Viewing** 8:00 pm-9:00 pm: "C" Poster Authors 9:00 pm-10:00 pm: "B" Poster Authors 10:00 pm-11:00 pm: "A" Poster Authors	Franklin Hall Level Four

SATURDAY, MARCH 10		
Time	Event	Location
8:30 am - 4:00 pm	**Registration & Book Pickup**	Franklin Hall Foyer Level Four
8:30 am - 10:15 am	**Concurrent Platform Sessions V**	
	Pattern Formation I	Grand Ballroom Salon E Level Five
	Cell Division and Growth Control	Grand Ballroom Salons H-J Level Five
	Signal Transduction II	Grand Ballroom Salons G, K-L Level Five
9:00 am - 5:00 pm	**FlyBase Demonstrations** **Demo Room Open for Tutorials and Discussions** 1:30 pm-2:00 pm Presentations: Navigating the new FlyBase website. Accessing the dozen fly genomes. Increasing your query power: TermLink & QueryBuilder. 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website. Accessing the dozen fly genomes. Increasing your query power: TermLink & QueryBuilder.	Room 401-403 Level Four
9:00 am - 5:00 pm	**FlyMine Drop-in Demonstrations** **Presentations at 10:30 am and 2:15 pm**	Room 414-415 Level Four
10:15 am - 10:45 am	**Break**	Grand Ballroom Foyer Level Five
10:45 am - 12:30 pm	**Concurrent Platform Sessions VI**	
	Pattern Formation II	Grand Ballroom Salon E Level Five
	Cell Division and Growth Control	Grand Ballroom Salons H-J Level Five
	Techniques and Genomics	Grand Ballroom Salons G, K-L Level Five

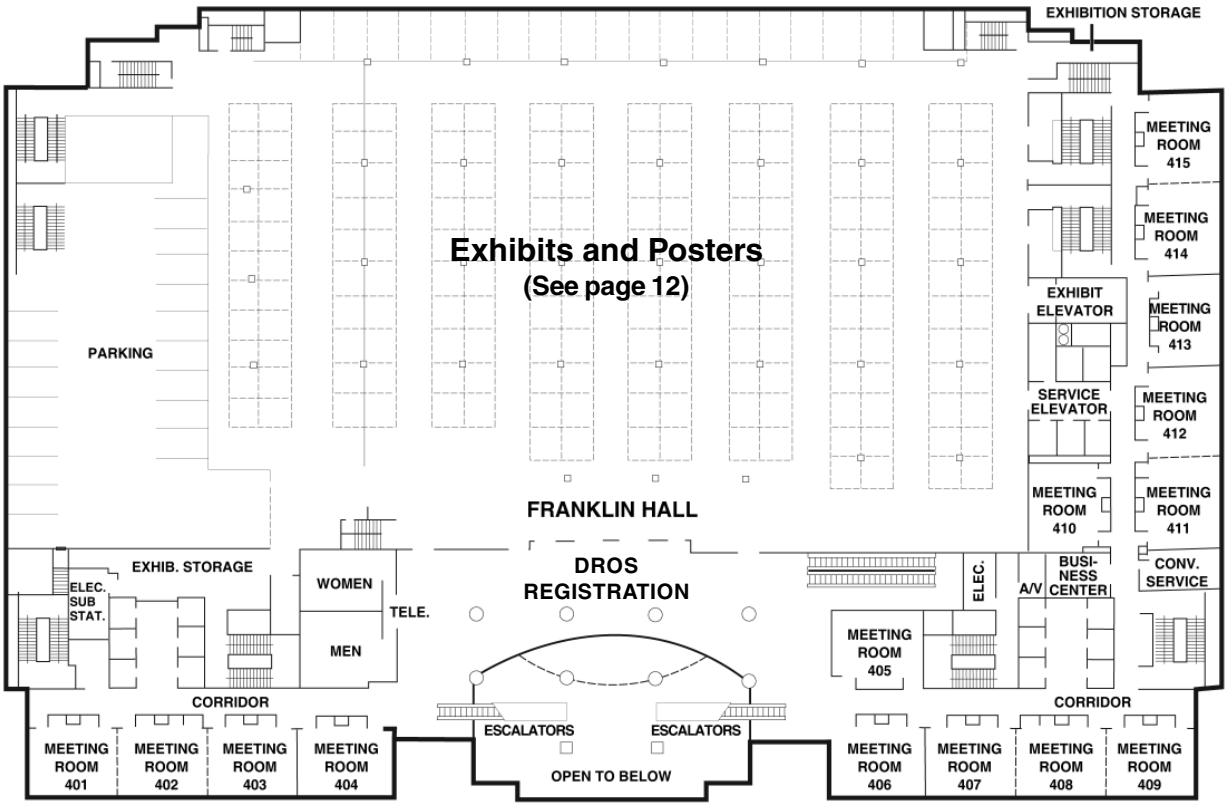
Time	Event	Location
12:45 pm - 1:45 pm	**Joint Steering Committee for Public Policy** Limited attendance. Sign up by 5:00 pm on March 9 at DROS registration desk, Level 4.	Allie's American Grill Lobby Level
1:30 pm - 3:30 pm	**Exhibits & Poster Viewing** 1:30 pm-2:30 pm: Odd-Numbered Poster Authors 2:30 pm-3:30 pm: Even-Numbered Poster Authors	Franklin Hall Level Four
4:00 pm - 6:00 pm	**Concurrent Platform Sessions VII**	
	Genome and Chromosome Structure	Grand Ballroom Salon E Level Five
	Neurogenetics and Neural Development	Grand Ballroom Salons H-J Level Five
	Physiology and Aging	Grand Ballroom Salons G, K-L Level Five
6:45 pm - 8:45 pm	**Concurrent Workshops**	
	Immunity, Hematopoiesis, and Pathogenesis *Organizers:* Todd Schlenke, Emory University, Atlanta, Georgia; and Brian Lazzaro, Cornell University, Ithaca, New York	Grand Ballroom Salon E Level Five
	Cell Cycle Checkpoints *Organizers:* Tin Tin Su, University of Colorado, Boulder; and Claudio Sunkel, University of Porto, Portugal	Grand Ballroom Salons G, K-L Level Five
	RNAi High-Throughput Screening *Organizers:* Bernard Mathey-Prevot, Harvard Medical School, Boston, Massachusetts; and Steven Suchyta, Ambion Inc., Austin, Texas	Grand Ballroom Salons H-J Level Five
8:00 pm - 11:00 pm	**Exhibits and Open Poster Viewing**	Franklin Hall Level Four
9:30 pm - 11:30 pm	**Concurrent Workshops**	
	Workshop on RNA Biology *Organizer:* A. Javier Lopez, Carnegie Mellon University, Pittsburgh, Pennsylvania	Grand Ballroom Salon E Level Five
	Extracellular Matrix Interactions and Signaling *Organizer:* Halyna Shcherbata, University of Washington, Seattle	Grand Ballroom Salons G, K-L Level Five

SUNDAY, MARCH 11		
Time	Event	Location
8:30 am - 12:00 noon	**Plenary Session II** *Moderator:* Jessica Treisman, NYU Medical Center, New York, New York	Grand Ballroom Salons A-F Level Five
8:30 am	**GSA Poster Award Presentation** Jessica Treisman, NYU Medical Center, New York, New York	
8:35 am	**Evolution of Gene Regulation in Drosophilids** Michael Eisen, University of California, Berkeley	
9:00 am	**Signatures of Speciation in the Pseudoobscura Species Group** Mohamed Noor, Duke University, Durham, North Carolina	
9:30 am	**Glial Function in Nervous System Development** Ulrike Gaul, Rockefeller University, New York, New York	
10:00 am	**Break**	
10:30 am	**Color Vision** Claude Desplan, New York University, New York	
11:00 am	**Regulatory Mechanisms Controlling Directed Cell Migration** Pernille Rørth, EMBL, Heidelberg, Germany	
11:30 am	**Origin of the Brain Endocrine Axis: Insights for Type 1 Flyabetes** Eric Rulifson, University of Pennsylvania, Philadelphia	

MARRIOTT PHILADELPHIA DOWNTOWN
LEVELS FOUR AND FIVE MEETING SPACE

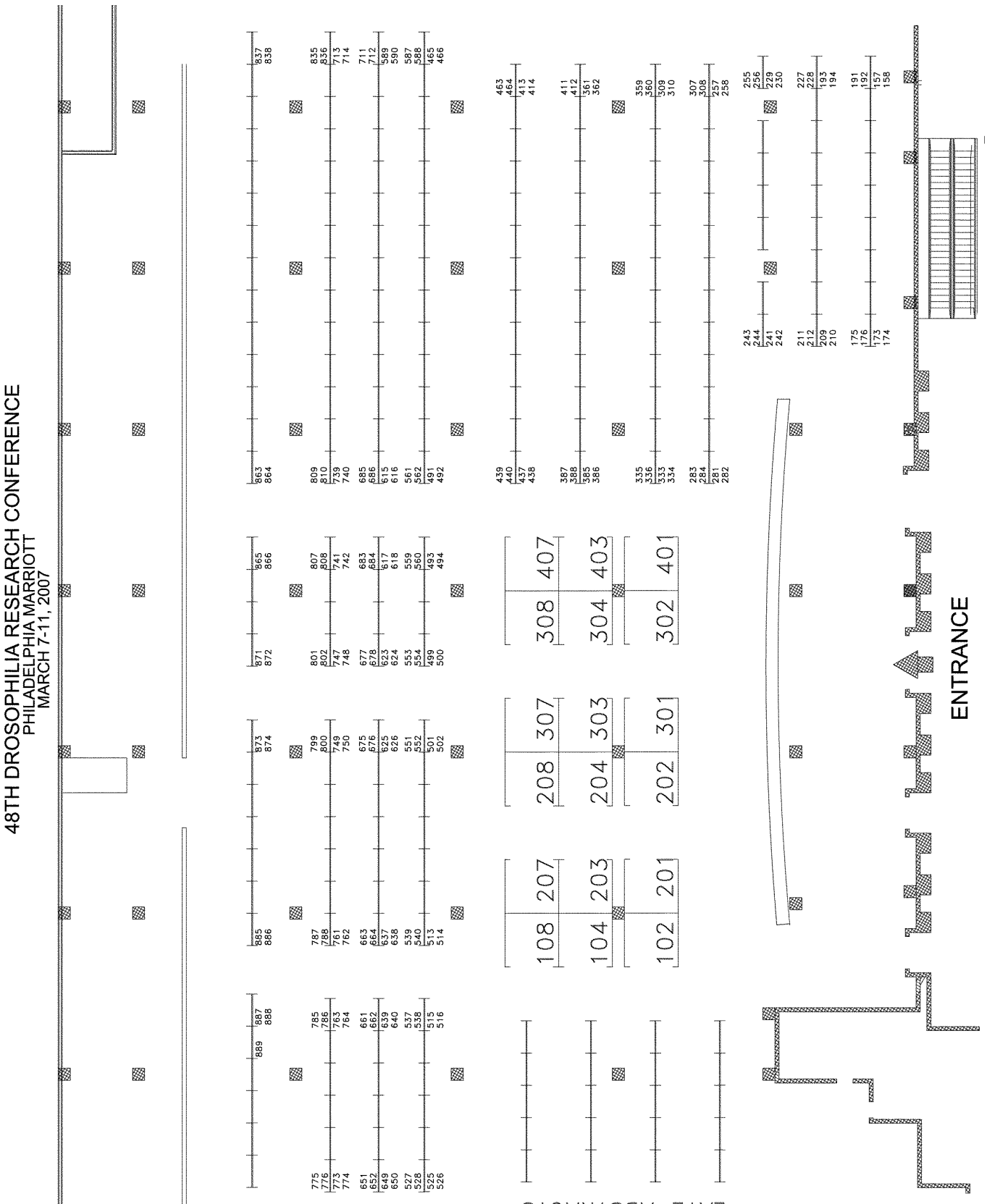


LEVEL FIVE



LEVEL FOUR

MARRIOTT PHILADELPHIA DOWNTOWN
LEVEL FOUR, POSTERS AND EXHIBIT HALL



GENERAL INFORMATION

- **Badges**

  Badges are **required** for admission to all sessions, the opening mixer, and the posters and exhibits in the Exhibition Hall. Security officers will not allow individuals without badges to enter the Exhibition Hall. If you lose your badge, a replacement may be requested at the Registration and Information Counters during posted registration hours. Badges may not be used by anyone other than the registered attendee. Each attendee must have his/her own badge.

- **Employment Opportunities/Seeking Employment Notices – Level Four – Franklin Hall Foyer**

  Individuals and institutions offering or seeking employment may post notices and résumés on the "Employment Opportunities" bulletin boards set up near the Registration and Information Counters in the Franklin Hall Foyer.

- **FlyBase Demonstrations – Level Four – Room 401-403**

  This has been a year of major changes for FlyBase, with an entirely new web interface and many new kinds of data. FlyBase invites all Conference registrants to come to the demo room to learn how to make the best use of the new FlyBase tools and features for your research and teaching. Throughout the day, other than the scheduled group presentations noted below, FlyBase personnel are available in the demo room for one-on-one tutorials, troubleshooting and discussions. Any thoughts on improvements to FlyBase are gratefully appreciated.

Thursday, March 8	1:00 pm–6:00 pm	Demo room open for tutorials and discussions 1:30 pm-2:00 pm Presentations: Navigating the new FlyBase website Accessing the dozen fly genomes Increasing your query power: TermLink & QueryBuilder 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website Accessing the dozen fly genomes Increasing your query power: TermLink & QueryBuilder
Friday, March 9	9:00 am–6:00 pm	Demo room open for tutorials and discussions 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website Accessing the dozen fly genomes Increasing your query power: TermLink & QueryBuilder
Saturday, March 10	9:00 am–5:00 pm	Demo room open for tutorials and discussions 1:30 pm-2:00 pm Presentations: Navigating the new FlyBase website Accessing the dozen fly genomes Increasing your query power: TermLink & QueryBuilder 3:00 pm-3:30 pm Presentations: Navigating the new FlyBase website Accessing the dozen fly genomes Increasing your query power: TermLink & QueryBuilder

- **FlyMine Drop-in Demonstrations – Level Four – Room 414-415**

Thursday, March 8	1:00 pm–5:00 pm	Drop-in Demonstrations Presentation: 2:15 pm
Friday, March 9	9:00 am–5:00 pm	Drop-in Demonstrations Presentations: 10:30 am and 2:15 pm
Saturday, March 10	9:00 am–5:00 pm	Drop-in Demonstrations Presentations: 10:30 am and 2:15 pm

- **GSA Mentor Roundtable – Level Four – Room 413**

  This session will be held on Friday, March 9, from 12:45-1:45 pm. Principal investigators will discuss their work and give valuable advice and input on career opportunities. For interested graduate and post docs. Sign up at the Level Four registration desk by Thursday, March 8, at 5:00 pm. *Limited Attendance.*

- **Joint Steering Committee for Public Policy (JSC) – Allie's American Grill**

  On Saturday, March 10, from 12: 45-1:45 pm, Lynn Marquis of the JSC will lead a discussion on why grants are in jeopardy and how scientists can tell their stories to the Congressional representatives. Sign up at the Level Four registration desk by Friday, March 9 by 5:00 pm. *Limited Attendance.*

Meals

Please note that **only two meals are included in the meeting registration**: the Opening Mixer on Wednesday night (with heavy hors d'oeuvres and a cash bar) and the Continental Breakfast on Thursday morning. All other meals are not part of your conference registration. For all other meals you may choose from options within the hotel or at nearby locations. Starbucks is located within the hotel along with Allie's American Grill. Both serve breakfast, lunch and dinner. Champions and the Sushi Bar are also open for lunch and dinner. For fine dining, go to JW's Steakhouse. If you would like to venture out of the hotel you don't have to go very far. The Reading Terminal Market is right next to the hotel and offers dozens of options for a quick meal.

Message Boards – Level Four – Franklin Hall Foyer

Message boards will be located near the Registration and Information Counters.

Parking

Parking is available at the Philadelphia Marriott Downtown at a rate of $35 per day. There is also parking nearby. Contact the Philadelphia Marriott Downtown (1-800-320-5744) for other parking options.

Poster Sessions and Exhibits – Level Four – Franklin Hall

All posters will be displayed in the Exhibition Hall. The Hall will be open to Conference registrants on a 24-hour basis beginning at 5:00 pm, Wednesday, March 7, until 10:00 AM, Sunday, March 11. **Security will be posted at the entrance to the Hall and only individuals with official Drosophila Conference registration badges will be admitted**. Guest pass applications may be made at the registration counter during regular registration hours.

Exhibit representatives will be in their booths:

Thursday, March 8:	2:00 pm–4:00 pm and 8:00 pm–11:00 pm
Friday, March 9:	2:00 pm–4:00 pm and 8:00 pm–11:00 pm
Saturday, March 10:	1:30 pm–3:30 pm and 8:00 pm–11:00 pm

Authors are expected to be present at their boards according to the following schedule:

Thursday, March 8:	2:00 pm–3:00 pm	Even-numbered Posters
	3:00 pm–4:00 pm	Odd-numbered Posters
	8:00 pm–9:00 pm	"A" posters
	9:00 pm–10:00 pm	"B" posters
	10:00 pm–11:00 pm	"C" posters
Friday, March 9:	8:00 pm–9:00 pm	"C" posters
	9:00 pm–10:00 pm	"B" posters
	10:00 pm–11:00 pm	"A" posters
Saturday, March 10:	1:30 pm–2:30 pm	Odd-numbered Posters
	2:30 pm–3:30 pm	Even-numbered Posters
	8:00 pm–11:00 pm	Open poster viewing (Authors not required to be present)

All posters must be removed from poster boards no later than 10:00 am on Sunday, March 11. After that time, remaining posters will be removed by vendors and may be lost or thrown away. The GSA Administrative Office does not take responsibility for posters that are not removed by 10:00 am on Sunday.

Registration and Book Pickup – Level Four – Franklin Hall Foyer

Conference registration and book pickup will be open on Level Four in the Franklin Hall Foyer, as follows:

Wednesday, March 7	3:30 pm–9:00 pm
Thursday, March 8	8:00 am–5:00 pm
Friday, March 9	8:30 am–5:00 pm
Saturday, March 10	8:30 am–4:00 pm
Sunday, March 11	Closed

Note that attendees must be registered before attending the Opening General Session on Wednesday, March 7, 7:00 pm.

Smoking

Smoking is allowed only in designated pre-function areas and outdoors.

Larry Sandler Memorial Lecture

The Larry Sandler Memorial Lecture was established in 1988 by the colleagues, friends and students of Dr. Larry Sandler after his untimely death in February 1987. The award serves to honor Dr. Sandler for his many contributions to Drosophila genetics and his exceptional dedication to the training of Drosophila biologists. Any student completing his Ph.D. in an area of Drosophila research in the calendar year preceding the annual Drosophila Research Conference is eligible and may be nominated by his/her dissertation advisor. The Selection Committee for 2006 includes Chair Helen Salz, R. Scott Hawley, Mariana Federica Wolfner, and James W. Erickson. The Committee reviews nominations, reads the dissertations of the finalists, and selects the awardee. Past recipients of this honor are:

Bruce Edgar, 1988
Kate Harding, 1989
Michael Dickinson, 1990
Maurice Kernan, 1991
Doug Kellogg, 1992
David Schneider, 1993
Kendal Broadie, 1994
David Begun, 1995
Chaoyong Ma, 1996
Abby Dernburg, 1997
Nir Hacohen, 1998
Terence Murphy, 1999
Bin Chen, 2000
James Wilhelm, 2001
Matthew C. Gibson, 2002
Sinisa Urban, 2003
Sean McGuire, 2004
Elissa Hallem, 2005
Daniel Ortiz-Barrientos, 2006

The DeLill Nasser Award for Professional Development in Genetics

The DeLill Nasser Award for Professional Development in Genetics was established by The Genetics Society of America in mid-2001 in honor of the late DeLill Nasser, who served for 22 years as the program director of the Eukaryotic Genetics Section at the National Science Foundation.

The award fund, made possible by contributions from her family and friends, is growing steadily. It recognizes Dr. Nasser's contributions to the field of genetics and her strong support of young scientists. Travel and tuition awards will be made annually to allow graduate students and postdoctoral trainees to attend meetings or enroll in laboratory courses.

Since 2002, deserving young investigators have been awarded to attend genetics research conferences.

2002 Awardees
Amy Rice, Indiana University
Kristin L. Latham, Oregon State University
Joshua Chern, Baylor College of Medicine
Tim Christensen, Cornell University

2003 Awardee
Sandra M. Leal, Saint Louis University Medical School

2004 Awardee
Sue L. Jaspersen, University of Colorado – Boulder

2005 Awardees
Joshua C. Mell, University of California, Davis
Honorable Mention
Elena A. Repnikova, Texas A&M University

2006 Awardees
Anjon Audhya, Ludwig Institute for Cancer Research
Gil B. Carvalho, California Institute of Technology
Atina G. Cote, Hospital for Sick Children, University of Toronto
Elissa P. Lei, The Johns Hopkins University
Kirki Tsigari, Alexander Fleming Biomedical Research Institute

2007 Awardees
William W. Ja, California Institute of Technology
Ya-Chieh Hsu, Baylor College of Medicine

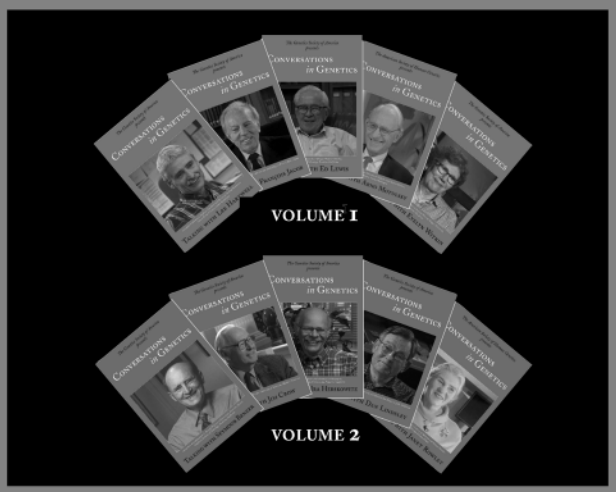
GSA strongly encourages all of its members and friends to donate. To date over $41,000 has been raised. Up to five percent of the fund amount will be awarded each year. Please make your check payable to The Genetics Society of America and send it to Elaine Strass, GSA, 9650 Rockville Pike, Bethesda, MD 20814-3998. Please write "Nasser Fund" on the bottom left of the check.

Volume III

Conversations *in* Genetics

AN ORAL HISTORY OF OUR INTELLECTUAL HERITAGE IN GENETICS

The Genetics Society of America and The American Society of Human Genetics are developing a collection of videotaped conversations with geneticists who have made major contributions to the conceptual foundations of modern genetics. "Conversations in Genetics" promises to become a rich resource for anyone interested in the history of genetics and the evolution of scientific ideas. The individual presentations reflect the thoughts and feelings of accomplished researchers as they recall their research achievements and describe the paths they took during various phases of their lives.



Volume 3

CONVERSATIONS WITH:	INTERVIEWED BY:
Elizabeth Blackburn	*Joe Gall*
Sydney Brenner	*Barbara Meyer*
Victor McKusick	*Judith Hall*
Piotr Slonimski	*Giuseppe Attardi*
Charles Yanofsky	*David Botstein*

And More To Come!

For further information and to order, visit our website: *www.genestory.org*

Executive Producer & Scientific Editor Rochelle Easton Esposito.

Video Production Aaron Stadler and Fat Cat Vdo.

GUIDELINES FOR MAKING PLATFORM PRESENTATIONS

IMPORTANT:

Each platform presenter has a total of 15 minutes —12 minutes to speak plus 3 minutes for questions/answers/discussion.

Each platform presenter **MUST BRING HIS OR HER OWN LAPTOP COMPUTER** TO MAKE THE PRESENTATION. You may be asked to e-mail your final presentation in advance of the conference. If so, you will be notified in February.

Please follow the instructions below **PRECISELY**:

1. **You must supply your own laptop computer.** A data projector (with necessary cables) will be supplied. IMPORTANT: Those using a Macintosh computer must bring their own MAC to VGA adaptor (usually supplied with computer).

2. **You must test/preview your computer-generated presentation** at the meeting, in the meeting room, two hours prior to the beginning of your assigned platform session time block (note: this is not the beginning of your presentation but rather the beginning of the block of time in which your presentation will be given).

3. **You must bring a back-up of the presentation on a portable CD or drive in case your laptop should fail.** If a presenter's laptop fails, the presenter is responsible to find a replacement, perhaps by borrowing a friend's.

4. **If you must cancel your presentation, or you wish to change presenters**, please notify Suzy Brown at the GSA Administrative Office sbrown@genetics-gsa.org no later than January 30. Updated information received by that date will be included in the program addendum.

5. Please disable any password protection or automatic timeout on your laptop.

6. Please pay close attention to your time. There are other presenters during the same time slot and we want all presenters to be able to have their full 12 minutes of presenting time. If your Q&A time runs over, please meet with participants in the lobby outside of the meeting room to continue your conversation so that the program can stay on schedule.

HELPFUL TIPS FOR EFFECTIVE PRESENTATIONS

Your presentation should help clarify ideas, emphasize key points, show relationships, and provide the visual information your audience needs to understand your message. Please consider the following suggestions as you plan your presentation:

A. Keep visuals clear and easy to read. Abbreviate your message. Simple graphs, charts and diagrams are much more meaningful to an audience than complex, cluttered ones. When preparing your presentation, limit the information on each screen to a single point or idea, and ideally, not more than 5 lines of text per screen. Keep each screen simple with plenty of open space.

B. Avoid using too many patterns and graphics in one frame.

C. Use a minimum of words for text and title frames. Five to eight lines per frame and five to seven words per line are the maximum–fewer is better.

D. Choose upper and lower case lettering, which is more legible than all capital letters.

E. Vary the size of lettering to emphasize headings and subheadings, but avoid using more than three sizes per frame.

F. Select sans serif type (example: Arial), which projects better and is easier to read than serif type.

G. Maintain the same or similar type sizes from frame to frame, even if some frames have less copy than others.

H. Keep all type horizontal, even in charts.

I. Consider color with care. A dark background with highly contrasting text and graphics is most readable. Cool colors (example: deep blue, turquoise, purple) appear to recede and make white or light colored text more readable. In one study, blue was found to be the most effective background color for projection. Do not use red for text; it is extremely difficult to read.

J. Highlight your main point or heading with a dominant color (example: yellow for the heading, white for body text). Avoid the use of intensely bright or saturated colors that compete with the text.

K. Maintain a consistent color scheme. Use no more than six colors throughout your presentation.

L. Select backgrounds to enhance your text or graphics. A background that transitions smoothly from lighter to darker shades of the same hue can be effective. Some software packages permit the gradation from one color to another. A textured background can be effective, but it should not detract from or compete with text or images.

M. Consider photographs for added interest. Combined with simple, straightforward graphics, illustrations, cartoons and artwork, photos can bring another dimension to your presentation.

N. Remember the basics of good design: Plan a template. Use colors consistently with light fonts on a dark background. Keep text clear and easy to read.

GUIDELINES FOR MAKING POSTER PRESENTATIONS

All posters will be located in Franklin Hall on Level Four of the Philadelphia Marriott. Authors may mount their posters on Wednesday, March 7, from 5:00 PM until 11:00 PM. **You must be wearing your official meeting badge to gain entry to the Exhibition Hall.**

IMPORTANT: POSTER BOARD SIZE AND FORMAT

Each author is allotted one-half of a 4'h x 6'w board, or net space of 3'8" (111.8 cm) HIGH by 2'10" (86.36 cm) WIDE and posters should be formatted in "PORTRAIT" format. Posters using more space than allotted will be removed. Posters will be displayed throughout the duration of the meeting and may be viewed 24 hours a day beginning Wednesday, March 7, at 5:00 PM until Sunday morning, March 11. **All posters must be removed from the boards by no later than 10:00 AM, Sunday, March 11.** The GSA Administrative Office staff, the hotel staff, and the personnel breaking down the boards will not be responsible for posters left on boards by their authors. Work crews will remove posters that remain on the poster boards after 10:00 AM Sunday.

Poster sessions are scheduled as follows:

Thursday, March 8

Poster Session

2:00 PM-3:00 PM	Even-numbered poster authors must be at boards
3:00 PM-4:00 PM	Odd-numbered poster authors must be at boards

Poster Session

8:00 PM-9:00 PM	"A" poster authors must be at boards
9:00 PM-10:00 PM	"B" poster authors must be at boards
10:00 PM-11:00 PM	"C" poster authors must be at boards

Friday, March 9

Poster Session

8:00 PM-9:00 PM	"C" poster authors must be at boards
9:00 PM-10:00PM	"B" poster authors must be at boards
10:00 PM-11:00 PM	"A" poster authors must be at boards

Saturday, March 10

Poster Session

1:30 PM-2:30 PM	Odd-numbered poster authors must be at boards
2:30 PM-3:30 PM	Even-numbered poster authors must be at boards

Open Viewing

8:00 PM-11:00 PM

As shown in the list above, for each of the poster sessions, authors have been assigned a one-hour time slot during which they are expected to be at their poster for discussion. Additional poster times also have been scheduled on Saturday evening, during which authors may choose to be present at their posters.

Poster Design and Preparation

The poster should be designed to summarize current research in graphic forms, i.e., charts, tables, graphs, and pictures. Simple use of color can add emphasis. Remember that the poster must be readable from a distance of at least 3 feet. Presentations should be self-explanatory so that the author is free to supplement and discuss particular points. For easy identification, provide a poster heading, listing its title and author(s). Lettering for the title should be at least 1" in height.

Poster materials may be mounted on thin poster paper or cardboard and attached to the poster board with push pins. **GSA will provide approximately 25 pins per board. They will be available near the entrance to the Exhibition Hall.** Do not mount your poster on heavy or thick backing, as it may be difficult to fasten to the board. Do not write or paint on the board. If you require assistance with mounting or removing your poster, please notify the GSA staff at the registration desk.

EXHIBITORS

As exhibitors at the 48th Annual Drosophila Research Conference, the following companies have contributed to the support of this Conference. Registrants are encouraged to visit the exhibits in the Exhibition Hall and to take advantage of this opportunity to see new products, publications and services available from these companies.

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- **WEDNESDAY, MARCH 7 7:00 pm–9:00 pm**

OPENING GENERAL SESSION

Room: Grand Ballroom Salons E-H
Moderator: Steve DiNardo, University of Pennsylvania Medical School, Philadelphia

Presentations:

7:00 pm **Welcome and Opening Remarks.** Steve DiNardo. University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

7:10 pm **Introduction of Historical Address Speaker.** Mark Fortini. National Cancer Institute, Frederick, Maryland.

7:20 pm **Historical Address.** Spyros Artavanis-Tsakonas. Harvard Medical School/MGH Cancer Center, Boston, Massachusetts.

8:10 pm **Introduction of Larry Sandler Memorial Lecture.** Helen Salz. Case Western Reserve University, Cleveland, Ohio.

- **THURSDAY, MARCH 8 8:30 am–12:00 noon**

PLENARY SESSION I

Room: Grand Ballroom Salons E-H
Moderator: Liz Gavis, Princeton University, New Jersey

Presentations:

8:30 am **Image Award Presentation.** Liz Gavis, Princeton University, New Jersey.

8:35 am **Circadian Clocks and Sleep.** Ravi Allada. Northwestern University, Evanston, Illinois.

9:00 am **Membrane and Organelle Traffic in Neurons.** Thomas Schwarz. Children's Hospital, Boston, Massachusetts.

9:30 am **Taste Recognition in Drosophila.** Kristin Scott. University of Iowa, Iowa City.

10:00 am Break

10:30 am **Regulation of Cell Surface Mechanics Underlying Tissue Morphogenesis.** Thomas Lecuit. IBDM, Marseille Cedex, France.

11:00 am **Progressive Heterochromatin: Players and Pathways.** Lori Wallrath. University of Iowa, Iowa City.

11:30 am **Mechanisms of P Element Transposition and Alternative Pre-mRNA Splicing.** Donald Rio. University of California, Berkeley.

- **SUNDAY, MARCH 11 8:30 am–12:00 noon**

PLENARY SESSION II

Room: Grand Ballroom Salons A-F
Moderator: Jessica Treisman, NYU Medical Center, New York, New York

Presentations:

8:30 am **GSA Poster Award Presentation.** Jessica Treisman, NYU Medical Center, New York, New York.

8:35 am **Evolution of Gene Regulation in Drosophilids.** Michael Eisen. University of California, Berkeley.

9:00 am **Signatures of Speciation in the Pseudoobscura Species Group.** Mohamed Noor. Duke University, Durham, North Carolina.

9:30 am **Glial Function in Nervous System Development.** Ulrike Gaul. Rockefeller University, New York, New York.

10:00 am Break

10:30 am **Color Vision.** Claude Desplan. New York University, New York.

11:00 am **Regulatory Mechanisms Controlling Directed Cell Migration.** Pernille Rørth. EMBL, Heidelberg, Germany.

11:30 am **Origin of the Brain Endocrine Axis: Insights for Type 1 Flyabetes.** Eric Rulifson. University of Pennsylvania, Philadelphia.

- **WEDNESDAY, MARCH 7 12:00 noon–6:00 pm**

ECDYSONE WORKSHOP

Room: Grand Ballroom Salons A-D
Organizers: **Robert Ward**, University of Kansas, Lawrence, and **Craig Woodward**, Mount Holyoke College, South Hadley, Massachusetts

Summary: The Ecdysone Workshop welcomes all those interested in the biochemistry, molecular biology, and physiology of insect hormones. As such, we will discuss mechanisms of 20-hydroxyecdysone, juvenile hormone, and other peptide hormone signaling in Drosophila, Manduca, Aedes, and other insect species. Platform talks of approximately 15 minutes will be grouped into sessions according to topic. Topics for presentation include (but will not be limited to): receptor complex/target gene interactions, downstream tissue-specific responses, and global effects of hormone exposure. Programs will be supplied upon request by email (robward@ku.edu or cwoodard@mtholyoke.edu) after March 1.

- **FRIDAY, MARCH 9 1:45 pm–3:45 pm**

CELL DEATH

Room: Grand Ballroom Salon E
Organizers: **Rebecca Hays**, University of Kansas, Lawrence, and **Andreas Bergmann**, University of Texas, Houston, and **Jamie Rusconi**, SUNY, Albany, NY

Summary: The Cell Death Workshop is a forum for the discussion of Drosophila apoptosis. The workshop is intended to highlight recent advances in apoptosis research and to foster communication and collaboration between individuals working in the area. Topics may include all aspects of apoptosis regulation and the importance of apoptosis in Drosophila biology.

Presenters:

1) Ionizing radiation induces caspase-dependent but Chk2- and p53-independent cell death. Anita Wichmann[1], Burnley Jaklevic[2], Tin Tin Su[1]. 1) Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; 2) Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley.

2) The Drosophila SUMO conjugase Lesswright regulates apoptosis and cell survival during larval hematopoiesis. Jinu Abraham[1], Liang Huang[1,2], Soichi Tanda[1]. 1) Department of Biological Sciences and MCB Program, Ohio University, Athens; 2) Present Address: National Institute of Health, Bethesda, Maryland.

3) SCF ubiquitin ligase complex mediates phagocytosis through the novel F-box domain protein, Pallbearer. Nathalie Franc, Connie Au-Yeung, Emeline van Goethem, Elizabeth Silva. MRC LMCB & CBU, Univ Col London, London, Great Britain.

4) The Morgue F-box protein is required for mitochondrial membrane depolarization and commitment to apoptosis. Rebecca Hays, John Means. Department of Biological Sciences, University of Kansas, Lawrence.

5) A new type of apoptosis-induced compensatory proliferation requires activity of effector caspases. Yun Fan, Andreas Bergmann. Department of Biochemistry & Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston.

6) klumpfuss, the fly homologue of Wilm's Tumor Supressor 1 and apoptosis. Jamie Rusconi, Department of Biological Sciences, State University of New York, Albany.

7) The caspases Strica and Dronc function redundantly during programmed cell death in oogenesis. Kim McCall[1], Jason S. Baum[1], B. Paige Bass[1], Jeanne S. Peterson[1], Antony Rodriguez[2], John M. Abrams[2]. 1) Department of Biology, Boston University, Massachusetts; 2) Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas.

8) Shaggy is required for ethanol-induced olfactory receptor neuron apoptosis. Rachael French, Ulrike Heberlein. Department of Anatomy, University of California, San Francisco.

- **FRIDAY, MARCH 9 1:45 pm–3:45 pm**

A DOZEN FLY GENOMES: WHAT HAVE WE LEARNED AND WHAT'S NEXT?

Room: Grand Ballroom Salons H-J
Organizers: **William M. Gelbart**, Harvard University, Cambridge Massachusetts, and **Thomas C. Kaufman**, Indiana University, Bloomington

Topics covered in the workshop will be:

1. What is the current state of analysis of the 12 genomes with regard to:

   a. Using comparative approaches to annotate D. melanogaster?

   b. Identifying the gene sets in the other species, including lineage-specific genes?

   c. Understanding evolution of the genes and genomes of the genus Drosophila?

2. What data sets or analytical tools are most needed to move these analyses forward?

3. Is there a compelling case for sequencing additional Drosophila species, and if so, why and which species?

- **FRIDAY, MARCH 9 1:45 pm–3:45 pm**

DROSOPHILA RESEARCH AND PEDAGOGY AT PRIMARILY UNDERGRADUATE INSTITUTIONS

Room: Grand Ballroom Salons G, K-L
Organizer: **Don Paetkau**, Saint Mary's College, Notre Dame, Indiana

Summary: This workshop focuses on increasing visibility of research performed at primarily undergraduate institutions and to facilitate faculty and students in their endeavors. The goals include:

1) encouraging undergraduate research by providing a forum in which undergraduate students make oral presentations.

2) connecting people interested in this career path with people already in primarily undergraduate institution (PUI) faculty positions.

3) connecting PUI faculty for discussions and support on professional issues that differ from those at large institutions.

4) sharing ideas for using Drosophila as teaching tools in the classroom and laboratory.

5) providing resources for PUI investigators. The workshop is divided into two components: Research presentations made by undergraduate students followed by break-out discussion groups.

- **SATURDAY, MARCH 10 6:45 pm–8:45 pm**

IMMUNITY, HEMATOPOIESIS, AND PATHOGENESIS

Room: Grand Ballroom Salon E
Organizers: **Todd Schlenke**, Emory University, Atlanta, Georgia, and **Brian Lazzaro**, Cornell University, Ithaca, New York

Summary: This workshop will focus on the cells and molecules that control humoral and cellular immune responses in Drosophila, on the pathology of microbial disease in Drosophila, and on pathogen mechanisms for defeating the fly immune system. Topics covered will include natural pathogens of Drosophila and their virulence mechanisms, molecular biology of hematopoiesis and blood clot formation, and transcriptional regulation of immunity signaling cascades.

Presentations:

Undertaker encodes a junctophilin-related molecule required for phagocytosis of apoptotic cells. Nathalie C. Franc, Leigh Cuttell, Emeline Van Goethem, Claire Escaron, Christina Bakatselou, Mark Lavine, Magali Quirin.

Rel signaling guides immune homeostasis in Drosophila. Nina Matova, Kathryn V. Anderson.

The Salmonella effector AvrA modulates JNK pathway signaling in Drosophila. Rheinallt Jones, Christy Wentworth, Andrew S. Neish.

A comparative dissection of innate immune pathways in Drosophila melanogaster using RNA interference. David Kuttenkeuler and Michael Boutros

Innate immunity and circadian rhythm. Mimi Shirasu-Hiza, Marc Dionne, Linh Pham, Janelle Lamberton, David Schneider.

Analysis of Drosophila TAB2 mutants reveals that IKK, but not JNK pathway activation, is essential in the host defense against *Escherichia coli* infections. Dominique Ferrandon.

- **SATURDAY, MARCH 10 6:45 pm–8:45 pm**

CELL CYCLE CHECKPOINTS

Room: Grand Ballroom Salons G, K-L
Organizers: **Tin Tin Su**, University of Colorado, Boulder, and **Claudio Sunkel**, University of Porto, Portugal

Summary: This workshop will concentrate on mechanisms that regulate the cell cycle in response to external and internal signals.

Preliminary Program:

Dissecting checkpoint pathways controlling meiotic progression. Vitor Barbosa, NYU Medical Center.

BubR1, but not Mad2, is required for recruiting and localizing of Fzy to the kinetochores in Drosophila melanogaster. Junyong Huang, University of Newcastle upon Tyne.

Mad2 plays an essential role in timing progression through mitosis. Claudio Sunkel, University of Porto

Chk2 and p53 in DNA damage responses. Tin Tin Su, University of Colorado, Boulder.

miRNA control of stem cell division. Hannele Ruohola-Baker, University of Washington, Seattle.

Drosophila Myb regulates chromosome condensation and spindle assembly checkpoint components. Joseph Lipsick, Stanford University School of Medicine.

Checkpoint responses to DNA re-replication. Brian Calvi, Syracuse University.

- **SATURDAY, MARCH 10 6:45 pm–8:45 pm**

RNAi HIGH-THROUGHPUT SCREENING

Room: Grand Ballroom Salons H-J
Organizers: **Bernard Mathey-Prevot**, Harvard Medical School, Boston, Massachusetts, and **Steven Suchyta**, Ambion Inc., Austin, Texas

Summary: The goal of the workshop is to discuss the use of RNAi technology, covering advances made in high throughput screens enabling discovery and characterization of gene function, both in tissue culture and *in vivo*. The focus will be placed on the availability and use of different dsRNA libraries for HTS in tissue culture, discussing inherent issues with the use of dsRNAs, as well as on recent efforts to design efficient vectors and comprehensive short hairpin collections for RNA interference *in vivo*.

In Vivo RNAi

The NIG RNAi fly bank - a resource for functional genomics. Ryu Ueda, Kuniaki Takahashi, Yukiko Sado, and Kazuko, Fujitani.Genetic Strains Research Center, National Institute of Genetics(NIG), Japan.

Optimizing in vivo RNAi with site specific integration, flexible UAS sites, introns, and insulators. Michele Markstein[1], Jianquan Ni[1], Luping Ni[1], Rich Binari[1], Christians Villalta[1], Barret Pfeiffer[2], Todd Laverty[2], Gerry Rubin[2], Norbert Perrimon[1]. 1) Harvard Medical School, Boston MA 02115, and Howard Hughes Medical Institute; 2) Janelia Farm, Ashburn, VA 20147 and Howard Hughes Medical Institute).

The Vienna Drosophila RNAi library and stock center. Krystyna Keleman[1,2], Georg Dietzl[1], Doris Chen[2], Michaela Fellner[1], Kaolin Kinsey[1], Sylvia Oppel[1], and Barry J. Dickson[1]. 1) Research Institute of Molecular Pathology (IMP); 2) Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Austria.

HTS Screens

Genome-wide RNAi screening for factors targeting Smad into the nucleus. Lan Xu, Xiaohao Yao, Xiaochu Chen. Program in Molecular Medicine, Univ. of Massachusetts Medical School, Worcester, MA 01605

Functional genomics of stress-induced apoptosis. Su Kit Chew, Kristi R. Pogue and John M. Abrams. Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75390

Chemical genomics and genome-wide RNAi or how to learn more about your favorite drugs. Marc Hild, Ranjani Padmanabhan, Xiaoying Shi, Qiong Wang, Haidi Yang, Fred Harbinski, Vic Myer, John Tallarico and Dan Garza. Developmental and Molecular Pathways; Drosophila Genetics Novartis Institutes for BioMedical Research Cambridge, MA 02139

A 5 minute period is reserved after each talk for questions.

- **SATURDAY, MARCH 10 9:30 pm–11:30 pm**

WORKSHOP ON RNA BIOLOGY

Room: Grand Ballroom Salon E
Organizer: **A. Javier Lopez**, Carnegie Mellon University, Pittsburgh, Pennsylvania

Summary: RNA plays essential roles as a target and mediator of regulation in diverse biological processes. This workshop will bring together investigators who study different aspects of RNA biology for informal presentations and discussions of common interest. Topics covered will include the localization of RNA, translational control, RNA-protein interactions, RNA-mediated interference and related phenomena, and processing of pre-mRNA.

Presentations:

The dynamics of fluorescently labelled endogenous gurken mRNA during Drosophila oogenesis. Angela M. Jaramillo, Timothy T. Weil, Elizabeth R. Gavis, Trudi Schupbach. HHMI/Department of Molecular Biology, Princeton University.

An in vivo imaging system for visualizing Fragile X protein and associated mRNA in Drosophila neurons. Marianna Pinter, Patty Estes, Adeel Yang, Daniela Zarnescu Department of Molecular and Cellular Biology, University of Arizona.

Understanding the role of the RNA-binding protein Orb2 in Drosophila development. Nathaniel Hafer, Shuwa Xu, Paul Schedl. Department of Molecular Biology, Princeton University.

Cup antagonizes Orb-mediated translational activation. Li Chin Wong[1], Alexandre Costa[2], Ian McLeod[3], John Yates III[3], Paul Schedl[1]. 1) Department of Molecular Biology, Princeton University; 2) Stanford University; 3) The Scripps Research Institute.

Molecular basis of RNA recognition by the translational repressor and hnRNP F/H homolog Glorund. Yossi Kalifa, Elizabeth Gavis Department of Molecular Biology, Princeton University.

RISC in Sex. Erica Kleinman, Jamila Horabin Biomedical Sciences Department, Florida State University.

Interactions between PIWI and HP1 suggest a link between the RNAi system and heterochromatin formation in Drosophila. S.C.R. Elgin[1], B. Brower-Toland[1], S. Findley[2], and H. Lin[3]. 1) Washington University in St Louis, 2) University of Missouri, 3) Yale University.

Evolutionary dynamics of recursive splicing. A. Javier Lopez, Panagiotis Papasaikas, Michael Chen, Aly Khan, Russell Schwartz. Department of Biological Sciences, Carnegie Mellon University.

- **SATURDAY, MARCH 10 9:30 pm–11:30 pm**

EXTRACELLULAR MATRIX INTERACTIONS AND SIGNALING

Room: Grand Ballroom Salons G, K-L

Organizer: **Halyna Shcherbata**, University of Washington, Seattle

Summary: Recent findings discussed in this workshop will show that the extracellular matrix proteins do not act simply as structural proteins or as migratory substrates, but also as sources of signaling information that regulate cell morphology, cytoskeletal rearrangements, polarity, morphogenesis, and signal transduction. The presentations in this workshop will focus on extracellular matrix molecules and their cell-surface receptors that play important roles in a broad array of developmental processes.

THURSDAY, MARCH 8 4:30 pm–6:30 pm
Grand Ballroom Salon E

Immune System and Cell Death

Moderator: David Schneider, Stanford University, California

1 - 4:30
Epidemiology of bacterial disease in wild *D. melanogaster*. **Punita Juneja, Brian P. Lazzaro.** Department of Entomology, Cornell University, Ithaca, NY.

2 - 4:45
The relationship between immunity and microbial community richness across natural populations of *D. melanogaster*. **Vanessa Corby-Harris, Daniel E. L. Promislow.** Dept Genetics, Univ Georgia, Athens, GA.

3 - 5:00
The Drosophila immune response exhibits specificity and memory. **Linh Pham, Marc Dionne, David Schneider.** Dept Microbiology & Immunology, Stanford University, Stanford, CA.

4 - 5:15
Using Drosophila to uncover host factors involved in viral pathogenesis. **Sara Cherry, Spencer Shelly, Terri Moser, Marta White.** Department of Microbiology, Penn Genomics Institute, University of Pennsylvania, Philadelphia, PA.

5 - 5:30
Functional genomics of stress-induced apoptosis. **Su Kit Chew, Kristi R. Pogue, John M. Abrams.** Department of Cell Biology, Univ Texas Southwestern Medical Center, Dallas, TX.

6 - 5:45
Live Imaging Programmed Cell Death in the Extraembryonic Amnioserosa. **Bruce H. Reed[1], Nilufar Mohseni[1], Howard Lipshitz[2,3].** 1) Department of Biology, University of Waterloo, 200 University Ave. W., Waterloo, Ontario N2L 3G1, Canada; 2) Program in Developmental Biology, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; 3) Department of Molecular and Medical Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.

7 - 6:00
The leucine zipper transcription factor Bunched regulates cell survival. **Xiaodong Wu[1], Megumu Mabuchi[1], Silvia Gluderer[3], Erick Morris[2], Hugo Stocker[3], Nick Dyson[2], Ernst Hafen[3], Laurel Raftery[1].** 1) Cutaneous Biology Research Center, Massachusetts General Hosp, Charlestown, MA; 2) MGH Cancer Center, Massachusetts General Hosp, Charlestown, MA; 3) Zoological Inst., Univ. Zurich, Zurich, Switzerland.

8 - 6:15
Determining the role of the Drosophila Bcl-2 proteins in mediating autophagy and apoptosis. **Jessica P. Monserrate, Yenyun Chen, Evgueni A. Sevrioukov, Carrie B. Brachmann.** Dev Cell Biol, Univ California, Irvine, Irvine, CA.

THURSDAY, MARCH 8 4:30 pm–6:30 pm
Grand Ballroom Salons H-J

Organogenesis

Moderator: Mary Baylies, Memorial Sloan-Kettering Cancer Center, New York, New York

9 - 4:30
Homeostatic control of germ cell numbers in the Drosophila gonad. **Lilach Gilboa[1], Ruth Lehmann[1,2].** 1) Developmental Genetics, NYU Med Ctr, Skirball Inst, New York, NY; 2) Howard Hughes Medical Institute.

10 - 4:45
Transcriptional Control of Apical Mechanics During Tube Morphogenesis. **Bilal E. Kerman[1,5], Alan M. Cheshire[1,2,5], Warren R. Zipfel[3], Monn Monn Myat[4], Alexander A. Spector[2], Deborah J. Andrew[1].** 1) Dept. of Cell Biology, Johns Hopkins SOM, Baltimore, MD; 2) Dept. of Biomed. Engineering, Johns Hopkins SOM, Baltimore, MD; 3) Developmental Resource for Biophysical Imaging Opto-Electronics, Cornell Univ., Ithaca, NY; 4) Dept. of Cell and Dev. Biology, Weill Medical College of Cornell Univ., New York, NY; 5) These authors contributed equally.

11 - 5:00
Communication and rearrangement among tracheal epithelial cells during branching morphogenesis. **Amin Ghabrial, Mark Krasnow.** Biochemistry and HHMI, Stanford Univ., Stanford, CA.

12 - 5:15
The Archipelago tumor suppressor protein regulates Drosophila tracheal development via the HIF-1α homolog Trachealess. **Nathan T. Mortimer, Kenneth H. Moberg.** Department of Cell Biology, Emory University, Atlanta, GA.

13 - 5:30
A hematopoietic niche defined by Antennapedia expression uses Hedgehog for the maintenance of blood cell precursors in Drosophila. **Lolitika Mandal, Julian Agosto- Martinez, Cory Evans, Utpal Banerjee, Volker Hartenstein.** Dept MCD Biol, Univ California, Los Angeles, Los Angeles, CA.90095.

14 - 5:45
Daughterless dictates Twist activity in a context dependent manner during somatic myogenesis. **Ming-Ching Wong[1], Mary Baylies[2].** 1) Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

15 - 6:00
Perdido encodes a component of a protein complex required for muscle guidance in Drosophila embryonic muscles. **Beatriz Estrada[1], Stephen S. Gisselbrecht[1], Alan M. Michelson[1,2].** 1) Dept Medicine/Genetics, BWH/HMS, Boston, MA; 2) National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

16 - 6:15
Requirement of the LIM homeodomain transcription factor Tailup for normal heart and hematopoietic organ formation in Drosophila. **Ye Tao, Jianbo Wang, Tsuyoshi Tokusumi, Kathleen Gajewski, Robert Schulz.** Department of Biochemisry & Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

THURSDAY, MARCH 8 4:30 pm–6:30 pm
Grand Ballroom Salons G, K-L

Neurophysiology and Behavior

Moderator: Justin Blau, New York University, New York

17 - 4:30
PI3K regulates neuronal excitability and axonal growth and arborization via distinct effector pathways. **Eric Howlett, William Lavery, Michael Stern.** Biochemistry & Cell Biology, Rice University, Houston, TX.

18 - 4:45
Peripheral multi-dendritic sensory neurons are necessary for locomotive pattern generation in Drosophila larvae. **Wei Song, Maika Onishi, Lily Jan, Yuh-Nung Jan.** HHMI, Dept. of Physiology and Biochemistry, UCSF, San Francisco, CA.

19 - 5:00
Odor coding and discrimination by the entire repertoire of larval receptors. **Dennis Mathew, Scott A. Kreher, John R. Carlson.** MCD Biology, Yale University, New Haven, CT-06511.

20 - 5:15
Identification and characterization of novel genetic pathways underlying feeding motivation in Drosophila. **Benjamin Parrott[1,2], Ping Shen[1,2].** 1) Dept. of Cellular Biology, University of Georgia, Athens, GA; 2) Biomedical and Health Science Institute, University of Georgia, Athens, GA.

21 - 5:30
Interactions between functionally coupled circadian neurons control temperature synchronization of Drosophila behavior. **Patrick Emery, Ania Busza.** Neurobiology, U.. Massachusetts Med. School, Worcester, MA.

22 - 5:45
A new behavior paradigm to study social behavior. **Anne F. Simon, Evelyn Salazar, Man-Ting Chou, David E. Krantz.** Psy. and Biobehavior. Sciences, UCLA, Brain Res Inst, Los Angeles, CA.

23 - 6:00
Specific subgroups of Fru^M expressing neurons control the sexually dimorphic patterns of aggression in *D. melanogaster*. **Yick-Bun Chan, Edward A. Kravitz.** Dept of Neurobiology, Harvard Medical School, Boston, MA.

24 - 6:15
Odor-evoked activities in Drosophila mushroom bodies before, during and after odor-electric shock pairing. **Akira Mamiya, Yalin Wang, Yi Zhong.** Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

FRIDAY, MARCH 9 8:30 am–10:15 am
Grand Ballroom Salon E

Evolution and Quantitative Genetics

Moderator: David Stern, Princeton University, New Jersey

25 - 8:30
Genomic analysis of adaptive differentiation between *D. melanogaster* populations. **Thomas Turner, Mia Levine, David Begun.** Population Biology, Univ California Davis, Davis, CA.

26 - 8:45
7 Mbp of Genomic Polymorphism in *D. melanogaster*. **Michael E. Zwick[1], David Begun[2], David J. Cutler[4], Pieter De Jong[3], Maxim Koriabine[3], Charis Marston[2], Shoshona Lee[1], David Okou[1], Kazutoyo Osoegawa[3], Kristian Stevens[2], Janet A. Warrington[5], Charles H. Langley[2].** 1) Department of Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Section of Evolution and Ecology, University of California at Davis; 3) Children's Hospital Oakland Research Institute, Oakland, CA 94609; 4) McKusick-Nathans Inst of Genetic Medicine, Johns Hopkins Univ School of Medicine, Baltimore, MD 21287; 5) Affymetrix, Inc., Santa Clara, CA.

27 - 9:00
Large-scale turnover of functional transcription factor binding sites in Drosophila. **Daniel Pollard[1,2], Alan Moses[1,2], Stewart MacArthur[1], David Nix[1], Venky Iyer[1,3], Xiao-Yong Li[1], Mark Biggin[1], Michael Eisen[1,2,3].** 1) Dept Genome Sci, LBNL, Berkeley, CA; 2) Biophys Grad Group, UC Berkeley, CA; 3) Mol Cell Bio Dept, UC Berkeley, CA.

28 - 9:15
Evidence for canalization of embryonic pattern formation in *D. melanogaster* and its evolution among closely related species. **Susan E. Lott[1], Michael Z. Ludwig[2], Arnar Palsson[2], Martin Kreitman[1,2].** 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

29 - 9:30
War and Peace in a Fly: How to Resolve a Genetic Conflict? **Yun Tao, Hailian Xiao.** Department of Biology, Emory Univ, Atlanta, GA.

30 - 9:45
Rapid evolution of smell and taste receptor genes during host specialization in *D. sechellia*. **Carolyn McBride.** Center for Population Biology, University of California, Davis, CA.

31 - 10:00
High variation in putative pheromone binding receptors Gr68a and Gr32a in *D. virilis* species group and its role in evolution of mate recognition. **Nikolai Mugue.** Institute of Developmental Biol, Moscow, Russian Federation.

FRIDAY, MARCH 9 8:30 am–10:15 am
Grand Ballroom Salons H-J

Regulation of Gene Expression

Moderator: Stephen Small, New York University, New York

32 - 8:30
Posttranscriptional regulation by the Pan gu kinase in the early Drosophila embryo. **Wael Tadros[1,2], Aaron Goldman[2], Fiona Menzies[1,2], Craig A. Smibert[3], Howard D. Lipshitz[1,2].** 1) Dept Molecular & Medical Genetics, University of Toronto, Toronto, ON, Canada; 2) Dept Developmental Biol, Hosp Sick Children, TDMT, Toronto, ON, Canada; 3) Dept Biochemistry, University of Toronto, Toronto, ON, Canada.

33 - 8:45
Unmasking transcriptional activation during the maternal to zygotic transition. **Stefano De Renzis[1], Olivier Elemento[2], Saeed Tavazoie[2], Eric Wieschaus[1].** 1) Department of Molecular Biology, Princeton University; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University.

34 - 9:00
A "bottoms-up" approach to deciphering transcriptional cis-regulatory grammar in the embryo. **David Arnosti[1,3], Ahmet Ay[2], Chichia Chiu[2], Walid Fakhouri[1].** 1) Dept Biochemistry & Molecular Biology; 2) Dept. of Mathematics; 3) Program in Genetics Michigan State Univ, East Lansing, MI.

35 - 9:15
The dCtBP corepressor attenuates DNA-binding of the Dorsal activator in the Drosophila embryo. **Yutaka Nibu, Hitoshi Aihara, Mark Stern.** Dept Cell & Developmental Biol, Cornell Univ/Weill Medical Col, New York, NY.

36 - 9:30
Molecular logic of ventral appendage formation. **Carlos Estella, Daniel McKay, Richard Mann.** Columbia University, New York, NY.

37 - 9:45
Localized recruitment of TCF/Armadillo induces widespread chromatin remodeling. **David Parker[1], Yunyun Ni[1], Zhenglong Li[2], Ken Cadigan[1].** 1) Dept MCDB, Univ Michigan, Ann Arbor, MI; 2) Biologic & Materials Sci, Ann Arbor, MI.

38 - 10:00
Multiple microRNAs act cooperatively in the developing nervous system to regulate the temporal and spatial expression dynamics of the Drosophila Nerfin-1 protein. **Alexander Kuzin, Mukta Kundu, Thomas Brody, Ward F. Odenwald.** Neural Cell-Fate Determinants Section, NINDS/NIH, Bethesda, MD.

FRIDAY, MARCH 9 8:30 am–10:15 am
Grand Ballroom Salons G, K-L

Cytoskeleton and Cell Biology

Moderator: Jennifer Zallen, Memorial Sloan-Kettering Cancer Center, New York, New York

39 - 8:30
Visualizing changes in *bicoid* mRNA localization dynamics during oogenesis. **Timothy Weil[1], Richard Parton[2], Ilan Davis[2], Elizabeth Gavis[1].** 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Wellcome Trust Centre for Cell Biology, School of Biological Sciences, The University of Edinburgh, Edinburgh, UK.

40 - 8:45
Trailer Hitch is part of a RNA-protein complex that is required for efficient ER exit. **James Wilhelm.** Biological Sciences, UCSD, La Jolla, CA.

41 - 9:00
Analysis of Dynamin function in cytoskeletal remodeling during Drosophila embryogenesis by time-lapse imaging. **Richa Rikhy, Manos Mavrakis, Jennifer Lippincott-Schwartz.** CBMB, NICHD, NIH, Bethesda, MD.

42 - 9:15
Translocation of RhoGEF2 to the furrow canal during cellularization. **Christian Wenzl, Jörg Grosshans.** ZMBH Zentrum für Molekulare Biologie Heidelberg, Heidelberg, Germany.

43 - 9:30
Planar cell polarity and the organization of cell behavior during axis elongation. **Todd Blankenship, Stephanie Backovic, Justina Sanny, Ori Weitz, Jennifer Zallen.** Dept. of Developmental Biology, Memorial Sloan-Kettering, New York, NY.

44 - 9:45
The hh pathway induces non-muscle Myosin II-driven apical epithelial cell constriction and groove formation during organogenesis. **Franck Pichaud.** LMCB, MRC Cell Biology Unit, Dept Anatomy & Dev Biol, University College London, London, UK.

45 - 10:00
Moesin regulates apoptosis by blocking activation of Rho1 and JNK signaling. **Amanda Neisch[1], Olga Speck[2], Richard Fehon[1].** 1) Committee on Developmental Biology and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Department of Biology, Duke University, Durham, NC.

FRIDAY, MARCH 9 10:45 am–12:30 pm
Grand Ballroom Salon E

Evolution and Quantitative Genetics

Moderator: David Stern, Princeton University, New Jersey

46 - 10:45
A sperm's eye view of evolution: Lessons from the Drosophila sperm proteome. **Timothy Karr, Steve Dorus, Ursula Gerike.** Dept Biol & Biochemistry, Univ Bath, Bath, GB.

47 - 11:00
Widespread adaptive evolution of Drosophila genes with sex-biased expression. **John Parsch, Zhi Zhang, Matthias Proeschel.** Biology, University of Munich, Germany.

48 - 11:15
Gain and loss of sex-specific expression of a HOX gene is associated with rapid morphological evolution. **Artyom Kopp, Olga Barmina.** Dept Evolution & Ecology, University California - Davis, Davis CA 95616.

49 - 11:30
New candidate genes for sex comb divergence between *D. mauritiana* and *D. simulans*. **Rita M. Graze[1], Olga Barmina[3], Daniel Tufts[2], Elena Naderi[2], Kristy L. Harmon[2], Maria Persianinova[2], Sergey V. Nuzhdin[2].** 1) Genetics Graduate Group, University of California, Davis, CA 95616; 2) Center for Population Biology, Section of Evolution and Ecology, University of California, Davis, CA 95616; 3) Center for Genetics and Development, University of California, Davis, CA 95616.

50 - 11:45
Genetic basis of pigmentation differences within and between Drosophila species. **Patricia Wittkopp, Belinda Haerum, Emma Stewart, Gabriel Smith-Winberry.** Ecology & Evolutionary Biol, Univ Michigan, Ann Arbor, MI.

51 - 12:00
Comparative genomics of innate immune pathways in Drosophila. **Timothy Sackton[1], Brian Lazzaro[2], Todd Schlenke[3], Jay Evans[4], Dan Hultmark[5], Andrew Clark[1].** 1) Molecular Biology and Genetics, Cornell Univ, Ithaca, NY; 2) Dept of Entomology, Cornell Univ, Ithaca NY; 3) Dept of Biology, Emory Univ, Atlanta GA; 4) Bee Research Lab, USDA ARS, Beltsville MD; 5) UCMP, Umeå Univ, Umeå, Sweden.

52 - 12:15
Ancestral repeats in Drosophila. **Anat Caspi[1], Lior Pachter[2].** 1) Joint Graduate Group Bioengineering, UC Berkeley/UCSF, Berkeley, CA; 2) Department of Mathematics, University of California, Berkeley, CA.

FRIDAY, MARCH 9 10:45 am–12:30 pm
Grand Ballroom Salons H-J

Regulation of Gene Expression

Moderator: Stephen Small, New York University, New York

53 - 10:45
The JAK/STAT pathway regulates heterochromatin formation. **Song Shi, Kimberly Larson, Fan Xia, Willis X. Li.** Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY.

54 - 11:00
Epigenetic switches between precursor cells and differentiation cells during spermatogenesis of Drosophila. **Xin Chen, Margaret Fuller.** Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

55 - 11:15
Ecdysone Receptor, homolog of FXR/RXR, functions in Drosophila as a Type 1 nuclear receptor. **Danika M. Johnston, Yurii Sedkov, Svetlana Petruk, Kristen M. Riley, Miki Fujioka, James B. Jaynes, Alexander Mazo.** Biochemistry, Thomas Jefferson University, Philadelphia, PA.

56 - 11:30
Cell fate in the Drosophila embryo depends on regulation of transcriptional elongation. **Xiaoling Wang, John P. Gergen.** Dept Biochem & Cell Biol, SUNY-Stony Brook, Stony Brook, NY.

57 - 11:45
Transcriptional elongation of non-coding bxd RNAs promoted by the Trithorax TAC1 complex represses Ubx by a transcriptional repression mechanism. **Svetlana Petruk[1], Yurii Sedkov[1], Kristen Riley[1], Jacob Hodgson[2], Francois Schweisguth[3], Susumu Hirose[4], James Jaynes[1], Hugh Brock[2], Alexander Mazo[1].** 1) Department of Biochemistry, Thomas Jefferson University, Philadelphia, PA 19107; 2) Department of Zoology, University of British Columbia, 6270 University Boulevard, V6T 1Z4, Vancouver, BC, Canada; 3) Ecole Normale Superieure, CNRS UMR 8542, Paris, France; 4) Department of Developmental Genetics, National Institute of Genetics, and Graduate University for Advanced Studies, Mishima, Shizuoka-ken 411-8540, Japan.

58 - 12:00
Re-coding of mRNA transcripts in Drosophila: functional consequences and the identification of 27 new targets of ADAR. **Mark Stapleton, Joseph W. Carlson, Susan E. Celniker.** Genome Biology, Lawrence Berkeley Nat'l Lab, Berkeley, CA.

59 - 12:15
In vivo Screening for Nuclear Receptor Agonists and Antagonists in Drosophila. **Aleksandar S. Necakov, Heidi M. Sampson, Henry M. Krause.** The Centre for Cellular and Biomolecular Research, The University of Toronto, Toronto, Ontario, CA.

FRIDAY, MARCH 9 10:45 am–12:30 pm
Grand Ballroom Salons G, K-L

Cytoskeleton and Cell Biology

Moderator: Jennifer Zallen, Memorial Sloan Kettering Cancer Center, New York, New York

60 - 10:45
Antagonistic functions of Par-1 kinase and protein phosphatase 2A are required for localization of Bazooka and photoreceptor morphogenesis in Drosophila. **Sang-Chul Nam[1,2], Bibhash Mukhopadhyay[3], Kwang-Wook Choi[2,3,4].** 1) Department of Biology, Baylor University, Waco, TX; 2) Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Ophthalmology, Baylor College of Medicine, Houston, TX.

61 - 11:00
Recruitment of the cellular actin polymerization machinery is essential for myoblast fusion in Drosophila embryos. **Eyal Schejter, R'ada Massarwa, Shari Carmon, Benny Shilo.** Dept Molecular Genetics, Weizmann Inst Science, Rehovot 76100, Israel.

62 - 11:15
Characterization of Drosophila WIP reveals a critical function of the actin cytoskeleton in myoblast fusion. **Elizabeth H. Chen.** Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

63 - 11:30
Fwd/PI4Kβ binds Rab11 and acts in a common pathway with Rab11 during cytokinesis. **Gordon Polevoy, Ho-Chun Wei, Julie A. Brill.** Developmental & Stem Cell Biol, The Hospital for Sick Children, Toronto, Ontario, Canada.

64 - 11:45
Drosophila post embryonic neuroblasts undergo a unique asynchronous centrosome maturation cycle. **Nasser M. Rusan, Mark Peifer.** Biology, University of North Carolina, Chapel Hill, NC.

65 - 12:00
CRM1 mediates regulation of oxygen-dependent HIF1/SIMA subcellular localization. **Pablo Wappner[1], Maximiliano Irisarri[1], Peggy Roth[2], Christos Samakovlis[2], Nuria M. Romero[1].** 1) Fundacion Instituto Leloir, Buenos Aires, Argentina; 2) Department of Developmental Biology, Wenner-Gren Institute, Stockholm University, S-106 96 Stockholm, Sweden.

66 - 12:15
The *Grainy head* downstream gene *Dret2* is required for efficient wound repair in Drosophila embryos. **Shenqiu Wang[1], Vasilis Tsarouhas[1], Nikos Xylourgidis[1], Nafiseh Sabri[1], Marco Gallio[2], Katarina Tiklova[1], Christos Samakovlis[1].** 1) Department of Developmental Biology, Wenner-Gren Institute, Stockholm University, S-10691, Stockholm, Sweden; 2) Howard Hughes Medical Institute and Department of Neurobiology, University of California at San Diego, La Jolla, California 92093-0649.

FRIDAY, MARCH 9 4:30 pm–6:30 pm
Grand Ballroom Salon E

Gametogenesis and Sex Determination

Moderator: Mark Van Doren, Johns Hopkins University, Baltimore, Maryland

67 - 4:30
Zfh1 is required in the somatic stem cells of Drosphila testes, and can non-autonomously cause accumulation of excess early-stage germ cells. **Judith Leatherman, Steve DiNardo.** Dept Cell & Developmental Biol, Univ Pennsylvania, Philadelphia, PA.

68 - 4:45
Socs36E mediated JAK/STAT signal attenuation regulates the balance of germline and somatic stem cells in the Drosophila testis niche. **Melanie Issigonis[1], Natalia Tulina[2], Laurel Mellinger[1], Crista Brawley[1], Erika Matunis[1].** 1) Cell Biology Department, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Genetics and Gene Regulation Program, University of Pennsylvania Medical School, Philadelphia, PA.

69 - 5:00
Analysis of Modulo-dependent transcription factors identifies essential regulators of testes specific gene expression. **Dmitry Nurminsky, Lyudmila Mikhaylova.** Dept Anatomy & Cellular Biol, Tufts Univ Sch Medicine, Boston, MA.

70 - 5:15
Defects in the individualization step of spermatogenesis produced by depression of Androcam expression with RNAi. **Kathleen Beckingham, Robert Y. S. Lee, Rebecca A. Simonette, William Deery.** Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

71 - 5:30
Prostaglandins mediate Drosophila egg maturation in vitro. **Tina L. Tootle, Allan Spradling.** HHMI/Carnegie Institution, Baltimore, MD.

72 - 5:45
The *stall* gene is an extra-ovarian regulator of follicle formation that encodes an ADAM-TS metalloprotease. **Emily F. Ozdowski, Claire Cronmiller.** Univ Virginia, Biology, Charlottesville, VA.

73 - 6:00
Ecdysone Receptor Activity Regulates Cell Migration in the Ovary. **Jennifer F. Hackney, Leonard Dobens, Benjamin D. Levine, Angela Truesdale.** Molecular Biology and Biochemistry, University of Missouri-Kansas City, 5007 Rockhill Rd, Kansas City, MO.

74 - 6:15
Intersexuality in the gonadal soma provides surprising insights into *transformer(tra)* and *doublesex(dsx)* function. **Scott Siera, Thomas W. Cline.** Department of Molecular Cell Biology, University of California Berkeley, Berkeley, CA.

FRIDAY, MARCH 9 4:30 pm–6:30 pm
Grand Ballroom Salons H-J

Drosophila Models of Human Diseases

Moderator: Nancy Bonini, University of Pennsylvania, Philadelphia

75 - 4:30
The tumor suppressor Lgl controls the distribution of Fragile X protein in developing neurons. **Daniela Zarnescu, Marianna Pinter, Patty Estes, Subha Srinivasan, Adeel Yang.** Dept Mol & Cell Biol, Univ Arizona, Tucson, AZ.

76 - 4:45
Minocycline blocks oxidative stress and confers neuroprotection against paraquat induced Parkinson model. **Arati Inamdar, Anathbandhu Chaudhuri, Amellia Cannon, J. Barne, Janis O'Donnell.** Dept Biological Sci, Univ Alabama, Tuscaloosa, AL.

77 - 5:00
Hsp70 protects Drosophila brain neurons against Prion-dependent neurodegeneration. **Pedro Fernandez-Funez, Sergio Casas-Tinto, Ana Cepeda-Nieto, Claudio Soto, Diego Rincon-Limas.** Dept Neurology, Univ Texas Medical Branch, Galveston, TX.

78 - 5:15
pink1 and parkin function in the same pathway to regulate mitochondrial function. **Ming Guo, Mark Dodson, Ira Clark, Changan Jiang, Renny Feldman, Joseph Cao.** Dept Neurology, Univ California, Los Angeles, Los Angeles, CA.

79 - 5:30
Mitochondrial encephalomyopathy in Drosophila: Pathogenic mechanisms and therapeutic approaches. **Michael Palladino[1,2], Alicia Celotto[1,2], Nicole Kotchey[1,2], Adam Frank[1,2].** 1) Department of Pharmacology, Univ Pittsburgh SOM, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, Univ Pittsburgh, Pittsburgh, PA.

80 - 5:45
Ataxin-2 is a critical modulator of neurodegeneration induced by the pathogenic polyglutamine protein Ataxin-3. **Derek Lessing, Nancy Bonini.** Department of Biology & Howard Hughes Medical Insititute, University of Pennsylvania, Philadelphia, PA.

81 - 6:00
Induction of autophagy and impairment of the proteasome in Drosophila model of spinal and bulbar muscular atrophy. **Udai Pandey[1], Zhiping Nie[1], Yakup Batlevi[2], Stephanie Schwartz[1], Deborah Berry[2], Oren Schuldiner[3], Eric Baehrecke[2], J. Paul Taylor[1].** 1) Dept Neurology, 233 Stemmler HI, Univ Pennsylvania, Philadelphia, PA; 2) Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742; 3) Department of Biological Sciences, Stanford University, Stanford CA 94305.

82 - 6:15
Genetic modifiers of Drosophila palmitoyl-protein thioesterase 1 induced degeneration. **Christopher Korey, Haley Buff, Alexis Smith.** Department of Biology, College of Charleston, Charleston, SC.

FRIDAY, MARCH 9 4:30 pm–6:30 pm
Grand Ballroom Salons G, K-L

Signal Transduction I

Moderator: Ilaria Rebay, University of Chicago, Illinois

83 - 4:30
Actin related protein-3(Arp3) is required for Delta trafficking during Notch signaling. **Akhila Rajan[1,4], An-Chi Tien[2,4], Karen L. Schulze[3], Hugo J. Bellen[1,2,3].** 1) Dept of Molecular and Human Genetics; 2) Program in Developmental Biology; 3) HHMI,Baylor College of Medicine; 4) Equal Contribution.

84 - 4:45
The *O*-fucosyltransferase *O-fut1* is an extracellular component that is essential for the constitutive endocytic trafficking of Notch in Drosophila. **Kenji Matsuno, Takeshi Sasamura, Hiroyuki Ishikawa, Nobuo Sasaki, Tomonori Ayukawa, Kenta Yamada, Tomoko Yamakawa.** Dept. Biol. Sci./Tec., Tokyo University of Science, Noda, Chiba, Japan.

85 - 5:00
Lethal giant discs, a novel C2-domain protein, restricts Notch activation during endocytosis. **Jennifer Childress[1,2], Melih Acar[3], Chunyao Tao[1], Georg Halder[1,2,3].** 1) Dept Biochem & Molecular Biol, Univ Texas, Houston, Houston, TX; 2) Program in Genes and Development, Univ Texas, MD Anderson Cancer Center, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX.

86 - 5:15
Analysis of the function of Presenilin during the development of Drosophila. **Gunter Merdes[1,2], Denise Stempfle[1,2], Alexandra Wojtalla[1,2], Abil Saj[1,2], Renato Paro[1,2].** 1) BSSE, ETH Zuerich, Basel, Switzerland; 2) ZMBH, University of Heidelberg, Heidelberg, Germany.

87 - 5:30
The roles of the D-cbl long and short isoforms in the regulation of MAPK. **Hannah Robertson, Gary Hime.** Department of Anatomy & Cell Biology, University of Melbourne, Melbourne, VIC, Australia.

88 - 5:45
The exon junction complex component Mago-nashi is required for EGF receptor signaling. **Jean-Yves Roignant[1], Florence Janody[2], Jessica Treisman[1].** 1) Dept Developmental Genetics, Skirball Inst, New York, NY; 2) Instituto Gulbenkian de Ciência Rua da Quinta Grande, 6 P-2780-156, Oeiras, Portugal.

89 - 6:00
Evidence for intrinsic signaling differences between EGF and FGF receptor tyrosine kinases. **Marc S. Halfon[1,2,3], John Leatherbarrow[1].** 1) Dept. of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics and the Life Sciences, Buffalo, NY; 3) Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY.

90 - 6:15
Ninjurin A Signaling Regulates Cell Adhesion. **Andrea Page-McCaw, Shuning Zhang, Bernadette Glasheen, Caitlin Piette, Nicholas Simms, Aashish Kabra.** Dept Biol, Rensselaer Polytechnical Inst, Troy, NY.

SATURDAY, MARCH 10 8:30 am–10:15 am
Grand Ballroom Salon E

Pattern Formation I

Moderator: Angelike Stathopoulos, California Institute of Technology, Pasadena, California

91 - 8:30
BICAUDAL-C regulates *nos* expression during oogenesis. **Chiara Gamberi, Paul Lasko.** Department of Biology, McGill University, Montreal, PQ, Canada.

92 - 8:45
Regulation of *nanos* mRNA by Drosophila hnRNP M. **Roshan A. Jain, Elizabeth R. Gavis.** Dept of Molecular Biology, Princeton University, Princeton, NJ.

93 - 9:00
Precision and reproducibility of biological patterning. **Thomas Gregor[1,2,3,4], William Bialek[1,2], David W. Tank[1,2,3], Eric F. Wieschaus[3,4].** 1) Lewis-Sigler Institute for Integrative Genomics; 2) Joseph Henry Laboratories of Physics; 3) Department of Molecular Biology; 4) Howard Hughes Medical Institute, Princeton University, Princeton, NJ.

94 - 9:15
Quantitative study of the terminal system in drosophila embryo. **Matthieu Coppey, Alistair N. Boettiger, Stanislav Y. Shvartsman.** Carl Ichan Lab, Princeton Univ, Princeton, NJ.

95 - 9:30
Anterior patterning independent of the Bicoid gradient. **Ulrike Lohr[1], Ho-Ryun Chung[2], Mathias Beller[1], Herbert Jäckle[1].** 1) Molecular Developmental Biol., MPI for Biophysical Chemistry, Goettingen, Germany; 2) Computational Molecular Biol., MPI for Molecular Genetics, Berlin, Germany.

96 - 9:45
Oriented cell divisions contribute to germ band extension in Drosophila embryos. **Sara Morais da Silva, Jean-Paul Vincent.** Developmental Neurobiology, NIMR, London, UK.

97 - 10:00
The Role of D-Ets4 in Primordial Germ Cell Migration. **Anita Hsouna, Dennis Watson, Tien Hsu.** Hollings Cancer Ctr, Medical Univ South Carolina, Charleston, SC.

SATURDAY, MARCH 10 8:30 am–10:15 am
Grand Ballroom Salons H-J

Cell Division and Growth Control

Moderator: Kenneth Moberg, Emory University, Atlanta, Georgia

98 - 8:30
The *lines* gene controls growth by repression of *bowl* and *wingless* in the Drosophila wing. **Victor Hatini, David Nusinow.** Dept Anatomy & Cellular Biol, Tufts Univ, Boston, MA.

99 - 8:45
Regulation of spindle stability and chromosome segregation by dephosphorylation of the Microtubule-Associated Protein dTACC. **Shengjiang Tan, Ekaterina Lyulcheva, Jon Dean, Daimark Bennett.** Department of Zoology, University of Oxford, South Parks Road, Oxford, UK.

100 - 9:00
Activated ERM control cortex dynamics and cell shape throughout mitosis. **Sebastien Carreno[1], Ilektra Kouranti[2], Edith Szafer Glusman[3], Margareth Fuller[3], Arnaud Echard[2], Payre Francois[1].** 1) CBD - CNRS UMR5547, Toulouse, France; 2) Institut CURIE - CNRS UMR144, Paris, France; 3) Stanford University School of Medicine, Stanford, CA.

101 - 9:15
dp53 coordinates tissue repair through a novel, DNA-damage independent pathway. **Brent Wells, Laura Johnston.** Dept Genetics & Development, Columbia Univ, New York, NY.

102 - 9:30
Fat Cadherin Modulates Organ Size in Drosophila via the Salvador/Warts/Hippo Signaling Pathway. **Kieran F. Harvey, F. Christian Bennett.** Cancer Cell Biology, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.

103 - 9:45
Drosophila ALS regulates animal growth rate and metabolism through direct and functional interaction with Drosophila insulins. **Nathalie Arquier[1], Charles Géminard[1], Marc Bourouis[1], Gisèle Jarretou[1], Basil Honneger[2], Alexandre Paix[1], Pierre Leopold[1].** 1) ISBDC, CNRS UMR 6543, Nice, France; 2) Zoologisches Institut, Universitat Zurich, Zurich, Switzerland.

104 - 10:00
The cytohesin Steppke is essential for insulin signalling in Drosophila. **Ingo Zinke, Bernhard Fuss, Thomas Becker, Michael Hoch.** University Bonn, LIMES (Life and Medical Sciences), Program Unit Development & Genetics, Bonn, Germany.

SATURDAY, MARCH 10 8:30 am–10:15 am
Grand Ballroom Salons G, K-L

Signal Transduction II

Moderator: Kristi Wharton, Brown University, Providence, Rhode Island

105 - 8:30
Characterization of a novel regulatory interaction in the Hedgehog pathway. **Anne Plessis[1], Sandra Claret[1], Matthieu Sanial[1], Sebastien Malpel[1], Tristan Piolot[2], Amira Brigui[1], Laurent Daviet[3].** 1) Génétique du Développement et Evolution , Institut Jacques Monod, UMR 7592, CNRS/Universités Paris6-Paris7, 2 Place Jussieu 75251 Paris cedex 05, France; 2) Imageries des Processus Dynamiques en Biologie Cellulaire et Biologie du Développement , IFR 117 Biologie Systémique -Institut Jacques Monod, 2 Place Jussieu 75251 Paris cedex 05, France; 3) Hybrigenics, 3/5 impasse Reille, 75014 Paris, France.

106 - 8:45
Lipids and Lipoproteins as carriers for Hedgehog spreading and reception. **Ainhoa Callejo, Joaquím Culi, Isabel Guerrero.** Centro de Biología Molecular (CSIC), Universidad Autónoma de Madrid, Madrid, ES.

107 - 9:00
Non-canonical *dpp* signalling mediates communication between R7 and R8 in the Drosophila eye. **Daniela Pistillo, Claude Desplan.** Dept Biol, New York Univ, New York, NY.

108 - 9:15
dSno facilitates Baboon signaling in the Drosophila brain by switching the affinity of Medea away from Mad and toward dSmad2. **Stuart Newfeld[1], Cathy Hyman-Walsh[2], Ying Ye[3], Robert Wisotzkey[1], Michael Stinchfield[1], Michael O'Connor[3], David Wotton[2], Norma Takaesu[1].** 1) Sch Life Sci, Arizona State Univ, Tempe, AZ; 2) Dept of Biochemistry and Molecular Genetics, Univ of Virginia, Charlottesville, VA; 3) Dept of Genetics, Cell Biology and Development and HHMI, Univ of Minnesota, Minneapolis, MN.

109 - 9:30
Drosophila miR-315 regulates Wingless signaling. **Eric Lai[1], Serena Silver[2], Joshua Hagen[1], Norbert Perrimon[2].** 1) Sloan Kettering Institute, Developmental Biology, New York, NY; 2) Harvard Medical School, Genetics Department, Boston, MA.

110 - 9:45
Canonical Wnt signaling depends on Evi/Wls, a conserved transmembrane protein required for Wnt secretion. **Kerstin Bartscherer, Nadège Pelte, Dierk Ingelfinger, Michael Boutros.** Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

111 - 10:00
The extracellular domain of the Frizzled receptor functions as a ligand by binding to Van Gogh/Strabismus during planar cell polarity signaling in Drosophila. **Jun Wu, Marek Mlodzik.** Department of Molecular, Cellular and Developmental Biology, Mount Sinai School of Medicine, New York, NY.

SATURDAY, MARCH 10 10:45 am–12:30 pm
Grand Ballroom Salon E

Pattern Formation II

Moderator: Kenneth Irvine, Rutgers University, Piscataway, New Jersey

112 - 10:45
Function of the Rho-GEF Pebble in FGF-dependent mesoderm migration. **Andreas van Impel, Sabine Schumacher, Arno Müller.** Division of Cell and Developmental Biology, College of Life Sciences, University of Dundee, Dundee, Scotland, UK.

113 - 11:00
The JAK/STAT pathway regulates proximal-distal patterning in Drosophila. **Aidee Ayala[1], Laura Ekas[1], Maria Sol Flaherty[1], Gyeong-Hun Baeg[2], Erika Bach[1].** 1) Department of Pharmacology, New York University School of Medicine, New York, NY; 2) Children's Cancer Research Laboratory, New York Medical College, Valhalla, NY.

114 - 11:15
Interaction between the nuclear import adapter importin-α3 and the Wnt antagonist Naked cuticle. **Chih-Chiang Chan[1,3], Raphaël Rousset[2], Keith Wharton, Jr.[3].** 1) Genetics and Development Graduate Program, UT Southwestern, Dallas, TX; 2) Institute of Signaling, Developmental Biology and Cancer, Centre de Biochimie, University of Nice, France; 3) Departments of Pathology and Molecular Biology, UT Southwestern, Dallas, TX.

115 - 11:30
Hedgehog restricts its expression domain in the Drosophila wing. **Fernando Bejarano[1], Lidia Perez[1], Yiorgos Apidianakis[2], Christos Delidakis[2], Marco Milan[1].** 1) Parc Cientific de Barcelona, Institut de Recerca Biomédica, Barcelona, Spain; 2) Institute of Molecular Biology and Biotechnology, Fo.R.T.H., and Department of Biology, University of Crete, 71110 Heraklion, Greece.

116 - 11:45
Identification of genes expressed in specific leg segments using microarray. **Reiko Tajiri[1], Tetsuya Kojima[1,2], Kaoru Saigo[1].** 1) Dept Biophys Biochemistry, Univ Tokyo Grad Sch Sci, Tokyo, Japan; 2) Dept. Integrated Biosci., Grad. Sch. Frontier Sci., Univ. Tokyo.

117 - 12:00
Dorsal-Ventral midline signaling and antagonistic transcription factors in the developing Drosophila eye. **Atsushi Sato, Andrew Tomlinson.** Dept Genetics & Development, Columbia Univ, New York, NY.

118 - 12:15
Spatial and temporal regulation of ecdysone signaling revealed through analysis of TAI, the Drosophila homolog of AIB1, a breast cancer oncogene. **Anna C.-C. Jang, Denise J. Montell.** Dept Biological Chemistry, JHMI, WBSB, Baltimore, MD.

SATURDAY, MARCH 10 10:45 am–12:30 pm
Grand Ballroom Salons H-J

Cell Division and Growth Control

Moderator: Kenneth Moberg, Emory University, Atlanta, Georgia

119 - 10:45
Specific role of the SR protein splicing factor B52 in cell cycle control in Drosophila. **Maxim Frolov, Vanya Rasheva, David Knight, Przemyslaw Bozko.** Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL.

120 - 11:00
A gradient of EGFR signaling determines the sensitivity of rbf1 mutant cells to E2F-dependent apoptosis. **Nam-Sung Moon, Luisa Di Stefano, Nicholas Dyson.** Cancer Research Ctr, Massachusetts General Hosp, Charlestown, MA.

121 - 11:15
In vivo and in vitro regulation of dMyc protein stability by Sgg/dGSK3 and Dco/CK1 kinases. **Margherita Galletti[1], Florenci Serras[2], Jin Jiang[3], Pier Giuseppe Pelicci[4], Daniela Grifoni[5], Paola Bellosta[6].** 1) Univ of Modena, Modena, Italy; 2) Uni of Barcelona, Spain; 3) UT Southwestern Medical Center, Dallas, TX; 4) IEO European Institute of Oncology Milan, Italy; 5) Uni of Bologna, Italy; 6) City Collage-CUNY New York, NY.

122 - 11:30
Mtrm: A Meiotic Inhibitor of Polo Kinase. **Youbin Xiang[1], Jeffrey Cotitta[1,2], Stacie Hughes[1], Sue Jaspersen[1], R. Scott Hawley[1,2].** 1) Stowers Inst Medical Research, Kansas City, MO 64110; 2) Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

123 - 11:45
The Role and Developmental Regulation of CORTEX, a Meiosis-Specific APC/C Activator. **Jillian A. Pesin, Terry Orr-Weaver.** Whitehead Institute and Department of Biology, M.I.T., Cambridge, MA.

124 - 12:00
A genetic screen reveals novel regulators of autophagy. **Andrew M. Arsham, Thomas P. Neufeld.** Genetics, Cell Biology & Development, and Developmental Biology Center, University of Minnesota, Minneapolis, MN.

125 - 12:15
Diverse signal transduction pathways link cell cycle progression and mitochondrial function. **Edward Owusu-Ansah, Amir Yavari, Sudip Mandal, Utpal Banerjee.** Dept MCDB, Univ California, Los Angeles, Los Angeles, CA.

SATURDAY, MARCH 10 10:45 am–12:30 pm
Grand Ballroom Salons G, K-L

Techniques and Genomics

Moderator: Ramanuj Dasgupta, New York University School of Medicine, New York

126 - 10:45
RNAi: Quest for the golden locus. **Michele Markstein[1], Chrysoula Pitsouli[1], Christians Villalta[1], Sue Celniker[2], Norbert Perrimon[1].** 1) Dept Genetics, Harvard Medical School, Boston, MA; 2) BDGP, Berkeley, CA.

127 - 11:00
Expanding the Gene Disruption Project collection with *Minos* insertion mutants. **Robert W. Levis[1], Yuchun He[2,3], Joseph W. Carlson[4], Martha Evans-Holm[4], Soo Park[4], Kenneth H. Wan[4], Karen L. Schulze[2,3], Koen J.T. Venken[2,3], P. Robin Hiesinger[5], Roger A. Hoskins[4], Allan C. Spradling[1,3], Hugo J. Bellen[2,3].** 1) Dept Embryology, Carnegie Inst of Washington, Baltimore, MD; 2) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute; 4) Dept Genome Biology, Lawrence Berkeley National Laboratory, Berkeley, CA; 5) Dept Physiology, UT Southwestern Medical Center, Dallas, TX.

128 - 11:15
Novel axon pruning mutants identified by a mosaic screen using *piggyBac*-based insertional mutagenesis. **Oren Schuldiner, Jonathan Levy, Daniela Berdnik, Joy Wu, David Luginbuhl, Liqun Luo.** Howard Hughes Medical Institute, Department of Biological Sciences, Stanford University, Stanford, CA.

129 - 11:30
Exploring the spatial and temporal diversity of embryonic gene expression patterns. **Benjamin P. Berman[1], Pavel Tomançak[1], Amy Beaton[1], Erwin Frise[2], Richard Weiszmann[2], Elaine Kwan[1], Michael B. Eisen[1,3], Volker Hartenstein[4], Gerald M. Rubin[1,2], Susan E. Celniker[2].** 1) Dept MCB, HHMI, UC Berkeley; 2) BDGP and; 3) Genome Sciences, LBNL; 4) Dept of MCDB, UCLA.

130 - 11:45
cgChIP: a novel technique for analyzing the chromatin structure of developmentally regulated genes. **Daniel J. McKay[1], Richard S. Mann[2].** 1) Integrated Program in Cellular, Molecular, & Biophysical Studies, Columbia Univ., New York, NY; 2) Dept. Biochemistry & Molecular Biophysics, Columbia Univ., New York, NY.

131 - 12:00
CisDECODER reveals the regulatory logic underlying coordinate gene expression. **Thomas Brody[1], Wayne Rasband[2], Kevin Baler[2], Alexander Kuzin[1], Mukta Kundu[1], Jermaine Ross[1], Ward Odenwald[1].** 1) Neural Cell-Fate Determinants, NINDS, NIH, Bethesda, MD; 2) Office of Scientific Director, IRP, NIMH, NIH, Bethesda, MD.

132 - 12:15
Automated Image analysis of Multiple Gene activity patterns in developing animals. **William Beaver[1], David Kosman[2], Gary Tedeschi[2], Adam Pare[2], Ethan Bier[2], William McGinnis[2], Yoav Freund[1].** 1) Department of Computer Science and Engineering, UCSD, La Jolla, CA 92093; 2) Cell and Developmental Biology Department, UCSD, La Jolla, CA 92093.

SATURDAY, MARCH 10 4:00 pm–6:00 pm
Grand Ballroom Salon E

Genome and Chromosome Structure

Moderator: Kami Ahmad, Harvard Medical School, Boston, Massachusetts

133 - 4:00
Small RNAS are involved in heterochromatin silencing in Drosophila. **Christophe Antoniewski[1], Bassam Berry[1], Olivier Voinnet[2], Delphine Fagegaltier[1].** 1) Dev Biology,Jacques Monod Bldg, Institut Pasteur, Paris, CDX 15, France; 2) IBMPC - CNRS, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France.

134 - 4:15
Identification of small RNAs associated with the *gypsy* chromatin insulator. **Elissa P. Lei.** Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD.

135 - 4:30
DmSETDB1 is a euchromatin and chromosome 4-specific histone H3 lysine 9 methyltransferase in *D. melanogaster*. **Carole Seum, Emanuela Reo, Pierre Spierer, Séverine Bontron.** Dept. Zoology & Animal Biology, University of Geneva, Geneva, Switzerland.

136 - 4:45
Using Drosophila as a model to understand human laminopathies: a study of dMAN1. **Belinda Pinto, Shameika Wilmington, Lori Wallrath, Pamela Geyer.** Molecular Biology Program, Department of Biochemistry, University of Iowa, Iowa City, IA.

137 - 5:00
D. melanogaster coilin. **Ji-Long Liu, Zheng'an Wu, Zehra Nizami, Joseph Gall.** Department of Embryology, Carnegie Institution, Baltimore, MD 21218.

138 - 5:15
Genetic analysis reveals different cellular requirements of CAF-1 during development. **Benjamin Klapholz, Bruce Dietrich, Catherine Schaffner, Jean-Pierre Quivy, Genevieve Almouzni, Nathalie Dostatni.** Institut Curie, CNRS UMR218, Paris, France.

139 - 5:30
Drosophila MCM10 in heterochromatin dynamics and DNA replication. **Tim Christensen, Helen Zhou, Jasmine Barrow, Gregory Kuzmik, Bike Tye.** Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

140 - 5:45
ATM/ATR kinase activity at Drosophila telomeres. **Sarah Oikemus[1], Daniel Savukoski[1], Joana Queiroz-Machado[2], Claudio Sunkel[2], Michael Brodsky[1].** 1) Dept PGF&E, University Massachusetts, Worcester, MA; 2) Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.

SATURDAY, MARCH 10 4:00 pm–6:00 pm
Grand Ballroom Salons H-J

Neurogenetics and Neural Development

Moderator: Wesley Grueber, Columbia University, New York, New York

141 - 4:00
Combinatorial codes specifying neuronal identities. **Magnus Baumgardt[1], Irene Miguel-Aliaga[2], Daniel Karlsson[1], Helen Ekman[1], Stefan Thor[1].** 1) Molecular Genetics, Linkoping University, Linkoping, Sweden; 2) Division of Mammalian Development, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom.

142 - 4:15
Notch signaling controls the differentiation of sibling neurons at the Drosophila CNS midline. **Scott R. Wheeler, Stephen T. Crews.** Program in Molecular Biology and Biotechnology, UNC-Chapel Hill, Chapel Hill, NC.

143 - 4:30
HOW mediates Ecdyson-dependent apoptosis of embryonic midline glia cells. **Adriana Reuveny, Talila Volk.** Dev. and Mol. Genetics, Weizmann Institute of Science, Rehovot, Israel.

144 - 4:45
Cytoplasmic and Mitochondrial Protein Translation in Development and Maintenance of Neuronal Terminal Arborization. **Takahiro Chihara[1,2], David Luginbuhl[1], Liqun Luo[1].** 1) Howard Hughes Medical Institute, Department of Biological Sciences, Stanford University, Stanford, California 94305; 2) Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

145 - 5:00
Dscam is required for self-avoidance and proper dendritic field organization of dendritic arborization neurons. **Peter Soba[1], Sijun Zhu[1], Kazuo Emoto[2], Susan Younger[1], Shun-Jen Yang[3], Hung-Hsiang Yu[3], Tzumin Lee[3], Lily Jan[1], Yuh Nung Jan[1].** 1) Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA; 2) National Institute of Genetics, Mishima, Japan; 3) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA.

146 - 5:15
A candidate regulator of the transition from axon pathfinding to branching in Drosophila neuronal development: the BTB/POZ Zinc finger transcription factor broad-Z3. **Janet Altman, James W. Truman.** Department of Biololgy, Univ. of Washington, Seattle, WA.

147 - 5:30
Functional characterization of Syndecan, a Heparan Sulphate Proteoglycan (HSPG), in Slit/Robo Signaling. **B. Chanana, P. Steigemann, H. Jäckle, G. Vorbrüggen.** Dept Mol Dev Bio, Max Planck Inst, Göttingen, Germany.

148 - 5:45
DE-cadherin/*shg* in Drosophila larval brain development. **Siau-Min Fung[1], Fay Wang[2], Volker Hartenstein[1].** 1) Dept MCDB, Univ California, Los Angeles, Los Angeles, CA; 2) Gladstone Institue of Neurological Disease, Univ California San Francisco, San Fancisco, CA.

SATURDAY, MARCH 10 4:00 pm–6:00 pm
Grand Ballroom Salons G, K-L

Physiology and Aging

Moderator: Marc Tatar, Brown University, Providence Rhode Island

149 - 4:00
HNF4 coordinates nutrition with fat metabolism in Drosophila. **Laura R. Palanker, Carl S. Thummel.** Dept of Human Genetics, University of Utah, Salt Lake City, UT.

150 - 4:15
Reduction of p53 activity in the brain of adult *D. melanogaster* extends life span as part of the Sir2 life span-extending pathway. **Johannes Bauer, Chenyi Chang, Suzanne Hozier, Siti Nur Sarah Morris, Sandra Andersen, Joshua Waitzman, Stephen Helfand.** Department of Molecular Biology, Cell Biology and Biochemistry, Division of Biology and Medicine., Brown University, 70 Ship Street, Room 408, Providence, RI 02903.

151 - 4:30
Germline Regulation of Aging in *D. melanogaster*. **Thomas Flatt[1], Michael Rocha[2], Kyung-Jin Min[1], Michael Grunwald[3], Ruth Lehmann[3], Leanne Jones[2], Marc Tatar[1].** 1) Dept Ecology & Evolution, Brown Univ, Providence, RI; 2) The Salk Institute for Biological Studies, Laboratory of Genetics, La Jolla, CA; 3) Howard Hughes Medical Institute, Developmental Genetics Program, Skirball Institute of Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, NY.

152 - 4:45
4EBP mediates alterations in translation and lifespan due to dietary restriction in Drosophila. **Brian Zid[1], Aric Rogers[2], Seymour Benzer[1], Pankaj Kapahi[2].** 1) Caltech, Pasadena, CA; 2) Buck Institute, Novato, CA.

153 - 5:00
Subcellular regulation allows the Drosophila PI3-kinase/Akt signalling to perform different metabolic and developmental functions. **Clive Wilson[1], Natalia Vereshchagina[1], Noelia Pinal[2], Lucy Collinson[2], Yasuyuki Fujita[2], Iain Cox[1], Franck Pichaud[2], Deborah Goberdhan[2].** 1) Dept Physiology, Anatomy & Genetics, Le Gros Clark Building, Univ Oxford, Oxford, UK; 2) MRC LMCB, Dept of Anatomy and Developmental Biology, UCL, Gower Street, WC1E 6BT, London.

154 - 5:15
Genetic analysis of AMPK and LKB1 function in *D. melanogaster*. **Michelle Bland, Julie Magallenes, Morris Birnbaum.** Dept. of Medicine, University of Pennsylvania, 415 Curie Blvd., Philadelphia, PA.

155 - 5:30
The Role of Autophagy in the Adult Nervous System During Oxidative Stress and Aging. **Kim Finley[1], Anne Simonsen[2], Robert Cumming[1], David Schubert[1].** 1) Cellular Neurobiology Lab, Salk Inst Biological Studies, La Jolla, CA; 2) Institute for Cancer Research Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway.

156 - 5:45
Lipid Storage Droplet (Lsd2) Mutants are Lean, Long-lived, and Benefit from Dietary Restriction. **Danielle Skorupa[1], Beverly Patuwo[2], Sergiy Libert[1], Jessica Zwiener[1], Scott Pletcher[1].** 1) Huffington Center on Aging, Baylor College of Medicine, Houston, TX; 2) Center for Educational Outreach, Baylor College of Medicine, Houston, TX.

Poster board is in bold above the title. See page 14 for presentation schedule. The first author is the presenter. Abstracts begin on page 77.

Cell Division and Growth Control

157A
An expression-based approach to identifying factors that mediate cell competition. **Claire de la Cova, Laura A. Johnston.** Genetics and Development, Columbia University, New York, NY.

158B
Characterization of a Mutation that Produces Cell Competition. **Yassi Hafezi, Iswar Hariharan.** Molecular and Cell Biology, Univ. of California, Berkeley, Berkeley, CA.

159C
A functional analysis of cell competition using Drosophila cell culture. **Nanami Senoo-Matsuda, Laura A. Johnston.** Department of Genetics & Development, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

160A
Studies on the regulation of dMyc expression by Insulin and Nutrients signaling. **Rajendra Chilukuri[1], Federica Parisi[2], Daniela Grifoni[2], Paola Bellosta[1].** 1) City College-CUNY,New York, NY; 2) University of Bologna, Italy.

161B
Drosophila TCTP is a new component of the TSC pathway. **Ya-Chieh Hsu[1], Kwang-Wook Choi[1,2].** 1) Program in Developmental Biology, Baylor College Med, Houston, TX; 2) Molec & Cell Biol, Baylor College Med, Houston, TX.

162C
The role of CUL4-DDB1 in the control of growth and CDT1/DUP levels during Drosophila development. **Hyun O. Lee, Sima Zacharek.** GMB, University of North Carolina, Chapel Hill, NC.

163A
Identification and characterization of novel regulators of insulin signalling. **Shivanthy M. Visvalingam, Deborah C. I. Goberdhan, Clive Wilson.** Department of Physiology, Anatomy & Genetics, Le Gros Clark Building, Oxford University, Oxford, UK.

164B
The Drosophila Hus1 is required for double strand DNA repair during meiosis. **Lihi Gur-Arie[1,2], Uri Abdu[1,2].** 1) Life Science, Ben-Gurion University, Beer-Sheva, Israel; 2) The National Institute for Biotechnology in the Negev, Beer-Sheva, Israel.

165C
BubR1, but not Mad2, is required for recruiting and localizing of Fzy to the kinetochores in *D. melanogaster*. **Deyu Li[1], Roger Karess[2], Michael Whitaker[1], Jun-Yong Huang*[1].** 1) Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle, UK; 2) CNRS, Centre de Génétique Moléculaire, Ave de la Terrasse, 91198 Gif sur Yvette, France.

166A
Activation of Mitotic checkpoints by impaired mitochondrial function. **Sudip Mandal, Kevin Yackle, Utpal Banerjee.** Dept MCDB, Univ California, Los Angeles, Los Angeles, CA.

167B
Drosophila MEK and ERK function in an intrinsic cell-cycle checkpoint pathway. **Vladic Mogila, Fan Xia, Willis X. Li.** Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642.

168C
The fruit fly *D. melanogaster* as a model system for studying the role of 9-1-1 complex in DNA damage checkpoints. **Ronit Tokarsky[1,2], Uri Abdu[1,2].** 1) Life science, Ben - Gurion University, Beer -Sheva, Israel; 2) The National Institute for Biotechnology in the Negev, Beer -Sheva, Israel.

169A
Fragile X mental retardation protein controls *trailer hitch* expression and cleavage furrow formation in Drosophila embryos. **K. Monzo[1], O. Papoulas[1], G. T. Cantin[2], Y. Wang[1], J. R. Yates III[2], J. C. Sisson[1].** 1) The Section of MCD Biology and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 2) The Department of Cell Biology, The Scripps Research Institute, La Jolla, CA.

170B
Mutation of the microRNA *let-7* in *D. melanogaster*. **Elizabeth E. Caygill, Laura A. Johnston.** Department of Genetics & Development, Columbia University College of Physicians and Surgeons, New York, NY.

171C
Cullin3 suppresses Hedgehog signaling to pattern the Drosophila retina. **Wang Chien-Hsiang[1,2], Ou Chan-Yen[1], Chien Cheng-Ting[1,2].** 1) Academia sinica, Institution of Molecular Biology, Taipei, Taiwan; 2) National Yang Ming University, Institution of Neuroscience, Taipei, Taiwan.

172A
Characterization of *whale* a spontaneous cell growth mutant. **Deborah K. Hoshizaki[1], Alia Jabali[1], Cheryl Gustafson[2], Paul Lawson[1].** 1) School of Life Sciences, University of Nevada, Las Vegas, NV; 2) Unversity of Nevada School of Medicine, Reno, NV.

173B
Control of proliferation during the transition from undifferentiated into progenitor cell state during Drosophila eye development. **Carla S. Lopes[1], Fernando Casares[1,2].** 1) CABD-Centro Andaluz de Biologia del Desarrollo, Universidad Pablo de Olavide, Seville, Spain; 2) IBMC-Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.

174C
Rbf1-independent termination of E2f1 target gene expression during early Drosophila embryogenesis. **Shusaku Shibutani, Lisa M. Swanhart, Robert J. Duronio.** Dept Biology, Univ North Carolina, Chapel Hill, NC.

175A
The proneural bHLH proteins Atonal/Daughterless and the canonical EGFR signaling coordinately regulate photoreceptor specification and cdk inhibitor expression. **Madina Z. Sukhanova, Dilip K. Deb, Gabriel M. Gordon, Miho Matakatsu, Wei Du.** Ben May Institute for Cancer Research, University of Chicago, Chicago, IL.

176B
Growth regulation in the *Minute* mutants of *D. melanogaster*. **Meng-Ping Tu, Debra A. Smith, Laura A. Johnston.** Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

177C
Characterization of a novel conserved cyclin in Drosophila. **Dongmei Liu, Russell Finley.** Dept CMMG, Wayne State Univ, Detroit, MI.

178A
MAPK activity during Drosophila egg activation. **Katharine Sackton, Norene Buehner, Mariana Wolfner.** Dept Molecular Biol & Genetics, Cornell Univ, Ithaca, NY.

179B
Shattered, Anaphase Promoting Complex-1, is indispensable for proper photoreceptor cell differentiation through cell cycle synchronization. **Miho Tanaka-Matakatsu[1], Barbara J. Thomas[2], Wei Du[1].** 1) BMICR, University of Chicago, Chicago, IL; 2) Genes, Genomes and Genetics IRG, CSR, OER, NIH.

180C
Cell biological analysis of Cyclin E/Cdk2-mediated replication-dependent histone mRNA biosynthesis. **Anne E. White[1], Michelle E. Leslie[2], Brian R. Calvi[5], William F. Marzluff[1,2,3,4], Robert J. Duronio[1,2,4].** 1) Department of Biology; 2) Curriculum in Genetics and Molecular Biology; 3) Department of Biochemistry and Biophysics; 4) Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, NC; 5) Department of Biology, Syracuse University, Syracuse, NY.

181A
An RNAi screen to identify regulators of MEI-S332 localization. **Hannah R. Cohen, Thouis Jones, Robert Lindquist, David Sabatini, Terry Orr-Weaver.** Whitehead Institute, Cambridge, MA.

182B
An analysis of chromosome pairing dynamics in meiosis and in the early embryo. **Justin Blumenstiel[1], William Theurkauf[2], R. Scott Hawley[1].** 1) Stowers Inst, Kansas City, MO; 2) Univ Mass Medical School, Worcester, MA.

183C
Why Don't Null Alleles of Axs have a Phenotype? **Susan M. Flynn, Stacie E. Hughes, R. Scott Hawley.** Stowers Institute for Medical Research, Kansas City, MO.

184A
The *ald/mps1* and *polo* Kinases Form Filaments in Drosophila Female Meiosis. **William Gilliland[1], Jeffrey Cotitta[1,2], Stacie Hughes[1], Youbin Xiang[1], R. Scott Hawley[1,2].** 1) Stowers Inst, Kansas City, MO; 2) U. Kansas Medical Center, Kansas City, KS.

185B
A work in progress: Live imaging of chiasmate and achiasmate co-orientation during Drosophila female meiosis. **Stacie E. Hughes, Jeffrey Cotitta, William Gilliland, R. Scott Hawley.** Stowers Institute for Medical Research, Kansas City, MO.

186C
Identification of proteins that interact with Nod, the chromokinesin-like protein essential for achiasmate chromosome segregation. **Li-Jun Huo, Youbin Xiang, Kimberly Collins, R. Scott Hawley.** Stowers Institute for Medical Research, Kansas City, MO.

187A
The fly that came in from the cold: *trade embargo* (*trem*), a new cold-sensitive meiotic mutant. **Cathleen M. Lake[1], Rachel J. Nielsen[1], Kathy Teeter[1], Scott Page[2], R. Scott Hawley[1].** 1) Stowers Inst Medical Research, Kansas City, MO; 2) Comparative Genomics Centre, James Cook Univ., Townsville, Australia.

188B
The chromatin insulator protein Dtopors is required for nuclear lamina assembly, centrosome regulation and meiotic chromosome segregation in males. **Maiko Matsui[1], Krishn Sharma[1], Barbara Wakimoto[2], John Tomkiel[1].** 1) Biology, UNC Greensboro, Greensboro, NC; 2) Zoology, University of Washington, Seattle, WA.

189C
The Cdc20/Cdh1-related protein, Cort, cooperates with Cdc20/Fzy in cyclin destruction and anaphase progression in meiosis I and II. **Andrew Swan[1], Trudi Schüpbach[2].** 1) Dept of Biological Sciences, University of Windsor, Windsor, Canada; 2) Dept of Molecular Biology, Princeton University, Princeton, NJ.

190A
A genetic screen for modifiers of *teflon*, a gene required for proper segregation of autosomes at meiosis I in males. **Amanda L. Thomas, John E. Tomkiel.** Dept Biol, The University of North Carolina at Greensboro, Greensboro, NC.

191B
Cdk1 phosphorylation sites on Cdc27 are required for correct chromosomal localization and APC/C function in syncytial Drosophila embryos. **Jun-Yong Huang, Gary Morley, Michael Whitaker.** Cell and Developmental Physiology, Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, University of Newcastle upon Tyne, Catherine Cookson Building, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK.

192C
A genetic analysis of the E2F1 mediated transcriptional activation. **Jun-yuan Ji, Fajun Yang, Anabel Herr, Anders Näär, Nick Dyson.** Massachusetts General Hospital Cancer Center, Charlestown, MA 02129.

193A
Loss of Drosophila Myb interrupts the progression of chromosome condensation. **Joseph Lipsick, John Manak, Hong Wen, Tran Van, Laura Andrejka, Wai Choi.** Dept Pathology & Genetics, Stanford Univ, Stanford, CA.

194B
Isolation and characterization of new maternal mutants defective for blastoderm cellularization. **Ana Rita Marques[1], Rui Tostões[1], Thomas Marty[2], Rui Gonçalo Martinho[1].** 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Skirball Institute, NYU, NY.

195C
Isolation and characterization of *D. melanogaster* kinetochore proteins. **Lucia Mentelova[1,3], Gonçalo Costa[1], Fatima Pereira[1], Ana Roque[1], Alvaro Tavares[1,2].** 1) Cell Division Group, Inst.Gulbenkian Ciencia, Lisboa, Portugal; 2) Chemical Eng. Inst. Superior Tecnico, Lisboa, Portugal; 3) Dept. of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

196A
Nopo is a candidate E3 ubiquitin ligase required for genomic stability during early embryogenesis in Drosophila. **Julie Merkle, Jamie Rickmyre, Audrey Frist, Erin Loggins, Laura Lee.** Department of Cell and Developmental Biology, Vanderbilt University Medical School, Nashville, TN.

197B
Microcephalin (MCPH1) is required for cell-cycle progression in the early Drosophila embryo. **Jamie L. Rickmyre, Audrey Y. Frist, Laura A. Lee.** Cell & Developmental Biology, Vanderbilt University Med Ctr, Nashville, TN.

198C
Characterization of Drosophila cyclin J. **Govindaraja Atikukke[1], Russell L. Finley, Jr.[1,2].** 1) Biochemistry and Molecular Biology, Wayne State University, Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI.

199A
The Role of Centriole and Centrosome in Cilium Inheritance and Formation. **Tomer Avidor-Reiss, Stephanie Blachon.** Cell Biology, Harvard Medical School, Boston, MA.

200B
Search for components of a nutrient sensor of the Drosophila larval fat body. **Marc Bourouis, Marianne Bjordal, Sophie Layalle, Pierre Léopold.** Institute for Signalling, Developmental Biology and Cancer, CNRS UMR 6543, Université de Nice, France.

201C
An interaction map guided screen for novel genetic interactions. **Stephen Guest[1], Jingkai Yu[1], Russell Finley, Jr.[1,2].** 1) Center for Molecular Medicine and Genetics; 2) Department of Biochemistry and Molecular Biology Wayne State University School of Medicine, 540 East Canfield, Detroit Michigan, 48201.

202A
The Drosophila MRL adapter protein Pico promotes insulin- and Egfr-dependent cell growth and proliferation. **Daimark Bennett, Ekaterina Lyulcheva.** Department of Zoology, Oxford University, Oxford, UK.

203B
Genetic and molecular analysis of *bene*, a glutamyl-tRNA (Gln) amidotransferase homolog required for growth and maturation in Drosophila. **Leah Bergman, Anna Kruyer, Mikhail Gertsberg, Adriana Guigova, Jason Z. Morris.** Dep't of Natural Sciences, Fordham University, New York, NY.

204C
Cell-autonomous growth suppression by the Drosophila *tsg101* ortholog *erupted* via cell polarity and cell cycle pathways. **M. Melissa Gilbert, Caroline Krisel, Kenneth H. Moberg.** Department of Cell Biology, Emory University, Atlanta, GA.

205A
JNK signaling is necessary for the activation of a developmental checkpoint in response to tissue damage. **Adrian Halme, Iswar Hariharan.** Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

206B
Fat acts through Hippo signalling to regulate tissue size. **Fisun Hamaratoglu[1,2], Maria Willecke[1,3], Madhuri Kango-Singh[1], Ryan Udan[1,2], Chiao-Iin Chen[1,4], Chunyao Tao[1], Xinwei Zhang[1], Georg Halder[1,2,4].** 1) Dept Biochem & Molec Biol, MD Anderson Cancer Ctr, Houston, TX; 2) Prog in Dev Biol, BCM, Houston, TX; 3) Interfakultäres Institut für Zellbiologie, Abt. Genetik der Tiere, Universität Tübingen, Tübingen, Germany; 4) Prog in Genes and Dev, UT MD Anderson Cancer Ctr, Houston, TX.

207C
Mutations in the novel gene *gang of four* deregulate growth in the Drosophila eye. **Carolyn A. Krisel, Kenneth H. Moberg.** Department of Cell Biology, Emory University, Atlanta, GA.

208A
Molecular, cellular and biochemical analysis of the function of *bene/gatA*, a gene required for growth and maturation in Drosophila. **Anna Kruyer, Leah Bergman, Mikhail Gertsberg, Grace Vernon, Jason Morris.** Department of Natural Sciences, Fordham University, New York, NY.

209B
Expression profiling of Bowl-induced hyperplastic wing imaginal discs reveals multiple deregulated processes. **Elzbieta Kula-Eversole[1], Victor Hatini[1,2].** 1) Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA; 2) Program in Cell, Molecular and Developmental Biology.

210C
The role of the JAK/STAT pathway in growth control in Drosophila. **Aloma Rodrigues, Erika Bach.** Pharmacology, New York Univ. School of Med., New York, NY.

211A
The Fat cadherin acts through the Hippo tumor-suppressor pathway to regulate tissue size. **Maria Willecke[1,3,5], Fisun Hamaratoglu[1,4,5], Madhuri Kango-Singh[1], Ryan Udan[1,4], Chiao-Iin Chen[1,2], Chunyao Tao[1], Xinwei Zhang[1], Georg Halder[1,2,4].** 1) Dept of Biochemistry & Molecular Biology; 2) Program in Genes and Development MD Anderson Cancer Center Houston, TX; 3) Interfakultäres Institut für Zellbiologie Abteilung Genetik der Tiere Universität Tübingen, Germany; 4) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 5) These two authors contributed equally to this work.

212B
Drosophila Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation. **Ryan O. Andersen[1], Cheng-Yu Lee[1,3], Clemens Cabernard[1], Laurina Manning[1], Khoa D. Tran[1], Marcus J. Lanskey[1], Arash Bashirullah[2], Chris Q. Doe[1].** 1) Institutes of Molecular Biology and Neuroscience, Howard Hughes Medical Institute, University of Oregon, Eugene, OR; 2) Department of Human Genetics, Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT; 3) Center for Stem Cell Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI.

213C
Fat tumor suppressor pathway regulates Warts stability and activity. **Yongqiang Feng[1], Eunjoo Cho[1], Cordelia Rauskolb[2], Kenneth Irvine[1].** 1) HHMI, Waksman Inst/Rutgers Univ, Piscataway, NJ; 2) Waksman Institute, Rutgers, The State University of New Jersey, Piscataway New Jersey 08854.

214A
The effector caspase *DCP-1* has functions in *Tor* mediated autophagic cell death in Drosophila. **Young-Il Kim, OokJoon Yoo.** Dept Life Sci, KAIST, DeaJeon, DeaJeon, Korea.

215B
Functional antagonism of E2F and armadillo/β-catenin signaling in apoptosis regulation. **Erick J. Morris, Nicholas J. Dyson.** Lab Molecular Oncology, Massachusetts Gen Hosp Cancer Ctr, Charlestown, MA.

216C
The effector caspase *dcp-1* has functions in *Beclin* mediated autophagic cell death in Drosophila. **JuHyun Shin, OokJoon Yoo.** Life Sci, KAIST, DeaJeon, Korea.

217A
Apoptosis activator *hid* is a target for Rb-E2F mediate transcriptional repression. **Miho Tanaka-Matakatsu, Jinhua Xu, Wei Du.** BMICR, University of Chicago, Chicago, IL.

218B
Mob as Tumor Suppressor is Directly Activated by Hippo Kinase for Growth Inhibition. **Xiaomu Wei[1], Takeshi Shimizu[2], Zhi-Chun Lai[1,2,3].** 1) Genetics Program, Pennsylvania State University, University Park, PA; 2) Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA; 3) Dept of Biology, Pennsylvania State University, University Park, PA.

Cytoskeleton and Cellular Biology

219C
Bcr-Abl interacts with Rho GTPases to alter cell migration during Drosophila development. **Nicholas B. Artabazon, Sara Tittermary, Katherine D. Miller, Traci L. Stevens.** Biology Department, Randolph-Macon College, Ashland, VA.

220A
Projectin assembly, domain interactions and elastic domain in IFM myofibril. **Agnes Ayme-Southgate, Danielle Adler, Richard Southgate.** Dept Biol, Col Charleston, Charleston, SC.

221B
Muscle LIM Protein cooperates with Titin to maintain the structural integrity of muscle. **Kathleen Clark[1,2], Jennifer Bland[1], Mary Beckerle[1,2,3].** 1) Huntsman Cancer Inst, University of Utah, Salt Lake City, UT; 2) Department of Biology, University of Utah, Salt Lake CIty, UT; 3) Department of Oncology, University of Utah, Salt Lake CIty, UT.

222C
Unexpected complexity in the mechanisms that target assembly of the spectrin cytoskeleton. **Amlan Das, Christine Base, Srilakshmi Dhulipala, Ron Dubreuil.** Biological Sciences, Univ of Illinois at Chicago, Chicago, IL.

223A
How Do Cells Shape Actin-Based Protrusions? **Stacie A. Dilks, Stephen DiNardo.** University of Pennsylvania, Philadelphia, PA.

224B
Trc/Fry and Wts/Mats have opposite effects on wing cell shape and timing of hair initiation in *D. melanogaster*. **Xiaolan Fang, Paul Adler.** Department of Biology, University of Virginia, Charlottesville, VA.

225C
Ena promotes actin filament elongation during epithelial morphogenesis. **Julie Gates[1], Connie Barko[1], Stuart Hollenshead[1], Justine Lu[1], Frank B. Gertler[2], Mark Peifer[3].** 1) Biology, Bucknell University, Lewisburg, PA; 2) Biology, MIT, Cambridge, MA; 3) Biology, UNC-CH, Chapel Hill, NC.

226A
The role of a short peptide gene, *polished rice*, during imaginal development. **Yoshiko Hashimoto[1], Takefumi Kondo[1], Yuji Kageyama[1,2].** 1) Nara Institute of Science and Technology, Ikoma, Nara, Japan; 2) PREST, Japan Science and Technology Agency.

227B
Diaphanous, a link between the actin and myosin cytoskeleton. **Catarina Homem, Mark Peifer.** Dept Biol, UNC, Chapel Hill, NC.

228C
Polycistronically-encoded small peptides regulate actin-based morphogenesis in Drosophila. **Takefumi Kondo[1], Kagayaki Kato[3], Yoshiko Hashimoto[1], Shigeo Hayashi[3], Yuji Kageyama[1,2].** 1) Grad. Sch. Biol. Sci., NAIST, Nara, Japan; 2) PREST, Japan Science and Technology Agency; 3) Riken Center for Developmental Biology, Kobe, Japan.

229A
Identification and characterization of the role of TRAF1 in Drosophila ventral furrow formation. **Sam J. Mathew, Thomas C. Seher, Maria Leptin.** Institute for Genetics, University of Cologne, Germany.

230B
Plasma membrane diffusion barriers in the precellularizing Drosophila embryo. **Manos Mavrakis[1], Richa Rikhy[1], Bob Phair[2], Jennifer Lippincott-Schwartz[1].** 1) Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892; 2) Integrative Bioinformatics Inc, Los Altos, CA 94024.

231C
The role of DRhoGEF2 during segmental groove formation in the Drosophila embryo. **Shai Mulinari, Mojgan Padash Barmchi, Udo Häcker.** Dept Exp Med Sci, Lund Strategic Research Center for Stem Cell Biology and Cell Therapy Lund Univ, Lund, Sweden.

232A
Characterization of PTEN and skittles function during photoreceptor morphogenesis. **Ella Palmer, Franck Pichaud.** MRC LMCB, UCL, Gower Street, London. WC1E 6BT.

233B
Characterization of mutants defective in salivary gland migration along the circular visceral mesoderm. **Unisha B. Patel.** Cell and Developmental Biology, WMC of Cornell University, New York, NY.

234C
Dissecting Rhodopsin 1 function in photoreceptor morphogenesis. **Noelia Pinal, Franck Pichaud.** Deot LMCB, MRC, London, UK.

235A
Rac GTPase regulation of E-cadherin-mediated cell-cell adhesion in epithelial migration. **Carolyn Pirraglia, Monn Monn Myat.** Department of Cell & Developmental Biology, Weill Medical College of Cornell University, New York, NY 10021.

236B
Actin capping proteins maintain epithelium integrity of *vestigial*-expressing cells in the wing blade epithelium. **Sofia R. P. Rebelo, Florence Janody.** Actin Dynamics Unit, Instituto Gulbenkian de Ciênca, Oeiras, Portugal.

237C
Myoblast fusion requires active remodeling of the actin cytoskeleton. **Brian Richardson[1], Mary Baylies[2].** 1) Program in Biochemistry, Molecular and Cell Biology, Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

238A
An RNAi screen for genes affecting the architecture and polarity of the Drosophila ovarian follicular epithelium. **Emily C. N. Richardson, Franck Pichaud.** MRC Laboratory for Molecular Cell Biology, University College London, London, United Kingdom.

239B
The Wiskott-Aldrich Syndrome Protein (WASP) is essential for Drosophila myoblast fusionä. **Gritt Schäfer[1], Susanne Weber[2], Anne Holz[3], Sven Bogdan[4], Renate Renkawitz-Pohl[1], Susanne Filiz Önel[1].** 1) Dept. for Developmental Biology, Philipps-Universität, Karl-von-Frisch Str. 8, D-35043 Germany; 2) Institute for Moleculare Biology and Tumor Research, Philipps-Universität Marburg, Emil-Mannkopff-Str. 2, D-35033 Marburg, Germany; 3) Institute for Allgemeine und Spezielle Zoologie, Stephanstr. 24, Justus-Liebig- Universität Giessen, D-35390 Giessen, Germany; 4) Institute for Neurobiologie, Universität Münster, Badestr. 9, D-48149 Münster, Germany.

240C
Activated myosin II is required for cell alignment in the epidermis. **Robert P. Simone.** Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

241A
Investigating the role of *crinkled* (*ck*) Myosin VIIA in the morphogenesis of actin-rich cellular projections in *D. melanogaster*. **Vinay Singh, Jody Franke, Mark Chee, Daniel P. Kiehart.** Department of Biology (DCMB), Duke University, Durham, NC.

242B
A molecular analysis of *Src64* during cellularization. **Taylor C. Strong, Jeffrey Thomas.** Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX.

243C
The STE20 kinase *misshapen* acts with the SH2/SH3 adapter *dPOSH* in patterning the rhabdomere. **Rhian Walther, Franck Pichaud.** MRC LMCB, University College London, London, United Kingdom.

244A
Cloning and characterization of *E(br)165*, a mutation that dominantly enhances *br*[1]. **Xiaochen Wang, Elspeth Pearce, Robert Ward.** Dept Molecular Biosciences, Univ Kansas, Lawrence, KS.

245B
An APC2-Diaphanous complex organizes actin in the Drosophila syncytial embryo. **Rebecca L. Webb, Jasper S. Weinberg, Meng-Ning Zhou, Sarah E. Clark, Brooke M. McCartney.** Dept Biological Sci, Carnegie Mellon Univ, Pittsburgh, PA.

246C
Rho family GTPases in the Drosophila larval cellular immune response. **Michael Williams, Dan Hultmark.** UCMP, Umea Univ, Umea, Sweden.

247A
Reciprocal regulation of Rho GTPase and Crumbs in epithelial polarity and contraction. **Na Xu, Benison Keung, Monn Myat.** Dept Cell & Developmental Biol, Weill Medical Col of Cornell, New York, NY.

248B
Cloning and characterization of a dominant modifier of *broad*. **Liang Zhang, Stefani Fontana, Ty Beaver, Kistie Patch, Robert Ward.** Dept Molecular Biosciences, Univ Kansas, Lawrence, KS.

249C
The transcription factor Broad mediates hormone regulated cell shape change during Drosophila pupariation. **Xiaofeng Zhou, Xiaoqun Zeng, Lynn M. Riddiford.** Department of Biology, University of Washington, Seattle, WA.

250A
Car/Vps33A is necessary for SNARE-mediated trafficking to Lysosomes and Lysosome-related organelles (LROs). **Mohammed Akbar, Sanchali Ray, Helmut Kramer.** Ctr Basic Neuroscience, Univ Texas Southwestern Med Ctr, Dallas, TX.

251B
Characterization of the Apical Microtubule Association of Klarsicht in the Developing Drosophila Eye Disc. **Susan Banks, Janice Fischer.** ICMB, Univ Texas, Austin, Austin, TX.

252C
The δ isoform of the transport regulator Klar plays a role in nuclear positioning within the eye disk and the ovary. **Sean Cotton[1], Dae-Hwan Kim[1], Amanda Norvell[2], Michael Welte[1].** 1) Dept Biol, Brandeis Univ, Waltham, MA; 2) Dept Biol, The College of New Jersey, Ewing, NJ.

253A
Hook-like is a regulator of endocytic trafficking and pigment granule function in Drosophila. **Adam Haberman, Helmut Krämer.** Cntr Basic Neurosci, UT Southwestern Med Cntr, Dallas, TX.

254B
In vivo engineering of *klarsicht*: a tool kit to dissect a complex gene. **Ankit Jain, Michael Welte.** Department of Biology, Brandeis University, Waltham, MA.

255C
Apical nuclear migration in the *D. melanogaster* 3rd instar eye imaginal disc requires the KASH protein Klarsicht and the SUN protein Klaroid. **M. Kracklauer, S. Banks, J. Fischer.** Dept MCD Biol, University of Texas, Austin, TX.

256A
The endocytic regulator dRabenosyn is a novel neoplastic tumor suppressor. **Holly A. Morrison, Heather Dionne, David Bilder.** Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

257B
Distinct functions for the Kinesin-1 tail in oogenesis and axonal transport. **Pangkong Moua, Debra J. Rose, Katherine M. Brendza, Rahul Warrior, William M. Saxton.** Biology, Indiana University, Bloomington, IN.

258C
Regulation of apical membrane architecture via Rab11-dependent intracellular trafficking. **Jeremiah Roeth, Danny Willner, Mark Peifer.** Department of Biology, UNC-CH, Chapel Hill, NC.

259A
Genetic Screens for Axonal and Dendritic Interactors of Kinesin Heavy Chain. **Kristina Schimmelpfeng, Cheryl Herrera, Meike Roux, Lawrence S. B. Goldstein.** Dept Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA 92093-0683.

260B
Determining the function of the intracellular domain of the cell adhesion molecule Echinoid. **Grant W. Simmons, Susan Spencer.** Biology, Saint Louis University, Saint Louis, MO.

261C
Partners and dependencies in the modulation of surface protein level by β[Heavy]-spectrin. **Graham Thomas, Mansi Khanna, Janice Williams, Elizabeth Klipfell, Krystal Sandilos.** Depts Biol & BMB, Penn State Univ, University Park, PA.

262A
Plus-end Transport of Lipid Droplets in the Drosophila Embryo is Driven by Kinesin I and Influenced by Levels of Halo and Dynein. **Susan Tran, Michael Welte.** Dept Biol, Brandeis Univ, Waltham, MA.

263B
A genetic screen for regulators of Amyloid Precursor Protein in axonal transport. **Carole Weaver, Kristina Schimmelpfeng, Cheryl Herrera, Lawrence S. B. Goldstein.** Department of Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA.

264C
Exploring a potential function of Drosophila Msp-300 in Nuclear Positioning. **Xuanhua Xie, Janice Fischer.** 2500 Speedway, MBB1 312, Austin, TX.

265A
Identification of protein interactors of Dribble—a single KH domain nucleolar protein in Drosophila. **Ching-Gee Choi[1,2], H. Y. Edwin Chan[1,2,3].** 1) Laboratory of Drosophila Research; 2) Molecular Biotechnology Program; 3) Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, China.

266B
Polyhomeotic functions in the maintenance of epithelial integrity in the *D. melanogaster* wing and ovarian follicle. **Pierre Gandille[1], Karine Narbonne-Reveau[2], Elisabeth Boissonneau[1], Denise Busson[3], Anne-Marie Pret[1].** 1) CGM-CNRS-UPR 2167, 91 198 Gif-sur-Yvette, France; 2) Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892; 3) Institut Jacques Monod (UMR 7592), Laboratoire de Génétique du Développement et Evolution, 75 251 Paris Cedex 05, France.

267C
Analysis of proteins required for CDM-meditated myoblast fusion in Drosophila. **Erika R. Geisbrecht[1], Mei-Hui Chen[1], Lakshmi Balagopalan[2], Susan M. Abmayr[1].** 1) Stowers Institute, Kansas City, MO; 2) NIH, Bethesda, MD.

268A
Identifying Interactors of Invadolysin: a novel metalloprotease required for mitosis and migration. **Shubha Gururaja Rao, Bryce Nelson, Margarete Heck.** Queen's Medical Research Institute, Centre for Cardiovascular Science, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK.

269B
Genetic analysis of an aseptic wound response pathway in epidermis. **Michelle T. Juarez, Joesph C. Pearson, William J. McGinnis.** Biological Sciences, UC San Diego, La Jolla, CA.

270C
Novel roles for the actin-binding protein Quail. **Dorothy A. Lerit, Elizabeth R. Gavis.** Dept. of Molecular Biology, Princeton University, Princeton, NJ.

271A
Function of Zona Pellucida proteins in epidermal cell morphogenesis. **Francois Payre[1], Isabelle Fernandes[1], Helene Chanaut-Delalande[1,2], Philippe Valenti[1], Serge Plaza[1].** 1) Centre de Biologie du Developpement, Universite P. Sabatier, Toulouse, France; 2) Biozentrum der Universitat Basel, Basel, Switzerland.

272B
Systematic analysis of phosphoinositide phosphates during morphogenesis. **Inês Ribeiro, Amy Kiger.** Division of Biological Sciences, UCSD, La Jolla, CA.

273C
Dissecting Muscle and Neuronal Disorders in Drosophila Muscular Dystrophy. **Halyna Shcherbata[1], Andriy Yatsenko[1,2], Maria Kucherenko[1,2], Uri Nudel[3], David Yaffe[3], David Baker[1], Hannele Ruohola-Baker[1].** 1) Dept Biochemistry, Univ Washington, Seattle, WA; 2) Ivan Franko Lviv University, Ukraine; 3) The Weizmann Institute of Science, Rehovolt, Izrael.

274A
Identification of substrates for the Drosophila ABC-transporter DMRP. **Jolene Tarnay[1], Steven Robinow[2].** 1) Cell & Molec Biol, Univ Hawaii - Manoa, Honolulu, HI; 2) Department of Zoology, University of Hawaii, Honolulu, HI.

275B
Roles for Myotubularin Phosphoinositide Phosphatase in Membrane Homeostasis and Cellular Morphogenesis. **Michaella Velichkova, Amy Kiger.** Division of Biological Sciences, UCSD, La Jolla, CA.

276C
awd, the homolog of the human *Nm23* metastasis suppressor gene, regulates epithelial integrity of follicle cells. **Julie A. Woolworth[1], Tien Hsu[2].** 1) Dept Molecular & Cellular Biology, Medical Univ South Carolina, Charleston, SC; 2) Dept Pathology & Laboratory Medicine, Medical Univ South Carolina, Charleston, SC.

277A
APC2, Armadillo and α-catenin form a complex in Drosophila syncytial embryos. **Meng-Ning Zhou, Andrea Blitzer, Brooke McCartney.** Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

278B
Interaction of Drosophila rab GDI with Gint3, a ubiquitin-domain-containing protein. **Clarissa Cheney, Palak Amin, Alyssa Cope, Michael Lawson, Brian Richardson, Alex Chen, Katherine Ayres, Alexis Moore, Naveen Sangji, Michelle Keese.** Dept Biol, Pomona Col, Claremont, CA.

279C
Orchestrating secretory machinery in the larval salivary gland. **Benjamin F. B. Costantino, Daniel Bricker, Andrew Andres.** Biological Sciences, UNLV, Las Vegas, NV.

280A
The role of an Arf-like GTPase in plasma membrane remodeling required for tracheal tube connection. **Ken Kakihara[1,2], Kagayaki Kato[1], Hosei Wada[1], Shigeo Hayashi[1,2].** 1) Morphogenetic Signaling, Riken CDB, Kobe, Hyogo, Japan; 2) Dept. Sci. Tech., Kobe Univ. Kobe, Hyogo, Japan.

281B
In vivo motor protein interaction revealed in spatial and temporal dynamics of vesicle transport in Drosophila segmental nerve axons. **Gerald F. Reis[1], Ge Yang[2], Sameer Shah[3], Lukasz Szpankowski[4], Gaudenz Danuser[2], Lawerence Goldstein[5].** 1) Neuroscience, UCSD, La Jolla, CA; 2) The Scripps Research Institute, La Jolla, CA; 3) Bioengineering, University of Maryland; 4) Bioinformatics, UCSD, La Jolla; 5) Cellular and Molecular Medicine, UCSD, La Jolla.

Genome and Chromosome Structure

282C
Epigenetic regulation of centromeres. **Sylvia Erhardt[1], Barbara Mellone[1], Craig Betts[2], Aaron Straight[2], Gary Karpen[1].** 1) Dept Genome Biol, Lawrence Berkeley National Lab, Berkeley, CA; 2) Beckman Center, Dept. of Biochemistry, Stanford University, Stanford, CA.

283A
Centromere formation and function in Drosophila. **Barbara Mellone[1,2], Sylvia Erhardt[1,2], Craig Betts[3], Aaron Straight[3], Gary Karpen[1,2].** 1) Dept. of Genome Sciences, Lawrence Berkeley Laboratory, Berkeley, CA; 2) MCB Dept., University of California at Berkeley, Berkeley, CA; 3) Dept. of Biochemistry, Stanford University School of Medicine, Stanford, CA.

284B
Lethal 6/neverland is an essential 3L heterochromatic gene involved in the ecdysone biosynthetic pathway in *D. melanogaster*. **Monika A. Syrzycka, Don A. R. Sinclair, Kathleen A. Fitzpatrick, Barry Honda.** Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

285C
The role of MT2 in *D. melanogaster*. **Catalina Alfonso, Keith Maggert.** Dept Biochemistry/Biophysics, Texas A&M Univ, College Station, TX.

286A
CHD1: A broadly expressed chromatin remodeling factor with a potential role in wing development. **Jennifer A. Armstrong, Ivy E. McDaniel, Jennifer M. Lee, Parimal A. Deodhar.** Joint Sci Dept, The Claremont Colleges, Claremont, CA.

287B
Requirements for HP1-mediated silent chromatin spreading. **Diane E. Cryderman, Karrie A. Hines, Lori L. Wallrath.** Department of Biochemistry, University of Iowa, Iowa City, IA.

288C
The histone-acetylase dGcn5 is involved in modulation of high order chromatin structures. **Caroline Jacquier[1], Clément Carré[1], Anita Ciurciu[2], Orban Komonyi[2], Delphine Fagegaltier[1], Josette Pidoux[1], Hervé Tricoire[3], Laszlo Tora[4], Imre Boros[2], Christophe Antoniewski[1].** 1) Developmental Biology, CNRS / Institut Pasteur, Paris, France; 2) Department of Genetics and Molecular Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary; 3) Institut Jacques Monod 2 place Jussieu 75251 Paris, France; 4) IGBMC/UMR 7104 CNRS, Parc d'Innovation,1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France.

289A
Genetic Interactions between RNAi components and RNA Polymerase II subunit in Drosophila. **Harsh Kavi, James Birchler.** Dept Biological Sci, Univ Missouri, Columbia, MO.

290B
High-Resolution Mapping of histone modifications in Drosophila Stage 5 embryos. **Sasha Langley, Gary Karpen.** Dept MCB, Univ California Berkeley/LBNL, Berkeley, CA.

291C
Chromatin structure of genes silenced by heterochromatin in *trans*. **Parul Nisha, Amy K. Csink.** Dept Biological Sci, Carnegie Mellon Univ, Pittsburgh, PA.

292A
Deciphering the roles of histone demethylases in chromatin-based mechanisms during development. **Neetu Singh, Felice Elefant.** Dept Bioscience/Biotechnology, Drexel Univ, Philadelphia, PA.

293B
Deciphering the effects of the amyloid precursor protein (APP) on the regulation of the histone acetyltransferase Tip60's target genes. **Meridith Toth, Felice Elefant.** Dept Bioscience/ Biotechnology, Drexel Univ, Philadelphia, PA.

294C
Poly ADP-ribose Polymerase in chromatin and transcriptional regulation. **Alexei Tulin, Natasha Naumova, Elena Kotova, Aaron Pinnola.** Dept Basic Sci, Fox Chase Cancer Ctr, Philadelphia, PA.

295A
Histone Acetyltransferase Human Homolog Dmel/TIP60 is Essential for Multicellular Development in Drosophila. **Xianmin Zhu, Felice Elefant.** Dept Bioscience/Biotechnology, Drexel Univ, Philadelphia, PA.

296B
Centrosomal localization is required for proper chromosomal distribution of a chromatin insulator component. **Omar Akbari, Daniel Oliver, Chi-Yun Pai.** Dept Biol, Univ Nevada, Reno, NV.

297C
Insulating activity analysis of the interbands of *D. melanogaster* polytene chromosomes. **Maria B. Berkaeva, Sergey A. Demakov, Igor F. Zhimulev.** Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia.

298A
Study of an endogenous insulator found downstream of the Drosophila mini-white gene. **Pavel G. Georgiev, Darya Chetverina.** Institute of Gene Biology RAS, Moscow, Russia.

299B
Dual functional activity of the Mcp insulator from the Drosophila bithorax complex and effects of insulator pairing on gene expression. **Pavel G. Georgiev, Olga Kyrchanova, Stepan Toshchakov.** Institute of Gene Biology RAS, Moscow, Russia.

300C
tRNA genes: a potential role as boundary elements in *D. melanogaster*. **Paola Guerrero, Keith Maggert.** Dept Biology, Texas A & M Univ, College Station, TX.

301A
Characterization of knockout mutations in the BEAF gene generated by homologous recombination. **Craig M. Hart, Matthew K. Gilbert, Swarnava Roy.** Dept Biological Sci, Louisiana State Univ, Baton Rouge, LA.

302B
Regulation of the Fab-8 Insulator of the Abd-B Gene Requires the Activities of dCTCF and Mod(mdg4). **Sheryl Smith, Qi Chen, Ian Thomas, Jamie Planck, Lan Lin, Jumin Zhou.** Gene Expression Program, The Wistar Institute, Philadelphia, PA.

303C
Zygotic gene activity is dispensable for the initiation of somatic homolog pairing. **Jack R. Bateman, Ting Wu.** Department of Genetics, Harvard Medical School, Boston, MA.

304A
Comprehensive analysis of the *Minute* loci in the Drosophila genome. **Kevin Cook[1], John Roote[2], Andrew Lambertsson[3], Gunter Reuter[4], Michael Ashburner[2], Gillian Millburn[2], Paul Harrison[5], Zhan Yu[5], Thomas Kaufman[1], Naoya Kenmochi[6], Sally Leevers[7], Steven Marygold[2,7].** 1) Bloomington Stock Center, Indiana University; 2) University of Cambridge; 3) University of Oslo; 4) Martin Luther University, Halle; 5) McGill University; 6) Miyazaki University; 7) Cancer Research UK, London Research Institute.

305B
A model of segmental duplication formation in *D. melanogaster*: impact of transposable elements. **Anna-Sophie A. S. Fiston[1], Dominique D. Anxolabéhère[2], Hadi H. Quesneville[1].** 1) Bioinformatics and Genomics, Jacques Monod Institut, Paris, France; 2) Dynamics of the Genome and Evolution, Jacques Monod Institut, Paris, France.

306C
Trans-regulatory effects at the *D. apterous* locus. **Daryl Gohl[1], Martin Müller[2], Paul Schedl[1].** 1) Dept. of Molecular Biology, Princeton University, Princeton, NJ; 2) Dept. of Cell Biology, University of Basel, Basel, Switzerland.

307A
The Drosophila Heterochromatin Genome Project. **Roger Hoskins, Christopher Smith, Cameron Kennedy, David Acevedo, Joseph Carlson, Susan Celniker, Gary Karpen.** Department of Genome Biology, Lawrence Berkeley National Laboratory, Berkeley, CA.

308B
The involvement of Drosophila error-prone DNA polymerases in DNA double-strand break repair. **Daniel P. Kane, Sarah Rubin, Justine Liepkalns, Mitch McVey.** Tufts University, Medford, MA.

309C
SUUR protein follows replication during the S-phase in *D. melanogaster* salivary gland polytene chromosomes. **Tatiana D. Kolesnikova, Eugenia N. Andreyeva.** Institute of Cytology And Genetics, Russian Academy of Sciences, Novosibirsk, Russia.

310A
Mapping the Y-to-autosome translocation in *D. pseudoobscura*. **Amanda M. Larracuente[1], Mohamed A. F. Noor[2], Andrew G. Clark[1].** 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) DCMB Group/Biology, Duke University, Durham, NC.

311B
Identifying Regulators of Chromosome Territories in *D. Melanogaster*. **Akiko Minoda, Gary Karpen.** Dept Genome Biol, Lawrence Berkeley Natl Lab, Berkeley, CA.

312C
Tarsal-less defines a new family of polycistronic genes in eukaryotes containing small ORFs with key functions during development. **Jose Pueyo-Marques, Maximo Galindo, Sylvaine Fouix, Juan Pablo Couso.** School of Life Sciences, Univ Sussex, Brighton, UK.

313A
Aberrant mono-methylation of histone H4 lysine 20 activates the DNA damage checkpoint in Drosophila. **Ayako Sakaguchi, Ruth Steward.** Waksman Institute, Rutgers University, Piscataway, NJ.

314B
Overexpression of the *D1* gene affects chromosome morphology and viability. **Marissa B. Smith, Karen S. Weiler.** Department of Biology, West Virginia University, Morgantown, WV.

315C
The Role of Drosophila Ligase III in DNA Break Repair. **Ilana Traynis, Mitch McVey.** Biology Department, Tufts University, Medford, MA.

316A
Endogenous interbands of *D. melanogaster* polytene chromosomes contain non-coding regions of genome and form ectopic interbands. **T. Yu Vatolina, S. A. Demakov, V. F. Semeshin, V. V. Shloma, I. F. Zhimulev.** Russian Academy of Sciences, Institute of Cytology and Genetics, Novosibirsk, Russian Federation.

317B
Tissue-specific roles of Drosophila DNA ligase 4 in suppressing large deletions during repair of complementary-ended DNA double strand breaks. **Amy M. Yu, Mitch McVey.** Department of Biology, Tufts University, Medford, MA.

318C
ESC-like forms the ESCL/E(Z) complex and functions during embryonic and postembryonic stages. **Feng Tie, Rebeccah Kurzhals, Carl Stratton, Jayashree Prasad-Sinha, Peter Harte.** Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

319A
Repetitious Element *1360* as a Target for Heterochromatin Formation in *D. melanogaster*. **Kathryn L. Huisinga, Alejandra Figueroa-Clarevega, Stephen McDaniel, Shachar Shimonovich, Amy Wu, Jo Wuller, Sarah C. R. Elgin.** Department of Biology, Washington University, Saint Louis, MO.

320B
Studies of the Preferential Male Lethality of Mutants for the HOAP Protein. **Hui Li, Michelle Collins, Rebecca Kellum.** Biology, University of Kentucky, Lexington, KY.

321C
Short and long-range trans-regulatory interactions mediated by reciprocal translocations with a heterochromatic break point on the fourth chromosome. **Martin Muller[1], Daryl Gohl[2], Henrik Gyurkovics[3], Olivier Cuvier[4], Markus Affolter[1], Paul Schedl[2].** 1) Dept. of Cellbiology Biozentrum CH-4056 Basel; 2) Dept. of Molecular Biology Princeton University Princeton NJ 08544; 3) Institute Of Genetics BRC H-6701 Szeged; 4) Institute of Human Genetics CNRS F-34396 Montpellier.

322A
Investigating the Role of *rDNA* in Genomic Imprinting. **Silvana Paredes, Keith Maggert.** Dept Biol, Texas A&M Univ, College Station, TX.

323B
Studies on the function of MU2. **Raghuvar G. Dronamraju, James M. Mason.** Laboratory of Molecular Genetics National Institute of Environmental Health Sciences, Research Triangle Park, NC.

324C
Rapid evolution of Drosophila telomere proteins. **Nels C. Elde, Harmit S. Malik.** Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

325A
Recycling the genome: Umbrea, a telomere-associated protein in Drosophila, arose from a duplicated HP1 protein. **Mary Alice Hiatt, Nels Elde, Danielle Vermaak, Harmit Malik.** Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

326B
Mutator2: A possible chromatin modulator at telomeres. **Sudha Prasad, James M. Mason.** Lab Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC. 27709.

327C
Epigenetic *trans*-silencing and *P* element repression in *D. melanogaster*. **Stéphane Ronsseray, Anne-Laure Todeschini, Thibaut Josse, Laure Teysset, Augustin de Vanssay, Dominique Anxolabéhère.** Dynamique du Génome, Inst. Jacques Monod, Paris, France.

328A
The multiplicity of Drosophila telomeric retrotransposons unveils extensive and rapid evolution of Drosophila telomeres. **Alfredo Villasante[1], Rosario Planelló[1], María Méndez-Lago[1], Susan Celniker[2], José P. Abad[1].** 1) Centro de Biologia Molecular, CSIC-Univ Autonoma, Madrid, Spain; 2) Department of Genome Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA.

Regulation of Gene Expression

329B
Nerfin-1: a novel binding partner of Scalloped. **Ankush Garg[1], Alexander Kuzin[2], Thomas Brody[2], Ward Odenwald[2], John Bell[1].** 1) Dept Biological Sci, University of Alberta, Edmonton, AB, Canada; 2) Neural Cell-Fate Determinants Section, National Institutes of Health, Bethesda, MD.

330C
Ash1, Pc and Pho influence embryonic transcriptional activation by Myc via a bivalent chromatin domain. **Julie Goodliffe[1], Michael Cole[2], Eric Wieschaus[3].** 1) Biology Department, UNC Charlotte, Charlotte, NC; 2) Dartmouth Medical School, Lebanon, NH; 3) HHMI, Princeton University, Princeton, NJ.

331A
Cell cycle regulation by retinal determination genes. **Jennifer Jemc[1], Ilaria Rebay[2].** 1) Dept. Biology MIT, Cambridge, MA; 2) Ben May Inst, Univ Chicago, Chicago, IL.

332B
Identifying the activator of early zen expression during early Drosophila embryos. **Chung-Yi Nien, Hsiao-Yun Liu, Nikolai Kirov, Christine Rushlow.** Department of Biology, New York University, New York, NY.

333C
Whole genome analysis of Bcd dependent transcriptional regulation in the early fly embryo. **Amanda Ochoa-Espinosa, Stephen Small.** Department of Biology, New York University, New York, NY.

334A
RNAi effects on Domino. **Jim Zhong, Wooly Pierre, Barry Yedvobnick.** Biology Department, Emory University, Atlanta, GA.

335B
Virtual embryos as tools for 3D gene expression analyses. **Cris L. Luengo Hendriks[1], Charless C. Fowlkes[2], Soile V. E. Keränen[3], Lisa Simirenko[3], Gunther H. Weber[4], Oliver Rübel[4], Min-Yu Huang[4], Angela H. DePace[3], Clara N. Henriquez[3], Xiao-Yong Li[3], Hou C. Chu[3], David W. Kaszuba[1], Amy Beaton[1], Susan E. Celniker[1], Bernd Hamann[4], Michael B. Eisen[3], Jitendra Malik[2], David W. Knowles[1], Mark D. Biggin[3].** 1) Life Sci. Div., Lawrence Berkeley Lab, Berkeley, CA; 2) Computer Sci. Div., UC Berkeley, CA; 3) Genomics Div., Lawrence Berkeley Lab, Berkeley, CA; 4) Inst. for Data Analysis and Visualization, UC Davis, CA.

336C
Elucidation of molecular processes involved in enhancer blocking by the Su(Hw) insulator protein. **Brian McCluskey[1], David Gilmour[2], Pamela Geyer[1].** 1) Biochemistry, University of Iowa, Iowa City, IA 52242; 2) Biochemistry, Pennsylvania State University, University Park, PA 16802.

337A
Bhringi, a highly conserved regulator of Twist transcription factor activity. **Scott J. Nowak[1], Katie Gonzalez[2], Mary K. Baylies[1].** 1) Baylies Laboratory/RRL 1065, Sloan Kettering Inst, New York, NY; 2) Scripps Research Institute, La Jolla, CA.

338B
Identification and characterization of Wingless Response Elements from a direct Wg-target gene, naked cuticle (nkd) in Drosophila. **Jinhee Chang[1], Mikyung Chang[1], Scott Barolo[2], Kenneth M. Cadigan[1].** 1) Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109; 2) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI 48109.

339C
3-Dimensional quantitative analysis of gene expression in multiple Drosophila species. **Angela H. DePace[1], Stewart MacArthur[2], Daniel Pollard[1], Venky Iyer[1], Soile Keränen[2], Clara N. Henriquez[2], Cris Luengo Hendriks[2], Charless Fowlkes[3], Lisa Simirenko[2], Jitendra Malik[3], David W. Knowles[2], Mark D. Biggin[2], Michael B. Eisen[1,2].** 1) Molecular and Cellular Biology, UC Berkeley, CA; 2) Genome Sciences, Lawrence Berkeley National Lab, CA; 3) Computer Science, UC Berkeley, CA.

340A
Phylogenetic Footprinting Analysis in the Regulatory Regions of the *D. Enhancer of split* Genes. **Deborah Eastman, Morgan Maeder, Bryanne Robson, Benjamin Polansky.** Dept Biol, Connecticut College, New London, CT.

341B
Coupling between SxlPe and SxlPm reinforces the female developmental switch. **Alejandra Gonzalez, James Erickson.** Dept Biol, Texas A&M Univ, College Station, TX.

342C
Evolution of *cis*-regulatory sequences in acalyptrate Cyclorrhapha: sequencing and analysis of 200 developmental loci in *Drosophilidae*, *Sepsidae*, and *Tephritidae*. **Emily Hare[1], Brant Peterson[1], Venky Iyer[1], Rudolf Meier[2], Rick Kurashima[3], Eric Jang[3], Brian Wiegmann[4], Michael Eisen[1,5].** 1) Dept Molecular & Cell Biol, Univ of California, Berkeley, Berkeley, CA; 2) Department of Biological Sciences, National University of Singapore, Singapore; 3) Pacific Basin Agricultural Research Center, UDSA Agricultural Research Service, Honolulu, HI; 4) Department of Entomology, North Carolina State University, Raleigh, NC; 5) Genome Sciences Department, Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

343A
Characterization of cis-regulatory elements of the glial gene *repo*. **Bradley W. Jones, Jamie W. Lamberton.** Department of Biology, The University of Mississippi, Oxford, MS.

344B
Transcriptional regulation of *nerfin-1* expression during Drosophila neurogenesis. **Alexander Kuzin, Mukta Kundu, Thomas Brody, Ward F. Odenwald.** Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, MD.

345C
Study of the Transcriptional Regulation of *homothorax* Expression in Drosophila Eye. **Wei-Wen Lan[1,2], Su-Yi Chang[1,2], Ju-Yu Wang[1], Y.Henry Sun[1,2].** 1) Institute of Molecular Biology, Taipei, Taiwan; 2) Faculty of Life sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

346A
Spatial regulation of achaete the leg microchaete primordia global activation and repression by Hairy and Delta. **Ji Inn Lee, Meghana Joshi, Teresa Orenic.** Biological Sciences, University of Illinois-Chicago, Chicago, IL.

347B
Genome Wide Mapping of the In Vivo DNA Binding Sites of Transcriptional Regulators of the Pregastrula Gene Network. **Xiao-Yong Li[1], S. MacArthur[1], R. Bourgon[2], D. Nix[1], N. Ogawa[1], H. C. Chu[1], L. Zeng[1], M. Stapleton[1], L. Simirenko[1], V. Iyer[1], D. Pollard[1], V. Sementchenko[3], T. R. Gingeras[3], M. D. Biggin[1], M. B. Eisen[1].** 1) Berkeley Drosophila Transcription Network Project, Lawrence Berkeley National Lab, Berkeley, CA; 2) Statistics Department, UC Berkeley; 3) Affymetrix, Inc.

348C
A combinatory mechanism sets the border of a low-level Dpp target gene, pannier, in early Drosophila embryos. **Hsiao-Lan Liang, Nikolai Kirov, Christine Rushlow.** Biology, New York Univ, New York, NY.

349A
Regulation of odor receptor genes in trichoid sensilla of Drosophila. **Carson J. Miller, John R. Carlson.** MCDB, Yale University, New Haven, CT.

350B
Harnessing natural sequence diversity to explore regulatory function. **Brant Peterson[1], Emily Hare[1], Rudolf Meier[2], Rick Kurashima[3], Brian Wiegmann[4], Michael Eisen[1].** 1) Dept Molecular & Cell Biol, Univ California, Berkeley, Berkeley, CA; 2) Dept of Biol Sci, Nat Univ Singapore, Singapore; 3) Pacific Basin Agricultural Research Center, UDSA ARS, Honolulu, HI; 4) Dept of Entomology, North Carolina State Univ, Raleigh, NC.

351C
Cis-regulatory control of slp1 expression during segmentation. **L. Prazak[1], M. Fujioka[2], J. Chang[1], X. Wang[1], J. P. Gergen[1].** 1) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, N.Y; 2) Thomas Jefferson University.

352A
A genetic selection to identify regulatory information driving the earliest zygotic transcription in *D. melanogaster*. **William J. Rowell, Warren C. Lee, Thomas W. Cline.** Dept Molecular & Cell Biology, Univ California, Berkeley, CA.

353B
Study of the transcriptional regulation of *unpaired* expression in Drosophila eye development. **Chuan-Ju Wang[1,2], Ya-Hsin Liu[1], Y. Henry Sun[1,2].** 1) Academia Sinica, Institute of Molecular Biology, Taipei, Taiwan; 2) Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

354C
Promotor analysis of Bällchen, a kinase specifically expressed in *D. melanogaster* embryonic neuroblasts. **Toma Yakulov, Alf Herzig, Herbert Jäckle.** Max-Planck-Institut für Biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, Am Fassberg 11, 37077 Göttingen, Germany.

355A
Evolution of the Ventral Midline in Insect Embryos. **Robert Zinzen[1], Jessica Cande[2], Matthew Ronshaugen[2], Dmitri Papatsenko[2], Michael Levine[2].** 1) Developmental Biology Unit, EMBL, Heidelberg, Germany; 2) MCB-GGD, University of California, Berkeley, CA.

356B
Role of oligomeric state in determining the transcriptional regulation of Wingless signaling targets by Drosophila CtBP. **Chandan Bhambhani[1], Ming Fang[2], Ken Cadigan[1].** 1) MCDB, University of Michigan, Ann Arbor, MI; 2) GDB, Southeast University Medical School, Nanjing, China.

357C
Sources of DNA-binding specificity in the Hox protein Ultrabithorax. **Sarah Bondos, Ying Liu, Kathleen Matthews.** Dept Biochemistry & Cell Biol, Rice Univ, Houston, TX.

358A
Genomic analysis of the ecdysone response. **Lucy Cherbas[1,2,3], Yi Zou[1,2,3], Philip Knollman[1,4], Tyler Iams[1], Peter Cherbas[1,2,3].** 1) Dept Biol, Indiana Univ, Bloomington, IN; 2) Drosophila Genomics Resources Center, Indiana University, Bloomington, IN; 3) Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN; 4) Notre Dame University, South Bend, IN.

359B
Hormonal regulation of tanning at eclosion. **Monica M. Davis[1], David A. Primrose[2], Sandra L. O'Keefe[1], Ross B. Hodgetts[1].** 1) Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; 2) Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada.

360C
Scr provides a new look for Hox specificity. **Rohit Joshi[1,3], Jonathan M. Passner[2,3], Alona Sosinsky[1], Remo Rehs[1], Barry Honig[1], Aneel K. Aggarwal[2], Richard Mann[1].** 1) Biochemistry and Mol. Biophy, Columbia University, New York, NY; 2) Department of Molecular Physiology and Biophysics Mount Sinai School of Medicine New York, NY; 3) Contributed equally to the work.

361A
Functional significance of miRNA sequence differences between species. **Supriya Kumar, Chung-I Wu.** Department of Ecology & Evolution, University of Chicago, Chicago, IL 60637.

362B
Molecular basis of the systemic RNAi response in a beetle *Tribolium castaneum*. **Sherry C. Miller, Susan J. Brown, Yoshinori Tomoyasu.** Biology, Kansas State University, Manhattan, KS.

363C
Identification of functional domains and target genes of the Hindsight zinc-finger protein. **Liang Ming[1,2], Ronit Wilk[1,2], Amanda Pickup[2], Howard Lipshitz[1,2].** 1) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Program in Developmental and Stem Cell Biology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

364A
Studies on the role of MLE in dosage compensation. **Rosa Morra, Edwin Smith, John Lucchesi.** Biology, Emory University, Atlanta, GA.

365B
In vitro search of the tissue-specific trans-acting factor interacting with regulatory sequences of the testis expressed *Stellate* genes in *D. melanogaster* . **Oxana M. Olenkina, Ludmila V. Olenina, Sergei A. Lavrov, Vladimir A. Gvozdev.** Institute of Molecular Genetics, Moscow, Russian Federation.

366C
A dMyc-Groucho complex regulates neuronal stem cell fate and mitosis. **Amir Orian[1], Jeffrey Delrow[2], Alicia Rosales Nieves[2], David Metzger[2], Mona Abed[1], Hanaa Knaneh[1], Ze'ev Paroush[3], Robert Eisenman[2], Susan Parkhurst[2].** 1) Rappaport Research Institute, Technion, Haifa, Israel; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) The Hebrew University, Jerusalem, Israel.

367A
The Role of E23 in Regulating Tissue-Specific Hormone Responses. **Elana Paladino[1], Andrew Andres[1], Dan Garza[2].** 1) Department of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) Novartis Institutes of Biomedical Research, Cambridge, Massachusetts.

368B
zucchini and *squash* encode two novel components required for RNAi processes in the Drosophila germline. **Attilio Pane, Kristina Wehr, Trudi Schupbach.** Dept. of Molecular Biology, Princeton University, Princeton, NJ.

369C
Non-coding RNAs in the Bithorax Complex. **Jessica C. Piel, Matthew Ronshaugen, Michael Levine.** Division of Genetics and Development, Department of Molecular Cell Biology, Center for Integrative Genomics, University of California, Berkeley, CA 94720.

370A
Identifying the Proteins Responsible for the Anti-Insulator Function of the Promoter Targeting Sequence in the Abdominal-B Locus. **Jamie L. Planck, Qi Chen, Kaycie Hopkins, Sheryl T. Smith, Jumin Zhou.** Wistar Institute, Philadelphia, PA.

371B
Genetical Genomics in Drosophila: Combined Microarray-QTL Studies Identify Several PbAc-induced Trans-expression-QTL Signaling Pathways. **Douglas M. Ruden[1], Grier Page[2], Parsa Rasouli[1], Daniel Shriner[2].** 1) Inst. for Environ. Health Sci., Wayne State Univ., Detroit, MI; 2) Department of Biostatistics, Univ. AL BHM, Birmingham, AL.

372C
Like its vertebrate homolog, Wilm Tumor Suppresor-1, klumpfuss is localized to both the nucleus and cytoplasm and may bind RNA. **Jamie C. Rusconi, Barbara Zaffo, Erica Hutchins, Kelly Romano.** Dept Biological Sciences, University at Albany, Albany, NY.

373A
Domain analysis of the dBlimp-1, an ecdysone inducible and labile transcription factor in *D. melanogaster*. **Moustafa M. Sarhan[2], Hitoshi Ueda[1,2].** 1) Department of Biology, Okayama University, Okayama, Okayma, Japan; 2) Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

374B
The role of RNA localization in controlling the translation of isoforms of the nuclear receptor E75. **Carol Schwartz[1], Henry Krause[1,2].** 1) Donnelly CCBR, Univ Toronto, Toronto, ON, Canada; 2) Dept. of Molecular and Medical Genetics.

375C
An interaction study of the male specific lethal (MSL) complex and trans-acting dosage effects in metafemales of *D. melanogaster*. **Xiaoping Sun, James Birchler.** Dept Biol, Univ Missouri, Columbia, MO.

376A
Drosophila Blimp-1 is a transient transcription repressor that controls timing of the ecdysone-induced developmental pathway. **Masayoshi Takai[2], Kazutaka Akagi[2], Moustafa Sarhan[2], Hitoshi Ueda[1,2].** 1) Department of Biology, Okayama University, Okayama, Japan; 2) Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

377B
Identifying novel gene targets of Hunchback that regulate early-born neuronal identity in the Drosophila CNS. **Khoa D. Tran, Chris Q. Doe.** Institutes of Neuroscience and Molecular Biology, Howard Hughes Medical Institute, University of Oregon 1254, Eugene Oregon 97403.

378C
Ecdysone signaling and microRNAs. **Jishy Varghese, Stephen Cohen.** Developmental Biology, EMBL, Heidelberg, Germany.

379A
Inhibition of RNA interference by cell death signaling. **Weiwu Xie, James A. Birchler.** Biological Sci Div, Univ Missouri-Columbia, Columbia, MO.

Poster board is in bold above the title. See page 14 for presentation schedule. The first author is the presenter. Abstracts begin on page 77.

380B
Regulation of Retinoblastoma protein stability and function by the COP9 signalosome. **Martin Buckley[1], Zakir Ullah[2], Geoffrey Williams[3], David Arnosti[1], R. William Henry[1].** 1) Department of Biochemistry and Molecular Biology, and Program in Genetics, Michigan State University, East Lansing, MI 48823; 2) The National Institutes of Health NICHD, Building 6/3A-15, 9000 Rockville Pike Bethesda, MD 20890; 3) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Box G-B187 Providence, RI 02912.

381C
Regulation of the Groucho corepressor by phosphorylation. **Einat Cinnamon, Ronny Helman, Ze'ev Paroush.** Department of Biochemistry, Faculty of Medicine, The Hebrew University, Jerusalem, Israel 91120.

382A
Maternally supplied hey protein is a repressor of early Sxl expression. **Elena Kozhina, Hong Lu, Dun Yang, James Erickson.** Dept Biol, Texas A&M Univ, College Station, TX.

383B
The hN13 RING finger protein is a Novel Cofactor for the HES Family of Transcription Factors Involved in Segmentation and Neurogenesis. **David Metzger[1], Dorit Kenyagin[2], Taryn M. Phippen[1], Amir Orian[2], Susan M. Parkhurst[1].** 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) The Rappaport Faculty of Medicine and Research Institute, Techion-Israel Institute of Technology, Haifa, Israel.

384C
Architectural principles shaping the formation of Hox-containing complexes. **Barbara Noro, Richard Mann.** Biochemistry/Molec Biophysics, Columbia University, New York, NY.

385A
Identification of Groucho as a component of the Knirps repressor complex. **Sandhya Payankaulam, David Arnosti.** Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48823.

386B
Analyzing the Effect of Loss of Drosophila SIN3. **Aishwarya Swaminathan, Lori Pile.** Biological Sciences, Wayne State University, Detroit, MI.

387C
Molecular and genetic characterization of Atrophin proteins, a novel class of nuclear receptor corepressors. **Lei Wang, Chih-Cheng Tsai.** Dept Physiology/Biophysics, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854.

388A
Mechanism of transcriptional repression by the ETS family transcription factor YAN. **Jie Zhang, Pavithra Vivekanand, Maureen Cetera, Ilaria Rebay.** Ben May Institute for Cancer Research, The University of Chicago, Chicago, IL.

389B
Tranposon born microRNAs in host-pathogen interaction. **Manika Pal Bhadra[2], L. Mamatha[1,2], Utpal Bhadra[1].** 1) Department of Chemical Biology, Indian Institute of Chemical Technology, Hyderabad 500007, India; 2) Functional Genomics & Gene Silencing Group, Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500 007 India.

390C
The Different Functions of Smaug in Post-Transcriptional Regulation. **Aaron L. Goldman[1], Fiona Menzies[1], Timothy Westwood[2], Howard Lipshitz[1].** 1) Developmental & Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) University of Toronto, Mississauga, Ontario, Canada.

391A
Live imaging of mRNA RNP particles in Drosophila ovaries. **Yiyin Ho, Elizabeth Gavis.** Molecular Biology, Princeton University, Princeton, NJ.

392B
Investigating the dynamics of localized fluorescently labeled *gurken* mRNA in Drosophila. **A. M. Jaramillo[1,2], T. T. Weil[1], E. R. Gavis[1], T. Schupbach[1,2].** 1) Howard Hughes Medical Institute; 2) Department of Molecular Biology, Princeton University, Princeton, NJ.

393C
Expression Patterns of Noncoding Transcripts in the Bithorax Complex. **Benjamin Pease, Welcome Bender.** Harvard Medical School, Dept of Biological Chemistry and Molecular Pharmacology, Boston, MA.

394A
Small interfering RNA induces transcriptional silencing in Drosophila independent to DNA methylation. **Utpal Bhadra[1], Pushpavalli Sncvl[1], Linga Mamatha[1,2], Manika Pal Bhadra[2].** 1) Functional Genomics & Gene Silencing Group, Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500007, India; 2) Department of Chemical Biology, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500007, India.

395B
An alternatively-spliced rnp-4f mRNA isoform retaining a 5'-UTR intron is more efficiently translated and acts upstream of genes important for Drosophila CNS development. **Jing Chen, Sunetra Bhatla, Malini Varadarajan, Jack C. Vaughn.** Zoology, Miami University, Oxford, OH.

396C
Role of Tis11 in *D. melanogaster*. **Robert Fedic[1], Perry J. Blackshear[2], Jasmin Kirchner[3], James M. Mason[1].** 1) Laboratory of Molecular Genetics, NIEHS/NIH, RTP, NC, 27709; 2) Laboratory of Neurobiology, NIEHS/NIH, RTP, NC, 27709; 3) Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK.

397A
Natural genetic variation in splice expression of the sex-determination pathway in *D. melanogaster* . **Brad R. Foley, Anne Genissel, Sergey V. Nuzhdin.** Dept of Evolution and Ecology, UC Davis, Davis, CA.

Poster board is in bold above the title. See page 14 for presentation schedule. The first author is the presenter. Abstracts begin on page 77.

398B
Enhancer and silencer elements associated with non-exonic recursive splice sites. **A. Javier Lopez, Panagiotis Papasaikas, Michael Chen.** Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

399C
Novel features in a Recursive Splice Site model generated by machine learning. **A. Javier Lopez[1], Panagiotis Papasaikas[1], Aly A. Khan[2], Russell Schwartz[1,2].** 1) Biological Sciences; 2) Computer Science, Carnegie Mellon University, Pittsburgh, PA.

400A
Dominant-negative mutation in Cdk9 reveals a role for the transcription elongation factor P-TEFb during oogenesis. **Denis Basquin, Daniel Pauli.** Dept of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland.

401B
Nanos downregulates transcription and modulates CTD phosphorylation in the soma of early Drosophila embryos. **Girish Deshpande, Gretchen Calhoun, Paul Schedl.** Dept Molecular Biol, Princeton Univ, Princeton, NJ.

402C
In Vitro Translation Experiments to Identify Mechanisms of nanos Translational Regulation. **Shane Andrews, Elizabeth Gavis.** Dept Molecular Biol, Princeton Univ, Princeton, NJ.

403A
Molecular basis of RNA recognition by the translational repressor and hnRNP F/H homolog Glorund. **Yossi Kalifa, Elizabeth R. Gavis.** Dept Molecular Biology, Princeton Univ, Princeton, NJ.

404B
Characterization of CG6770, a potential translational inhibitor. **Sara L. Naylor, Marc Tatar.** Ecology & Evolutionary Biology, Brown University, Providence, RI.

405C
The RNAi machinery represses nanos translation in the early embryo. **Ben D. Pinder[1], Wibke J. Meyer[2], H. Arno, J. Müller[3], Craig A. Smibert[1].** 1) Biochemistry, University of Toronto, Toronto, ON, Canada; 2) Institut für Genetik, Heinrich-Heine Universität, Düsseldorf, Germany; 3) Division of Cell and Developmental Biology, University of Dundee, Dundee, UK.

406A
Orb remodels the Cup-Bruno translational repression RNP complex to activate localized translation at the oocyte. **Li Chin Wong[1], Alexandre Costa[2], Ian McLeod[3], John Yates III[3], Paul Schedl[1].** 1) Dept of Molecular Biology, Princeton Univ, Princeton, NJ; 2) Stanford University, Stanford, CA; 3) The Scripps Research Institute, La Jolla, CA.

Signal Transduction

407B
Regulation of Wnt signaling by lipid modification. **Wendy Ching, Roel Nusse.** Dept Developmental Biol, Stanford Univ, Stanford, CA.

408C
Loss-of-function in a Delta allele (Dl^{RF}) is due to protein misfolding and failure to reach the cell surface. **Anton A. Delwig, Matthew D. Rand.** University of Vermont, Burlington, VT.

409A
Ninjurin A has two functions in regulating cell adhesion. **Bernadette Glasheen, Nicholas Simms, Shuning Zhang, Caitlin Piette, Andrea Page-McCaw.** Department of Biology, Rensselaer Polytechnic Institute, Troy, NY.

410B
Drosophila Importin-7/Moleskin alters Delta/Notch expression and activity in the developing wing. **Daniel Marenda.** Department of Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.

411C
Functional analysis in vivo reveals surprisingly robust asssembly of the Axin protein complex. **Marcel Wehrli, Wynne Peterson-Nedry, Naz Erdeniz.** Cell & Dev Biol/L215, Oregon Health & Sci Univ, Portland, OR.

412A
The HMG-box transcription factor, Sox-Neuro, acts with Tcf to control Wg/Wnt signaling activity. **Amy Bejsovec, Whitney Jones, Anna Chao.** Dept. of Biology, Duke University, Durham, NC.

413B
A quantitative assessment of the Hedgehog signaling pathway. **Shohreh Farzan[1], Melanie Stegman[2], Manuel Ascano[1], Stacey Ogden[1], David Robbins[1].** 1) Pharmacology & Toxicology, Dartmouth Medical School, Hanover, NH, 03755; 2) Microbiology & Immunology, Cornell Weill Medical College, New York, NY, 10021.

414C
Identification of JAK/STAT pathway target genes. **Maria Sol Flaherty[1], Jiri Zavadil[2], Aloma Rodrigues[1], Laura Ekas[1], Erika Bach[1].** 1) Pharmacology, New York Univ. School of Med., New York, NY; 2) Pathology, New York Univ. School of Med., New York, NY.

415A
S149 is a new Dpp target that acts as a corepressor with Brinker to promote cell death. **Offer Gerlitz, Yaron Suissa, Oren Ziv, Hadar Neuman, Tama Dinur.** Biochemistry, Faulty of Medicine, The Hebrew University, Jerusalem, Israel.

416B
The role of RacGap50C in the Wingless pathway. **Whitney Jones, Amy Bejsovec.** Biol, Duke Univ, Durham, NC.

417C
Negative regulation of Wingless signaling by a microRNA. **Jennifer Kennell, Kenneth Cadigan.** Dept of Mol, Cell & Dev Biol, Univ of Michigan, Ann Arbor, MI.

418A
The Jak/Stat pathway acts upstream of dpp for GSCs maintenance. **Lourdes López-Onieva, Ana Fernández-Miñan, Acaimo González-Reyes.** Centro Andaluz de Biologia del Desarrollo. CSIC-Universidad Pablo de Olavide, Seville, Spain.

419B
WntD pathway-mediated regulation of NF-κB activity during development and the innate immune response. **Mark A. McElwain, Dennis C. Ko, Michael D. Gordon, Michael A. Katsnelson, Roel Nusse.** Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

420C
A chemical genetic screen to identify new Par-1 targets. **Piyi Papadaki, Anne Ephrussi.** Developmental Biology Unit, EMBL, Heidelberg, Germany.

421A
Deciphering the role of signalling cascades in epidermis differentiation. **Francois Payre[1], Jennifer Zanet[1], Isabelle Delon[1,2], Philippe Valenti[1], Alistair Mac Gregor[3,4], David Stern[3], Serge Plaza[1].** 1) Centre de Biologie du Developpement, Toulouse, France; 2) The Gurdon Institute, Cambridge, UK; 3) Department of Ecology and Evolutionary Biology, Princeton, NJ; 4) Institute of Genetics, Cologne, Germany.

422B
Drosophila SnoN modulates growth and patterning by antagonizing TGF-β signalling. **Marie-Christine Ramel[1], Caroline S. Emery[1], Rebecca Foulger[2], Deborah C. I. Goberdhan[1], Marcel van den Heuvel[3], Clive Wilson[1].** 1) Dept Physiology, Anatomy & Gen, Univ Oxford, Oxford, UK; 2) Research School of Biosciences, Univ Kent, Canterbury, UK; 3) MRC Functional Genetics Unit, Univ Oxford, Oxford, UK.

423C
The roles of D-cblL and D-cblS in eye development. **Hannah Robertson, Jill Ackland, Rebecca Staehr, Adrian Monk, Gary Hime.** Department of Anatomy & Cell Biology, University of Melbourne, Melbourne, Australia.

424A
Fluorescent imaging of the Hedgehog transduction complex. **Matthieu P. Sanial, Anne D. Plessis.** Institut Jacques Monod, CNRS-Paris 7-Paris 6, Paris, France.

425B
Molecular genetic analysis of Drosophila COP9 Signalosome subunit 8. **Daniel Segal[1], Pazit Oren-Giladi[1], Daniel A. Chamovitz[2].** 1) Molecular Microbiol & Biotech; 2) Plant Sciences, Tel Aviv Univ, Tel Aviv, Israel.

426C
Hindsight Mediates the Role of Notch in Suppressing Hedgehog Signaling and Cell Proliferation. **Jianjun Sun, Wu-Min Deng.** Dept Biological Sci, Florida State Univ, Tallahassee, FL.

427A
Genetic mosaic analysis reveals effects of *APC2 APC1* double mutations during Drosophila wing development. **Sandra Zimmerman, Carolyn Mallozzi, Vilma Medrano, Lesley Holot, Lauren Thorpe, Brooke McCartney.** Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

428B
Examining the activation of Slipper, a JNKKK. **Rebecca Gonda, Beth Stronach.** Dept Biological Sci, Univ Pittsburgh, Pittsburgh, PA.

429C
The function of SOCS genes in Drosophila development and signaling pathways. **Qian Guo, Douglas Harrison.** Dept Biol, Univ Kentucky, Lexington, KY.

430A
Regulation of Dishevelled in Wnt/β-Catenin and Fz/planar cell polarity signaling. **Andreas Jenny[1], Thomas Klein[1], Michael Boutros[2], Marek Mlodzik[1].** 1) Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY 10029; 2) Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, D-69120, Germany.

431B
FERM domain specifies apical localization of protein tyrosine phosphatase Pez. **Troy M. Larson, Kavita V. S. Vadali, Kevin A. Edwards.** Biological Sciences, Illinois State University, Normal, IL.

432C
Phenotypic effect of the over-expression of Ras/MAPK components in the developing Drosophila wing. **Neena Majumdar, Daniel R. Marenda.** Department of Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.

433A
Downregulation of Slpr-dependent signaling by Alph phosphatase. **Beth Stronach[1], Caroline Baril[2], Marc Therrien[2].** 1) Dept Biological Sci, Univ Pittsburgh, Pittsburgh, PA; 2) IRIC, Laboratory of Intracellular Signaling, University of Montreal, Montreal, Quebec.

434B
Drosophila protein tyrosine phosphatase dPTP61F, an ortholog of human PTP1B and T cell PTP, modulates insulin signaling through DOCK engagement. **Chia-Lun Wu[1], Han Lee[1,2], Tzu-Ching Meng[1,2].** 1) Institute of Biological Chemistry, Acdemia Sinica, Taipei, Taiwan; 2) Institute of Biochemical Science, National Taiwan University, Taipei, Taiwan.

435C
Tyrosine kinase signaling regulates the retinal determination protein Eyes Absent. **Wenjun Xiong, Noura Dabbouseh, Ilaria Rebay.** Cancer Biology, The University of Chicago, Chicago, IL.

436A
Drosophila Sac1 role in eye development. **Amir A. Yavari, Gerald Call, Raghavendra Nagaraj, Edward Owusu-Ansah, Utpal Banerjee.** MCDB, Univ California, Los Angeles, Los Angeles, CA.

437B
Genetic analysis of the 31E genomic region of *D. melanogaster*: identification of Replication factor. **Amr Amin[1], Yuebing Li[2].** 1) Biol Dept, UAE Univ, Al-Ain, UAE; 2) Neurology Department, Cincinnati University, OH.

438C
Chifoumi is a novel negative regulator of JAK/STAT signalling. **Nadège Pelte[1], Patrick Mueller[2], Martin Zeidler[3], Michael Boutros[1].** 1) Research Group Signaling and Functional Genomics, German Cancer Research Center , Heidelberg, Germany; 2) Department of Molecular Developmental Biology, Max Planck Institute, Goettingen, Germany; 3) Department of Biomedical Science,The University of Sheffield, Sheffield, UK.

439A
Signaling functions of Kurtz in embryonic patterning. **Marla Tipping, Alexey Veraksa.** University of Massachusetts, Boston, Dorchester, MA.

440B
Upd/Jak/STAT signaling represses *wg* transcription to allow initiation of morphogenetic furrow in Drosophila eye development. **Yu-Chen Tsai[1], Jih-Guang Yao[2], Po-Hao Chen[1], Y. Henry Sun[2].** 1) Department of Life Science, Tunghai University, Taichung, Taiwan; 2) Institute of Mol. Biol., Academia Sinica, Taipei, Taiwan.

441C
Functional characterization of Neuralized isoforms and the NHR domain, a novel domain that mediates Delta binding and Notch signaling. **Cosimo Commisso[1,2], Gabrielle L. Boulianne[1,2].** 1) Developmental & Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

442A
The function of StIP in the JAK/STAT pathway. **Linzhu Han, Douglas Harrison.** Biol Dept, University of Kentucky, Lexington, KY.

443B
docked - a gene interacting with *dumpy* during wing development. **Suresh K. Kandasamy, Kiley Maguire, Justin Thackeray.** Biology Department, Clark University, Worcester, MA.

444C
Modulation of Notch signal transduction by endocytotic regulators Numb and the Nedd4 family of ubiquitin ligases. **Koji Kawahashi[1,2], Tadashi Sakata[3], Shigeo Hayashi[1,2].** 1) Laboratory for Morphogenetic Signaling, RIKEN, CDB, Kobe, Japan; 2) Graduate School of Science and Techonology, Kobe Univ; 3) Temasek Life Sciences Laboratory, Singapore.

445A
The Functional interaction of JAK/STAT pathway ligands. **Shanshan Pei, Doug Harrison.** Biology, University of Kentucky, Lexington, KY.

446B
Identification of regulators and interactors in the Delta-Notch signaling pathway using a transposon-based genetic screen. **Nevine A. Shalaby, Marisa C. Osswalt, Annette L. Parks, Eric J. Morreale, Marc A. T. Muskavitch.** Biology, Boston College, Chestnut Hill, MA.

447C
Molecular and genetic characterization of *upd*, *upd3* and *os*. **Liqun Wang, Douglas Harrison.** Dept Biology, Univ Kentucky, Lexington, KY.

448A
The alleles of *strawberry notch* and *discs large* have an altered role in lipid signaling. **Catherine Coyle-Thompson, Mary Lee Sparling.** Dept Biol, California State Univ, Northridge, CA.

Pattern Formation

449B
A multiplex in situ approach to define the precise contribution of the maternal BMP pathway in dorsal-ventral patterning of the early Drosophila embryo. **Katia Carneiro, Helena Araujo.** Histology and Embriology, UFRJ, Rio de Janeiro, Brazil.

450C
Formation of a proximo-distal axis in the absence of DPP signaling. **Carlos Estella, Richard Mann.** Columbia Univ, New York, NY.

451A
Weckle is required for the transcriptional activities of Dorsal in Drosophila. **Dechen Fu, Mike Levine.** Department of Molecular and Cell Biology, U. C. Berkeley, Berkeley, CA.

452B
Formation of a BMP gradient within the neuroectoderm. **Lisa Gunaydin[1,2], Claudia Mizutani[1], Ethan Bier[1].** 1) Div. of Biological Sciences, Univ. of California San Diego, La Jolla, CA; 2) Department of Biology, Swarthmore College, Swarthmore, PA 19081.

453C
Drosophila rasiRNA mutations disrupt axis specification through activation of a DNA damage checkpoint. **Carla Klattenhoff[1], Diana Bratu[1], Nadine McGinnis-Schultz[1], Birgit Koppetsch[1], Heather Cook[2], William Theurkauf[1].** 1) Program in Molecular Medicine and program in Cell Dynamics, University of Massachusets Medical School, Worcester, MA 01605; 2) Department of Biological Sciences, Wagner College, Staten Island, NY 10301.

454A
Characterization of the pleiotropic gene *Poly* in *D. melanogaster* oogenesis. **Stephen Klusza, Wu-Min Deng.** Dept Biological Sci, Florida State Univ, Tallahassee, FL.

455B
Roles of *single-minded* in the left-right asymmetric development and tissue specifications of the Drosophila embryonic gut. **Reo Maeda[1], Shunya Hozumi[1], Kiichiro Taniguchi[1], Takeshi Sasamura[1], Ryutaro Murakami[2], Kenji Matsuno[1].** 1) Dept. Biol. Sci/Tech., Tokyo Univ Science., Chiba, Japan; 2) Dept. Phy. Biol. Inf., Yamaguchi Univ., Yamaguchi, Japan.

456C
Expression of the *D. melanogaster* GADD45 homolog (CG11086) affects egg asymmetric development which is mediated by the p38/JNK pathway. **Gabriella Peretz[1,2], Uri Abdu[1,2].** 1) Life Sciences, Ben-Gurion University, Be'er Sheva, Israel; 2) The National Institute for Biotechnology in the Negev, Be'er-Sheva, Israel.

457A
Distribution of the potential morphogen Unpaired during oogenesis. **Travis Sexton, Doug Harrison.** Dept Biol, Univ Kentucky, Lexington, KY.

458B
Opposing interactions of homeodomain gene homothorax and Notch pathway genes Lobe and Serrate are required for ventral eye development. **Amit Singh[1], Kwang Wook-Choi[1,2,3].** 1) Dept Molecular & Cell Biol, Baylor Col Medicine, Houston, TX; 2) Dept of Ophthalmology, Baylor Col Medicine, Houston, TX; 3) Developmental Biology Program, Baylor Col Medicine, Houston, TX.

459C
The rearrangement of circular visceral musculature cells controlled by JNK signaling is involved in the left-right asymmetric looping of the anterior-midgut in Drosophila. **Kiichiro Taniguchi[1], Shunya Hozumi[1], Reo Maeda[1], Shuichi Shirakabe[1], Hiroo Fujiwara[1], Takeshi Sasamura[1], Aigaki Toshiro[2], Kenji Matsuno[1].** 1) Dept Biol Sci/Tech, Tokyo Univ Science, Chiba, Japan; 2) Tokyo Met.Univ., Dept.Biol.Sci., Hachioji, Tokyo, Japan.

460A
Lgl and its phosphorylation by aPKC regulate Par-1 localization and oocyte polarity. **Ai-Guo Tian, Wu-Min Deng.** Dept Biological Sci, Florida State Univ, Tallahassee, FL.

461B
Differential expression of Echinoid drives epithelial morphogenesis in Drosophila. **Caroline Laplante, Laura A. Nilson.** Dept Biol, McGill Univ, Montreal, PQ, Canada.

462C
Role of Nm23/Awd in border cell migration. **Gouthami Nallamothu, Tien Hsu, Vincent Dammai.** Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC.

463A
Characterization of a dorsal closure gene, *piragua (prg)* in *D. melanogaster*. **Nestor Nazario-Yepiz, Juan Rafael Riesgo-Escovar.** Dept Development Biol, UNAM, Inst de Neurobiologia, Queretaro, Querétao, Mexico.

464B
Regulation of planar cell polarity and proximodistal patterning by the DHHC transmembrane protein Approximated. **Hitoshi Matakatsu, Seth Blair.** Zoology, University of Wisconsin, Madison, WI.

465C
The function of *inturned*, *fuzzy* and *fritz* in controlling planar polarity. **Jie Yan, Chunming Zhu, Haeryun Lee, Paul Adler.** Dept Biol, Univ Virginia, Charlottesville, VA.

466A
Prepatterning the lateral thorax: specific roles of the *iroquois* genes. **Aissam Ikmi, Dario Coen.** Gènes, Développement, Neurogenèse, UMR 8080, CNRS et Université Paris Sud, 91405 Orsay, France.

467B
Novel Modifiers of the Hedgehog Signaling Pathway. **David J. Casso[1], Stacey K. Ogden[2], David Iwaki[1], Songmei Liu[1], David J. Robbins[2], Thomas B. Kornberg[1].** 1) Biochemistry and Biophysics, UCSF, San Francisco, CA; 2) 1Dartmouth Medical School, Department of Pharmacology and Toxicology, Hanover, NH.

468C
Epidermal Growth Factor Receptor (Egfr) regulated cell adhesion in *D. melanogaster* eye-antennal and wing imaginal discs. **Eduardo J. Gonzalez, Layne Dylla, Jennifer Curtiss.** Biology Department, New Mexico State University, Las Cruces, NM.

469A
The gene regulatory network involved in DV boundary formation in the Drosophila wing. **Héctor Herranz[1], Oriol Canela[2], Fransesc Sagués[3], Ramón Reigada[3], Javier Buceta[2], Marco Milán[1].** 1) ICREA and Institute for Research in Biomedicine (IRB), Parc Científic de Barcelona, Josep Samitier, 1-5, 08028 Barcelona, Spain; 2) Centre especial de Recerca en Química Teòrica (CeRQT), Parc Científic de Barcelona, Josep Samitier, 1-5, 08028 Barcelona, Spain; 3) Department de Química-Física, Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.

470B
Ubx controls Dpp mobility and haltere development through regulation of the glypican dally. **Michael Crickmore[1], Richard Mann[2].** 1) Deptartment of Biological Sciences, Columbia University, New York, NY; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

471C
A novel role for *bowl* in the patterning of the *Drosophila* antenna. **Catarina Bras-Pereira[1,3], Fernando Casares[1,2].** 1) Centro Andaluz de Biologia del Desarrollo (CABD)-Universidad Pablo de Olavide - CSIC, Seville, Spain; 2) Instituto de Biologia Molecular e Celular (IBMC) - Universidade do Porto, Porto, Portugal; 3) Programa Doutoral de Biologia Experimental e Biomedicina, Universidade de Coimbra, Coimbra, Portugal.

472A
Regulation and function of hairy in the Drosophila notum. **Denis Bulanin, Teresa Orenic.** Dept Biological Sci, Univ Illinois, Chicago, Chicago, IL.

473B
Characterizing the role of *distal antenna related* in regulating the expression of *atonal* during eye development. **Micheal Burnett, Erin Archuleta, Jennifer Curtiss.** Dept Biol, New Mexico State Univ, Las Cruces, NM.

474C
Defining Transcriptional Domains in the Undifferentiated Retina. **Lucy C. Firth, Nicholas E. Baker.** Molecular Genetics, Albert Einstein Coll. of Med, Bronx, NY.

475A
Characterization of the role of Homer in retinal apoptosis. **Erica J. Hutchins, Jamie C. Rusconi.** Department of Biological Sciences, University at Albany, Albany, NY.

476B
Induction of Eye Development in Drosophila. **Justin Kumar, Claire Salzer.** Dept Biol, Indiana Univ, Bloomington, IN.

477C
miRNA Regulation of the Eye Specification Cascade. **Justin Kumar, Arthur Luhur.** Dept Biol, Indiana Univ, Bloomington, IN.

478A
Molecular Dissection of Pax6 Proteins During Drosophila Eye Development. **Justin Kumar, Bonnie Weanser.** Dept Biol, Indiana Univ, Bloomington, IN.

479B
Six Class Transcription Factors in Drosophila Eye Development. **Justin Kumar, Brandon Weasner.** Dept Biol, Indiana Univ, Bloomington, IN.

480C
Evolution of the Eye Specification Cascade. **Justin Kumar, Rhea Datta.** Dept Biol, Indiana Univ, Bloomington, IN.

481A
Regulation of dachshund by the Hox Genes During Drosophila Development. **Justin Kumar, Jason Anderson.** Dept Biol, Indiana Univ, Bloomington, IN.

482B
Identification of Targets of the SIX Family of Transcription Factors. **Justin Kumar, Abigail Henderson.** Dept Biol, Indiana Univ, Bloomington, IN.

483C
A mosaic screen to identify X-linked genes required for the normal pattern of photoreceptor differentiation. **Kevin Legent, Josefa Steinhauer, Jessica Treisman.** Skirball Institute, NYU Medical Center, New York, NY.

484A
The transcriptional cofactor Chip sets the boundary of the eye field. **Jean-Yves Roignant, Kevin Legent, Florence Janody, Jessica Treisman.** Dept Developmental Genetics, Skirball Inst, New York, NY.

485B
Differential Delta expression underlies diversity of sensory organ patterns among the Drosophila legs. **Stuti Shroff, Teresa Orenic.** Dept Biological Sciences, Univ Illinois Chicago, Chicago, IL.

486C
The role of RBF and RNO in Drosophila eye development. **Latishya J. Steele, Jinhua Xu, Wei Du.** University of Chicago, Chicago, IL.

487A
Dip3: a regulator of Drosophila eye/antenna development that transforms eyes to antennae. **Cheng-wei Wang[1], Hao A. Duong[2], Y. Henry Sun[1], Albert J. Courey[2].** 1) Dept Academia Sinica, Inst Molecular Biology, Taipei, Taiwan; 2) Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569.

488B
Phylogenetic inference based on patterning mechanisms in flies. **Urs Schmidt-Ott[1,2], Steffen Lemke[1], Matteen Rafiqi[1], Michael Stauber[2], Sean Ferguson[1], Philip Shaw[2], Alexander Prell[2], Oliver Schön[3], Helmut Blöcker[3].** 1) Organismal Biol & Anatomy, Univ Chicago, Chicago, IL; 2) Max-Planck-Institute for Biophys. Chemistry, Göttingen, Germany; 3) Helmholtz Centre for Infection Research, Dept. Genome Analysis, Helmholtz Centre for Infection Research, Braunschweig, Germany.

489C
Conserved and divergent wing vein patterning mechanisms in the red flour beetle Tribolium castaneum. **Yoshinori Tomoyasu, Robin E. Denell.** Div Biol, Kansas State Univ, Manhattan, KS.

490A
Developmental changes of intracellular distribution of Dd4 protein and its homolog Tth in *D. melanogaster*. **Dina Kulikova[1,2], Denis Igumnov[2], Olga Simonova[1,2], Vladimir Buchman[3], Leonid Korochkin[1,2], Ilja Mertsalov[2].** 1) Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Vavilov St. 26, 119334, Moscow, Russia; 2) Institute of Gene Biology, Russian Academy of Sciences, Vavilov St. 34/5, 119334, Moscow, Russia; 3) School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK.

491B
Experimental and computational methods to determine the shape of the Spitz and Argos gradients. **Gregory T. Reeves[1,2], Stanislav Y. Shvartsman[1,2].** 1) Dept Chemical Engineering, Princeton Univ, Princeton, NJ; 2) Lewis-Sigler Institute of Integrative Genomics, Princeton Univ, Princeton, NJ.

492C
O-linked glycan expression during Drosophila development. **E. Tian, Kelly Ten Hagen.** Developmental Glycobiology, NIDCR/NIH, Bethesda, MD.

493A
Spatial control of BMP pathway by EGFR signaling in Drosophila oogenesis. **Nir Yakoby[1], Jessica Lembong[1], Christopher A. Bristow[1], Trudi Schupbach[2], Stanislav Y. Shvartsman[1].** 1) Dept of Genomics and Chemical Engineering, Princeton University, Princeton, NJ; 2) HHMI, Dept of Molecular Biology, Princeton University, Princeton, NJ.

494B
Characterization of klumpfuss expression in the cytoplasm during retinal development. **Barbara J. Zaffo, Barbara Zaffo, Jamie Rusconi.** Biological Sciences, University at Albany, Albany, NY.

495C
Sequential organization of leg segment patterning, growth and morphogenesis. **Lina Greenberg[1,2], Victor Hatini[1,2].** 1) Anatomy and Cellular Biology, Tufts University, Boston, MA; 2) Program in Cell, Molecular, & Developmental Biology.

496A
The role of Bicoid in the sharp border of anterior Hunchback: a deterministic and stochastic analysis. **Francisco Lopes[1,2,3], Carlos Vanario-Alonso[1,2,3], Alexander Spirov[1,2], Paulo Bisch[3], Fernando Vieira[4], David Holloway[5].** 1) Dept Applied Mathematics, Stony Brook Univ., Stony Brook, NY; 2) Developmental Genetics, Stony Brook Univ.; 3) Inst. de Biofisica, Univ. Fed. do Rio de Janeiro, Brazil; 4) Dept de Quimica, Univ. de Brasilia, Brazil; 5) Mathematics, British Columbia Institute of Technology, Burnaby; Chemistry, Univ. of Brit. Col., Vancouver; Biology, Univ. of Victoria, B.C. Canada.

497B
Characterization of *aaquetzalli (aqz)*, a gene required during embryogenesis in *D. melanogaster.*. **Miguel Mendoza-Ortiz, Juan R. Riesgo-Escovar.** Dept Developmental Biol, Inst Neurobiologia, UNAM, Queretaro, Querétaro, Mexico.

498C
Within-embryo noise of primary and secondary morphogenetic gradients in early Drosophila segmentation. **Alexander Spirov[1], Theodore Alexandrov[2], Nina Golyandina[2], David Holloway[3], Francisco Lopes[1].** 1) Developmental Genetics, State Univ of New York, Stony Brook, NY; 2) Statistical Simulation, St.Petersburg State University, Russia; 3) Mathematics, British Columbia Institute of Technology, Burnaby, Canada.

Gametogenesis and Sex Determination

499A
The structure and function of follicle cell ring canals. **Stephanie Airoldi, Lynn Cooley.** Dept Genetics, Yale University, New Haven, CT.

500B
The Tudor domain protein Montecristo is required for meiotic progression and microtubule-based transport to the Drosophila oocyte. **Vitor Barbosa, Caryn Navarro, Ruth Lehmann.** Developmental Genetics, NYU, Skirball Inst, New York, NY.

501C
Using an UV cross-linking assay to identify new components involved in regulating *dfmr* translation during oogenesis. **Rebecca W. Beerman, Thomas A. Jongens.** Genetics, University of Pennsylvania, Philadelphia, PA.

502A
Shaping cells and finding paths: The heterotrimeric G-protein subunit *Gβ13F* during dorsal appendage morphogenesis. **Michael J. Boyle[1,2], Celeste A. Berg[1,2].** 1) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

503B
Visualization of Gurken distribution in follicle cells. **Wei-Ling Chang[1], Willisa Liou[2], Hsiao-Chun Peng[1], Yu-Wei Chang[1], He-Yen Chou[1], Li-Mei Pai[1].** 1) Dept. of Biochem & Molecular Biol., Chang Gung University, Tao-Yuan, Taiwan; 2) Dept. of Anatomy, Chang Gung University, Tao-Yuan, Taiwan.

504C
The functional analysis of Endophilin B in EGFR signaling. **Yu-Wei Chang, Yi-Chen Li, Pei-Yu Wang, Li-Mei Pai.** Dept. Biochemistry & Molecular Biology, Chang-Gung Univ, Tao-Yuan, Taiwan.

505A
Histone methylation is required for oogenesis. **Emily Clough, Tulle Hazelrigg.** Dept Biol Sci, Columbia Univ, New York, NY.

506B
Analysis of an Orb related gene, orb2, during development of the germline and the nervous system in Drosophila. **Nathaniel Hafer, Shuwa Xu, Paul Schedl.** Dept Molecular Biol, Princeton Univ, Princeton, NJ.

507C
The role of Notch regulators in Drosophila oogenesis. **Yvonne Hung, Tanveer Akbar, Martin Baron.** Faculty Life Sciences, University of Manchester, Manchester, United Kingdom.

508A
Antagonistic roles between the Drosophila C/EBP and CDP homologs Slbo and Cut regulate centripetal migration. **Benjamin D. Levine, Leonard Dobens, Jennifer Hackney, Angela Truesdale.** Molecular Biology, University of Missouri KC, Kansas City, MO.

509B
Identification and genetic analysis of fusome-localized proteins. **Daniel Lighthouse[1,2], Michael Buszczak[1], Allan Spradling[1,2].** 1) Carnegie Institution/HHMI, Baltimore, MD; 2) Biology Dept, Johns Hopkins University, Baltimore, MD.

510C
Growl, a novel gurken RNA binding protein essential for dorsoventral patterning. **Shengyin Lin, Sui Zhang, Robert S. Cohen.** Molecular Bioscience, University of Kansas, Lawrence, KS.

511A
Investigating the role of *stonewall* in ovarian germline stem cell maintenance. **Jean Maines, Tiana Endicott, Tanya Robinson, Dennis McKearin.** Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX.

512B
Drosophila germline stem cell maintenance controlled by Loquacious-dependent miRNAs. **Joseph Park[1], Xiang Liu[2], Tamara Strauss[2], Qinghua Liu[2], Dennis McKearin[1].** 1) Molecular Biology, UT Southwestern, Dallas, TX; 2) Biochemistry, UT Southwestern, Dallas, TX.

513C
Template DNA strand retention in the stem cells of the Drosophila ovary. **Laura Ponting, Martin Baron.** Faculty of Life Sciences, University of Manchester, Manchester, UK.

514A
Missing oocyte and Nup44A/Seh-1 physically interact in the Drosophila germ line. **Stefania Senger, Mary Lilly.** CBMB, NICHD/NIH, Bethesda, MD.

515B
Mastermind, a member of the Notch signaling pathway, regulates somatic stem cells in the Drosophila ovary. **Cynthia Vied, Daniel Kalderon.** Dept Biological Sci, Columbia Univ, New York, NY.

516C
The golgin Lava Lamp is required for Drosophila oogenesis. **H. Wang[1], K. Monzo[1], J. T. Warren[2], L. I. Gilbert[2], J. C. Sisson[1].** 1) The Section of MCD Biology and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

517A
The role of Rab11 and endocytic recycling in maintaining cell polarity and suppressing cell motility in Drosophila epithelia. **Jiang Xu, Robert S. Cohen.** Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

518B
Requirements for the transcriptional regulator *lola* in adult stem cell maintenance. **Erin Davies[1], Leanne Jones[1,2], Margaret Fuller[1].** 1) Developmental Biology, Stanford University, Stanford, CA; 2) Laboratory of Genetics, Salk Institute La Jolla, CA.

519C
Testing the Role of the Maternal Pronucleus in Wolbachia-induced Cytoplasmic Incompatibility in *D. melanogaster*. **Patrick Ferree[1], William Sullivan[2].** 1) Dept of Mol Biol and Genetics, Cornell University, Ithaca, NY; 2) Dept of Mol Cell and Developmental Biology, UC Santa Cruz.

520A
Mating induces morphological changes in the Drosophila female reproductive tract. **Anat Kapelnikov[1], Patricia Rivlin[2], Ronald Hoy[2], Yael Heifetz[1].** 1) Dept Entomology, Hebrew Univ, Rehovot, Israel; 2) Dept. of Neurobiology and Behavior, Cornell Univ., Ithaca, NY.

521B
Ecdysone signalling during the development of the Drosophila female reproductive tract. **Vidya Nagalakshmi[1], Paul Mack[2], Anat Kapelnikov[1], Michael Bender[2], Yael Heifetz[1].** 1) Department of Entomology, The Hebrew University, Rehovot, Israel; 2) Department of Genetics, University of Georgia, Athens, GA.

522C
Specific microRNAs regulate GSCs through Dacapo/p21. **Steven Reynolds.** Dept Biochemistry, Univ Washington, Seattle, WA.

523A
Functional analysis of *CG3056*, the closest paralog of *Sex-lethal* in Drosophila. **Sha Sun, Thomas W. Cline.** Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

524B
Sperm of male sterile mutation *sheepish* fail to be stored in females. **Masatoshi Tomaru, Takashi Ohsako, Naoto Juni, Hiroshi Matsubayashi, Hiromi Sato, Masa-Toshi Yamamoto.** Drosophila Genetic Resource Center, Kyoto Institute of Technology, Kyoto, Japan.

525C
PPS, a novel protein required for establishment of the *Sxl* autoregulatory splicing loop. **Matthew Johnson[1], Alexis Nagengast[2], Helen Salz[1].** 1) Case Western Reserve University, Cleveland, OH; 2) Widener University, Chester, PA.

526A
Evidence that *transformer* is not the only gene target of *Sex-lethal* that directs female sexual differentiation in *D. melanogaster*. **Meghan R. Jones, Daniel S. Evans, Melissa M. Burns, Thomas W. Cline.** Dept. Molecular and Cell Biology, Univ. of California, Berkeley, CA.

527B
Deciphering the functions of *D. melanogaster* male accessory gland proteins using RNA interference. **Kristipati Ravi Ram, Mariana F. Wolfner.** Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY, 14853.

528C
Kokopelli: a novel, pleiotropic cyclin required for germline stem cell maintenance. **James D. Baker, Maurice J. Kernan.** Dept Neurobiology and Behavior & Center for Developmental Genetics, Stony Brook University, Stony Brook, NY.

529A
Direct Regulation of Germline and Somatic Stem Cell Maintenance by Jak-STAT Signaling in the Drosophila Testis. **Crista Brawley, Maggie de Cuevas, Erika Matunis.** Dept Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

530B
Characterization of *nmd* and its paralog in mitochondrial morphogenesis in Drosophila permatogenesis. **Bevin C. English, Sarah D. Durnbaugh, Kara M. Koehrn, Amanda C. Aldridge, Sara H. Holmberg, Karen G. Hales.** Department of Biology, Davidson College, Davidson, NC.

531C
Loss of Off-schedule, an eIF4G-like protein, causes arrest of meiosis and spermatid differentiation in the testis. **Tina Franklin-Dumont[1], Chandrima Chatterjee[1], Steve Wasserman[2], Steve DiNardo[1].** 1) Cell & Developmental Biol, Univ Pennsylvania Sch Med, Philadelphia, PA; 2) Cell & Developmental Biol, Univ of California at San Diego, La Jolla, CA.

532A
Roles for *mitoshell* in mitochondrial aggregation and meiotic cytokinesis during Drosophila spermatogenesis. **Karen G. Hales, Sarah E. Coffey, Sheena E. Favors, Amanda C. Aldridge.** Department of Biology, Davidson College, Davidson, NC.

533B
Candidate genes for elongated sperm, a microarray approach. **Sarah Kingan[1], Daniel Hartl[1], Scott Pitnick[2].** 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Department of Biology, Syracuse University, Syracuse, NY.

534C
The *D. melanogaster* SUN protein Giacomo is required for male fertility and links axonemal microtubules (MTs) to the spermatid tail plasma membrane. **M. Kracklauer[1], X. Chen[2], H. Wiora[1], J. Fischer[1], M. Fuller[2].** 1) Dept MCD Biol, Univ Texas, Austin, TX; 2) Dept Dev Biol, Stanford Univ School Med, Stanford, CA.

535A
Male-specific neurotransmitter transporter essential for spermiogenesis. **Janet Rollins, Nabanita Chatterjee, Chris Bazinet.** Biological Sciences, St. John's University, Queens, NY.

536B
Dedifferentiation of Spermatogonia in the Drosophila Germline Stem Cell Niche Involves Extensive Cellular Rearrangements. **Xuting Sheng, Crista Brawley, Erika Matunis.** Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

537C
A Novel Role for Integrins in Hub Cell Morphogenesis. **Guy Tanentzapf[1,2], Danelle Devenport[2,3], Nicholas H. Brown[2].** 1) CSB Department, University of Toronto, 25 Harbord Street, Toronto, Ontario, Canada M5S 3G5; 2) The Gurdon Institute and Department of Physiology, Development, & Neuroscience, University of Cambridge, Cambridge, UK, CB2 1QN; 3) Laboratory of Mammalian Genetics and Development, Rockefeller University, Box 300, 1240 York Avenue, New York NY 10021.

538A
The link between acrosome function and sperm plasma membrane breakdown during Drosophla fertilization. **Kathleen L. Wilson, Michelle K. Smith, Barbara T. Wakimoto.** Dept Biol, Univ Washington, Seattle, WA.

Organogenesis

539B
The LIM-HD gene *tailup* and *Iro-C* cooperate in Drosophila dorsal mesothorax specification. **Joaquin de Navascues, Juan Modolell.** Centro de Biología Molecular "Severo Ochoa" (UAM-CSIC). Cantoblanco, Madrid (SPAIN).

540C
Senz'aria, a MAGUK family adapter, is required for tracheal morphogenesis. **Katherine E. Moyer, J. Roger Jacobs.** Biology, McMaster University, Hamilton, ON, Canada.

541A
Spatio-temporal coordination of epithelial cell internalization by EGFR signaling. **Mayuko Nishimura[1,2], Yoshiko Inoue[1], Shigeo Hayashi[1,2].** 1) Riken CDB, Kobe, Japan; 2) Kobe Univ., Grad. Sch. Sci.Tech.

542B
Control of epidermal differentiation. **Francois Payre[1], Helene Chanut-Delalande[1,2], Isabelle Fernandes[1], Philippe Valenti[1], Severine Viala[1], Serge Plaza[1].** 1) Centre de Biologie du Developpement, Toulouse, France; 2) Biozentrum der Universitat Basel, Basel, Switzerland.

543C
Imaginal hindgut development in Drosophila. **Shigeo Takashima, Marianna Mkrtchyan, Volker Hartenstein.** Depertment of Molecular Cell, and Developmental Biology, UCLA, Los Angeles, CA.

544A
Regulation of the Notch pathway during stem cell division in the adult fly. **Allison Bardin, Francois Schweisguth.** Biology, Ecole Normale Superieure, Paris, France.

545B
FoxK, a new Fork Head protein required for Dpp-dependent midgut specification. **Sergio Casas-Tinto[1,2], Pedro Fernandez-Funez[1], Begona Granadino[2].** 1) Neurology, UTMB, Galveston, TX; 2) CIB, CSIC, Madrid, Spain.

546C
Dumpy interacts with a large number of proteins in the epithelial apical extracellular matrix. **Ross MacIntyre, Jeff Chien, Amber Carmon.** Dept Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

547A
The Expression, Function, and Regulation of Cad74A in Drosophila Oogenesis. **Jeremiah Zartman[1], Nir Yakoby[1], Chris Bristow[1], Trudi Schupbach[2], Stanislav Shvartsman[1].** 1) Lewis Sigler Institute and Dept Chemical Engineering, Princeton Univ, Princeton, NJ; 2) Howard Hughes Medical Institute and Department of Molecular Biology, Princeton Univ, Princeton, NJ.

548B
Examining the Requirement for Rac during Adult Myogenesis. **Krishan Badrinath, Allison Siebert, Joyce Fernandes.** Dept Zoology, Miami Univ, Oxford, OH.

549C
Regulation of Myoblast Proliferation during Adult Myogenesis in Drosophila. **Krishan Badrinath, Michael Gottlieb, Meatal Patel, Joyce Fernandes.** Dept Zoology, Miami Univ, Oxford, OH.

550A
Pox meso, a paired-box transcription factor, is required for development of the ventral somatic musculature. **Seth A. Brodie[1], Jonathan K. Kassel[1], Marc S. Halfon[1,2,3].** 1) Department of Biochemistry, State University of New York at Buffalo, Buffalo, NY; 2) Center of Excellence in Bioinformatics and the Life Sciences, State University of New York at Buffalo, Buffalo, NY; 3) Molecular and Cellular Biology Department, Roswell Park Cancer Institute, Buffalo NY.

551B
Founder cells regulate muscle pattern but not fiber formation during adult myogenesis in Drosophila. **Joyce Fernandes, Badrinath Krishan.** Zoology Dept, Miami Univ, Oxford, OH.

552C
Hemangioblast Differentiation: Asymmetric Division Versus Localized Signaling. **Melina Grigorian, Lolitika Mandal, Volker Hartenstein.** MCDB, Univ. California, Los Angeles, Los Angeles, CA.

553A
Investigation of the cellular dynamics of Drosophila myogenesis using primary cell culture. **Thomas J. Metzger[1,2], Mary K. Baylies[1].** 1) Dept Development, Sloan-Kettering Inst, New York, NY; 2) BCMB Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY.

554B
Drosophila GATA, Friend of GATA and Runx factors regulate *lozenge* expression in crystal cells. **Selen Muratoglu, R. Barry Hough, Soe T. Mon, Nancy Fossett.** Center for Vascular and Inflammatory Diseases, Department of Pathology, University of Maryland, Baltimore, MD.

555C
Defective Dpp signaling results in heart overgrowth and reduced cardiac output in Drosophila. **Stuart Newfeld, Aaron Johnson.** Sch Life Sci, Arizona State Univ, Tempe, AZ.

556A
Regulation of the transcription factor Hand in the visceral mesoderm. **Dmitry Popichenko[1], Julia Sellin[1], Marek Bartkuhn[2], Achim Paululat[1].** 1) Dept. of Zoology, University of Osnabrueck, D-49069 Osnabrueck, Germany; 2) Institute for Genetics, Justus-Liebig-University of Gieβen, D-35390 Gieβen, Germany.

557B
Fusion competent myoblasts require Loner activity for myoblast fusion. **Kate M. Rochlin[1], David Soffar[2], Mary K. Baylies[2].** 1) Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

558C
The Gli-like transcription factor Lame Duck is essential for correct cell fate decisions in the dorsal mesoderm of *D. melanogaster*. **Julia Sellin, Maik Drechsler, Achim Paululat.** Zoology Department, University of Osnabrueck, Osnabrueck, Germany.

559A
N-linked glycosylation requirements for SNS-Duf mediated cell adhesion. **Claude Shelton IV[1,2], Sandra Berger[3], Susan Abmayr[1,2].** 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS; 3) Institut National Agronomique Paris-Grignon, France.

560B
Organogenesis of the wing circulatory organs in Drosophila. **Markus Tögel[1,2], Günther Pass[2], Achim Paululat[1].** 1) Department of Zoology, University of Osnabrueck, 49069 Osnabrueck, Germany; 2) Department of Evolutionary Biology, University of Vienna, 1190 Vienna, Austria.

561C
Identifying the mechanisms of fusion and morphogenesis during myogenesis of *D. melanogaster*. **Mu Xu, Mary Baylies.** Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

562A
The Mevalonate Pathway Controls Heart Formation in Drosophila by Isoprenylation of Gγ1. **Peng Yi[1], Zhe Han[2], Xiumin Li[1], Eric Olson[1].** 1) Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept. of Internal Medicine, Dept. of Cell and Developmental Biology, Univ Michigan, Ann Arbor, MI.

563B
The role of Nedd4 family genes in *D. melanogaster*. **Tanveer Akbar, Ann Marie Carbery, Yvonne Hung, Martin Baron.** Faculty Life Sci, Univ Manchester, Manchester, UK.

564C
Functional analysis of the Iroquois complex genes. **Esther González-Pérez[1], Natalia Barrios[1], Annalisa Letizia[1,2], Sonsoles Campuzano[1].** 1) Centro Biología Molecular SO , CSIC-UAM, Madrid, Spain; 2) Instituto Biología Molecular, CSIC, Barcelona, Spain.

Neurogenetics and Neural Development

565A
The Liprin-α binding site, but not LAR phosphatase activity, is essential for LAR mediated R7 photoreceptor targeting. **Kerstin D. Hofmeyer[1], Corinne Maurel-Zaffran[2], Jessica E. Treisman[1].** 1) Skirball Institute, NYU Medical Center, New York, NY; 2) Institut de Biologie du Developpment de Marseille, Marseille.

566B
A screen for dominant enhancers of a *trio* mutant phenotype. **Eric Liebl[1], Colan Baldyga[1], Lindsay Bickel[1], Kathryn Dean[1], Morgan Kopeke[1], Rohan Manohar[1], Julianne McCall[1], Jenna McCroskey[1], Jessica Smith[1], Mark Seeger[2].** 1) Dept Biol, Denison Univ, Granville, OH; 2) Department of Molecular Genetics and Center for Molecular Neurobiology, The Ohio State University, Columbus, OH.

567C
Collapsin response mediator protein and dihydropyrimidinase are functionally divergent, alternatively splice products of the *crmp* gene. **Deanna Morris, John Rawls.** Department of Biology, University of Kentucky, Lexington, KY.

568A
Functional analysis of Golden goal, a novel transmembrane protein involved in photoreceptor axon guidance. **Tatiana Tomasi, Satoko Hakeda-Suzuki, Stephan Ohler, Takashi Suzuki.** Max-Planck-Institute of Neurobiology, Martinsried, Germany.

569B
Precise control of *Fasciclin2* expression is required for the adult mushroom body development in Drosophila. **Hidenobu Tsujimura, Kazuma Fushima.** Dept Developmental Biol, Tokyo Univ Agric & Technology, Tokyo, Japan.

570C
Regulation of Thoracic Neuroblast Proliferation by Bnl, Hh, and Trol in the Drosophila CNS. **Jonathan Lindner, Paul Hillman, Youngji Park, Sumana Datta.** Dept Biochemistry/ Biophysics, Texas A&M Univ, College Station, TX.

571A
Drosophila Insulin Receptor Signaling in the Embryonic Central Nervous System. **Tamar R. Sterling[1], Ronald A. Kohanski[2], Leslie Pick[1].** 1) Department of Entomology, University of Maryland, College Park, MD; 2) Departments of Pediatrics and of Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD 21205.

572B
Genes required for Drosophila midline cell development during late embryogenesis. **Yi Zhang, Warren Perry II, Kelly Daigle, Patricia Estes.** Department of Genetics, NC State University, Raleigh, NC. 27695.

573C
Regulation of gliogenesis by Rap/Fzr, an activator of the Anaphase Promoting Complex and Loco, an RGS protein. **Margarita Kaplow, Adam Korayem, Tadmiri Venkatesh.** Dept Biol, CCNY, New York, NY.

574A
Nonstop and Rap/Fzr/Cdh1 interact to regulate cell cycle progression and retinal axon targeting. **Margarita Kaplow, Tania Moin, Eliana Mino, Tadmiri Venkatesh.** Dept Biol, CCNY, New York, NY.

575B
Role of Glia in the Organization and Function of the Visual Nervous System of Drosophila. **Rosa Mino[1], Johanna Palacio[1], Margarita Kaplow[1], Jorge Morales[1], Peter O'Day[2], Tadmiri Venkatesh[1].** 1) Department of Biology, City College Of New York, New York, NY 10031; 2) Institute of Neuroscience, Huestis Hall, University of Oregon, Eugene, OR 97403.

576C
The Drosophila sialylation genes are expressed in a subset of neurons during embryonic development. **Ilhan Akan, Sundeep Singh, Chris Riling, Sheba Mathew, Karen Palter.** Department of Biology, Temple University, 1900 N. 12th St., Philadelphia, PA 19122.

577A
The role of Acj6 in odor receptor gene choice. **Lei Bai, Aaron Goldman, John Carlson.** MCDB, Yale University, New Haven, CT.

578B
Role of extra macrochaetae (emc) gene during retinal development. **Abhishek Bhattacharya, Hui Zhang, Nicholas E. Baker.** Dept Molecular Genetics, AECOM, Bronx, NY.

579C
Identification of novel genes involved in external sensory organ formation. **Nikolaos Giagtzoglou[1,2,4], Hillary Andrews[3,4], Karen L. Schulze[1,2], Shinya Yamamoto[3], Hugo Bellen[1,2,3].** 1) Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX; 4) equal contribution.

580A
Uncovering developmental gene regulatory networks in the Drosophila CNS midline. **Amaris Guardiola, Stephen Crews.** Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC.

581B
Initial insights into the mechanism controlling stochastic *spineless* expression required for the color vision retinal mosaic. **Robert Johnston, Claude Desplan.** Dept of Biology, New York University, New York, NY.

582C
Merlin/NF-2 and the Warts/Hpo/Sav pathway are required to specify R8 photorecepter subtypes. **David Jukam, Claude Desplan.** Dept Biology, 1009 Main Bldg, New York Univ, New York, NY.

583A
Regulation of Notch endosomal routing and γ-secretase function in various neurogenic backgrounds. **Ritu Kanwar, Mark Fortini.** CDBL, National Cancer Institute, NIH, Frederick, MD 21702.

584B
Control of neuronal cell fate specification by combinatorial patterning mechanisms. **Daniel Karlsson, Magnus Baumgardt, Stefan Thor.** Molecular Genetics, Linkoping University, Linkoping, Sweden.

585C
Interaction Patterns of Echinoid Homologous Protein, Friend of Echinoid (Fred). **Woongki Kim, Susan Spencer.** Biology, St. Louis University, 3507 Laclede Ave, St. Louis, MO. 63103.

586A
Characterizing the role of Dbx in the embryonic CNS development. **Haluk Lacin, Heather Broihier, Yi Zhu, Beth Wilson, Hemi Mistry, James Skeath.** Dept Genetics, Washington Univ, St Louis, St Louis, MO.

587B
Expression and functional requirements for the bangsensitive gene *easily shocked*. **Elaine R. Reynolds, Arda Hotz, Kristen Balsamo, Stephanie Cote.** Program in Neuroscience and Biol Dept, Lafayette Col, Easton, PA.

588C
Ero1L, a protein involved in disulfide bond formation, affects Notch signaling. **An-Chi Tien[1,4], Akhila Rajan[2,4], Karen L. Schulze[3], Hugo J. Bellen[1,2,3].** 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics, Baylor College of Medicine; 3) HHMI, Houston, TX; 4) Equal contribution.

589A
Functional analysis of the homeodomain protein Ind during embryonic CNS development. **Tonia L. Von Ohlen[1], Dervla M. Mellerick[2], Canda Harvey[1], Li-Jun Syu[2].** 1) Division of Biology, Kansas State University, Manhattan, KS; 2) Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI.

590B
The Role of Histone Deacetylase 1 (*Rpd3*) in Dendritic Targeting of Drosophila Olfactory Projection Neurons. **Takahiro Chihara[1,3,4], Joy S. Wu[1,2,4], Liqun Luo[1,2].** 1) Howard Hughes Medical Institute, Department of Biological Sciences; 2) Neurosciences Program, Stanford University, Stanford, CA 94305-5020; 3) Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; 4) These authors contributed equally to this work.

591C
Analysis of the Role of Rab11 in Nuclear Translocation in Drosophila. **Tarek Houalla, Yong Rao.** Dept Neurology & Neurosurgery, McGill Univ, Montreal, PQ, Canada.

592A
Drosophila models of human developmental brain disorders: comparative and cell culture approaches. **Katherine Olson[1,2], Robert Kraft[2], Jennifer Inlow[3], Linda Restifo[1,2].** 1) Graduate Interdisciplinary Program in Genetics, University of Arizona, Tucson, AZ; 2) ARL Division of Neurobiology, University of Arizona, Tucson, AZ; 3) Dept. of Chemistry, Indiana State University, Terra Haute, IN.

593B
Control of proximal-distal distribution of branching points in dendritic trees of Drosophila sensory neurons. **Daisuke Satoh[1], Daichi Sato[2], Taiichi Tsuyama[2], Motoki Saito[2], Fuyuki Ishikawa[2], Melissa M. Rolls[3], Chris Q. Doe[3], Hiroyuki Ohkura[4], Tadashi Uemura[2].** 1) Graduate School of Science, Kyoto University, Kyoto, Japan; 2) Graduate School of Biostudies, Kyoto University, Kyoto, Japan; 3) HHMI, University of Oregon, Eugene, OR; 4) The University of Edinburgh, Edinburgh, UK.

594C
Role of Wnt Signaling Pathway during Neuronal Remodeling. **Ajeet P. Singh[1], Roy Bidisha[2], K. VijayRaghavan[2], Veronica Rodrigues[1,2].** 1) Dept. of Biological Sciences, TIFR, Mumbai, India; 2) National Center for Biological Sciences, TIFR, Banglore, India.

595A
Restructuring of the abdominal neuromuscular system during metamorphosis. **Joyce Fernandes, Meredith Dorr, Camilo Molina, Sarita Hebbar, Aswati Subramanian.** Zoology Dept, Miami Univ, Oxford, OH.

596B
The Function of Bällchen in Neuronal Stem Cell Maintenance. **Ufuk Gunesdogan, Herbert Jackle, Alf Herzig.** Developmental Biology, MPI for Biophysical Chemistry, Goettingen, Germany.

597C
Targeting of Sanpodo to asymmetric pericentrosomal early endosomes regulates Notch signaling in sensory organ precursor cells. **Fabrice Roegiers, Xin Tong, Diana Zitserman.** Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA.

598A
Molecular mechanisms that underlie the transition of neuroepithelial cells to neuroblasts in the Drosophila optic lobe. **Daiki Umetsu, Tetsuo Yasugi, Makoto Sato, Tetsuya Tabata.** University of Tokyo, IMCB, Tokyo, Japan.

599B
Role for the JAK/STAT signaling pathway in the optic lobe development. **Tetsuo Yasugi[1], Daiki Umetsu[1], Satoshi Murakami[1], Kuniaki Takahashi[2], Kaoru Saigo[3], Ryu Ueda[2], Shoko Yoshida[1], Makoto Sato[1], Tetsuya Tabata[1].** 1) IMCB, Univ. Tokyo, Tokyo, Japan; 2) NIG, Shizuoka, Japan; 3) Dept. Biophys. Biochem., Grad. Sch. Sci., Univ. Tokyo, Tokyo, Japan.

600C
Two types of Drosophila R7 photoreceptor cells are arranged randomly: A model for stochastic cell-fate determination. **Steven G. Britt[1], Melanie L. Bell[2], James B. Earl[1].** 1) Department of Cell & Developmental Biology, University of Colorado at Denver & Health Sciences Center, Aurora, CO; 2) Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand.

601A
The intraflagellar transport protein REMPA/IFT140 is a component of the chordotonal ciliary dilation. **Eugene Lee[1,2], Elena Sivan-Loukianova[3], Daniel F. Eberl[3], Maurice Kernan[1].** 1) Dept of Neurobiology; 2) Program in Neuroscience, SUNY, Stony Brook, NY; 3) Department of Biology, University of Iowa, Iowa City, IA.

602B
Feedback from Rhodopsin 6 protein is required to maintain pR8 identity through inhibition of Rh5 expression. **Daniel Vasiliauskas[1], Esteban O. Mazzoni[2], Claude Desplan[1].** 1) Department of Biology, New York University, New York, NY; 2) Department of Pathology College of Physicians and Surgeons, Columbia University, New York, NY.

603C
The Role of Serine Protease Inhibitors in Nervous System Development. **You-Seung Kim, Thomas Osterwalder, Haig Keshishian.** Dept MCDB, Yale Univ, New Haven, CT.

Neural Physiology and Behavior

604A
Posttranslational Regulation of the Drosophila Circadian Clock Requires Protein Phosphatase 1 (PP1). **Yanshan Fang, Sriram Sathyanarayanan, Amita Sehgal.** Howard Hughes Medical Institute, Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

605B
JETLAG resets the Drosophila circadian clock by promoting light-induced degradation of TIMELESS. **Kyunghee Koh, Xiangzhong Zheng, Amita Sehgal.** HHMI, Neuroscience, University of Pennsylvania, Philadelphia, PA.

606C
An oenocyte clock regulates the expression of *desat1*, a gene required for sex pheromone biosynthesis. **Joshua J. Krupp, Clement Kent, Julia A. Schonfeld, Joel D. Levine.** Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario, Canada.

607A
logjam is expressed in a small number of cells in the CNS and is required for egg laying behavior. **Ginger Carney, Kara Boltz, Lisa Ellis.** Dept Biol, Texas A&M Univ, College Station, TX.

608B
D. melanogaster males can respond rapidly at the genetic level to courtship interactions. **Lisa L. Ellis, Ginger E. Carney.** Dept Biol, Texas A&M Univ, College Station, TX.

609C
The effects of interspecific courtship on mating success and learning in *D. melanogaster*. **Scott McRobert, Rebecca Dawson.** Dept Biol, St Joseph's Univ, Philadelphia, PA.

610A
Functional analyses of *fru*^{M}-expressing neurons for their role in regulating courtship initiation. **David Tran[1], Ulrike Heberlein[2], Bruce Baker[1].** 1) Dept Biological Sciences, Stanford Univ, Stanford, CA; 2) Dept. of Anatomy, UCSF, San Francisco, CA.

611B
Catsup Function in Dopamine Homeostasis. **Faiza Ferdousy, Hakeem Lawal, Zhe Wang, Iyare Izevbaye, Carrie Williams, Daniel Roberts, Janis M. O'Donnell.** Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

612C
Targeted Activation of CCAP Neurons Using the Cold-Sensitive TRPM8 Channel Reveals a Pre-eclosion Critical Period in Wing Expansion. **Nathan Peabody[1], Andrew Vreede[1], Fengqiu Diao[1], Elizabeth Dewey[2], Hans-Willi Honegger[2], Benjamin White[1].** 1) Lab of Molecular Biology, NIMH/NIH, Bethesda, MD; 2) Department of Biological Sciences, Vanderbilt University, Nashville, TN.

613A
Anatomical and Behavioral Defects in Drosophila Mushroom Body Mutants. **Brian S. Dunkelberger, Christine N. Serway, Nicole W. C. Nolan, J. Steven de Belle.** School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV.

614B
Differential Induction of Short-term and Medium-term Memories by Appetitive and Aversive Reinforcements in Drosophila Larvae. **Ken Honjo, Katsuo Furukubo-Tokunaga.** Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.

615C
Nemy, a cytochrome B561, is required for memory formation in Drosophila. **Konstantin Iliadi, Natalia Iliadi, Gabrielle Boulianne.** The Hospital for Sick Children, Toronto, Canada.

616A
Heat shock drastically shortens the onset of deficits in memory and locomotion in a model for age-dependent neurodegenerative disorders, the mutants of the kynurenine pathway. **Elena Savvateeva-Popova[1], Ekaterina Nikitina[1], Anna Medvedeva[1], Elena Tokmatcheva[1], Alexandr Peresleni[1], Andrei Popov[2], Peter Riederer[3].** 1) Pavlov Institute of Physiology, St Petersburg, Russia; 2) Sechenov Institute of Evolutionary Physiology and Biochemistry, St Petersburg, Russia; 3) Department of Clinical Neurochemistry, Clinic and Policlinic of Psychiatry and Psychotherapy, University of Würzburg, Germany.

617B
Exclusive requirement of NMDA receptors for long-term memory consolidation in Drosophila ellipsoid body. **Shouzhen Xia[1], Chia-Lin Wu[2,3], Tsai-Feng Fu[2,3], Huaien Wang[1], Ying-Hsiu Chen[2,3], Daniel Leong[1], Ann-Shyn Chiang[2,3], Tim Tully[1].** 1) Beckman Neuroscience Center, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Sprg Harbor, NY 11724; 2) Institute of Biotechnology and Department of Life Science, National Tsing Hua University, Hsinchu 30013, Taiwan; 3) Brain Research Center, National Tsing Hua University, University System of Taiwan, Hsinchu 30013, Taiwan.

618C
Dissection of a neuronal network required for wing expansion and tanning using the Split Gal4 System. **Haojiang Luan, Fengqiu Diao, Kevin Ho Wan, Nathan Peabody, Benjamin White.** Lab Molecular Biol, NIMH, Bethesda, MD.

619A
The proprotein convertase amontillado may function in larval growth and glucose homeostasis by processing Drosophila insulin-like peptides and adipokinetic hormone. **Jeanne Rhea[1], Lowell Rayburn[1], Christian Wegener[2], Michael Bender[1].** 1) Department of Genetics, University of Georgia, Athens, GA; 2) Department of Biology, Phipps-University, Marburg, Germany.

620B
Evidence for Regulatory Interactions between Key Enzymes in Dopamine Synthesis. **K. Bowling, C. Funderburk, D. Xu, Z. Huang, F. Ferdousy, J. O'Donnell.** Biological Sciences, University of Alabama, Tuscaloosa, AL.

621C
DSERT mutants display altered cocaine responses, circadian rhythmicity, and startle responses. **Noël C. Derecki, Erik Loken, Jay Hirsh.** Biology, University of Virginia, Charlottesville, VA.

622A
Monoamine neurotransmitter transporter expression in the Drosophila eye. **Bernhard Hovemann, Guido Uhlenbrock, Anna Ziegler.** Dept Chemistry, Ruhr Univ, Bochum, Bochum, Germany.

623B
The 5-HT$_2$Dro receptor is expressed in the larva and adult CNS and modulates aspects of circadian and aggressive behaviors. **Charles D. Nichols, Oralee Johnson.** Department of Pharmacology and Experimental Therapeutics, LSU Health Sciences Center, New Orleans, LA.

624C
Pleiotropic behavioral phenotype of mutants of the Vesicular Monoamine Transporter. **Anne F. Simon, Rafael Romero-Calderon, Anna Grygoruk, Hui-Yun Chang, Mordecai Solomon, David Shamouelian, Evelyn Salazar, David E. Krantz.** Psychiat.and Biobehavior. Sci., UCLA , Brain Res Inst, Los Angeles, CA.

625A
Genetic and physical mapping of two spontaneous mutants that affect wing-beat frequency in *D. melanogaster*. **Phillip T. Barnes, Justine Miller, Slavina Georgieva.** Biology Department, Connecticut College, New London, CT.

626B
Characterization of a fly Sensory Neuron Membrane Protein (SNMP) homolog. **Harbinder Singh Dhillon[1,2], Kenny Fernandez[2], Richard Vogt[2].** 1) Biological Sciences, Delaware State University, Dover, DE; 2) Biological Sciences, University of South Carolina, Columbia, SC.

627C
Quantitative genomics of aggressive behavior in *D. melanogaster*. **Alexis Edwards[1], Stephanie Rollmann[1], Theodore Morgan[2], Trudy Mackay[1].** 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Division of Biology, Kansas State University, Manhattan, KS.

628A
Mapping fly color-vision circuits. **Shuying Gao[1], Chun-Yuan Ting[1], Songling Huang[1], Ian A. Meinertzhagen[2], Chi-Hon Lee[1].** 1) Unit on Neuronal Connectivity, NICHD, NIH, Bethesda, MD; 2) Life Sciences Centre, Dalhousie University, Halifax, Canada.

629B
Energy stores are genetically correlated with sleep but not altered by long-term sleep deprivation in Drosophila. **Susan Harbison, Amita Sehgal.** Department of Neuroscience, University of Pennsylvania Medical School, Philadelphia, PA.

630C
Molecular and Behavioral Analyses of Transgenic α-Synuclein Flies. **Ralph Hillman, Natalie Jerome, Nneka Isamah, Connie Yang, Darryl L'Heureux, Robert Pendleton.** Dept Biol, Temple Univ, Philadelphia, PA.

631A
The CAFE assay allows precise measurement of ingestion in Drosophila. **William W. Ja[1], Gil B. Carvalho[1], Noelle N. de la Rosa[1], Elizabeth M. Mak[1], Jonathan Liong[1], Ted Brummel[2], Seymour Benzer[1].** 1) Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125; 2) Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341-2116.

632B
A mutation in a putative Ste20 family gene eliminates ethanol-induced hyperactivity. **Ian F. G. King[1], Linus Tsai[1], Ralf Pflanz[2], Herbert Jäckle[2], Ulrike Heberlein[1].** 1) Dept. of Anatomy, UCSF, San Francisco, CA; 2) Max-Planck Institute for Biophysical Chemistry, Gottingen.

633C
Mutations in the α2-6 sialyltransferase gene DSiaT cause nervous system functioning defects. **Kate Koles[1], Elena Repnikova[1], Yi Ren[2], Scott Selleck[2], Yi Zhou[3], Claire Haueter[3], Hugo Bellen[3].** 1) Dept Biochem & Biophysics, Texas A&M Univ, College Station, TX; 2) University of Minnesota Department of Genetics, Cell Biology, and Development, Minneapolis, MN; 3) HHMI and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

634A
Neuroanatomy of the central complex: a statistical approach. **Mark H. Longair[1], Dean A. Baker[2], J. Douglas Armstrong[3].** 1) Neuroinformatics DTC, University of Edinburgh, Edinburgh, UK; 2) Department of Genetics, University of Cambridge, Cambridge, UK; 3) Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK.

635B
Drosophila CG16801/NR2E3 modulates eclosion and wing expansion behaviors and fertility. **Steven Robinow, Qing Chang, Laura Wong, Gavin Ganzer, Nelson Lazaga, Elizabeth Nguyen, Michelle Varize, Carl Sung.** Dept Zoology, Univ Hawaii, Honolulu, HI.

636C
Neural control of respiration in Drosophila. **Vikram Sudarsan, Helen Wiersma, Mark Krasnow.** Department of Biochemistry/ HHMI, Stanford University School of Medicine, Stanford, CA.

637A
The genetic architecture of Drosophila locomotor behavior. **Akihiko Yamamoto[1,3], Robert Anholt[1,2,3], Trudy Mackay[2,3].** 1) Dept Zoology, North Carolina State Univ, Raleigh, NC; 2) Genetics, North Carolina State Univ, Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State Univ, Raleigh, NC.

638B
Two Genes Affecting Drosophila Gravitaxis. **Sonia Bjorum, Kathleen M. Beckingham.** Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

639C
Phospholipase A2 and acyltransferase enzymes involved in *D. melanogaster* olfaction and vision. **Ismael Josafat Gimate-Baños, Juan Rafael Riesgo-Escovar.** Development Biology, INB, UNAM, Querétaro, Querétaro, Mexico.

640A
Sweet and bitter taste profiling in Drosophila. **Beth Gordesky-Gold, Natasha Rivers, Osama Ahmed, Paul Breslin.** Monell Chemical Senses Ctr, Philadelphia, PA.

641B
Functional roles for β1,4-N-acetlygalactosa-minyltransferase-A in Drosophila Larval Neurons and Muscles. **Nicola Haines, Bryan A. Stewart.** Dept. Biology, University of Toronto, Mississauga, ON, Canada.

642C
touch insensitive larva B, A Gene Necessary for Hearing and Male Fertility Encodes a Conserved Ciliary Protein. **Ryan G. Kavlie[1,3], Maurice J. Kernan[2], Daniel F. Eberl[1,3].** 1) Interdisciplinary Ph.D. Program in Genetics, Univ of Iowa, Iowa City, IA; 2) Department of Neurobiology and Behavior, State Univ of New York at Stony Brook, Stony Brook, NY; 3) Department of Biological Sciences, Univ of Iowa, Iowa City, IA.

643A
Characterization of mutants for jog, a gene with a role in gravity perception in Drosophila. **Vanaja Konduri, Kathleen Beckingham.** Dept Biochemistry & Cell Biol, Rice Univ, Houston, TX.

644B
Two approaches to understanding the function of the RdgB protein. **Christin M. Molnar, Kate R. Muenzer, Don W. Paetkau.** Department of Biology, Saint Mary's College, Notre Dame, IN.

645C
Oxygen-sensitive soluble guanylyl cyclases mediate larval hypoxia escape responses. **David Morton, Anke Vermehren.** Integrative Biosciences, Oregon Health & Science Univ, Portland, OR.

646A
Larval lethality in mutations of *nervana 3*, which encodes the beta subunit of Na/K ATPase. **Madhuparna Roy[1], Ryan G. Kavlie[2], Daniel F. Eberl[1,2].** 1) Dept of Biological Sciences, University of Iowa, Iowa City, IA; 2) Genetics PhD Program, University of Iowa, Iowa City, IA.

647B
The gravitaxis-affecting protein Yuri interacts with the actin cytoskeleton. **Michael J. Texada[1], Cassidy B. Johnson[1], Rebecca A. Simonette[1], Ravi P. Munjaal[1], J. Douglas Armstrong[2], Kate M. Beckingham[1].** 1) Biochemistry and Cell Biology, Rice University, Houston, TX; 2) Bioinformatics, University of Edinburgh, Edinburgh, Scotland, UK.

648C
Identification of the adenylyl cyclase that mediates sugar perception in Drosophila. **Kohei Ueno[1], Yoshiaki Kidokoro[2].** 1) Department of Behavioral Sciences, Graduate School of Medicine Gunma University, Maebashi, Gunma, Japan; 2) Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Gunma, Japan.

649A
Atypical soluble guanylyl cyclases in Drosophila may be involved in feeding preference behaviors. **Anke Vermehren, David Morton.** Integrative Biosciences, OHSU, Portland, OR.

650B
Molecular and Neural Regulation of Social Response to Aversive Stimuli in *D. melanogaster*. **Jie Xu, Ping Shen.** Department of Cellular Biology, University of Georgia, Athens, GA.

651C
A Potential Role for the Cytoskeletal Linker Protein, Moesin, in Drosophila Neuromuscular Junction Morphology. **Sara Seabrooke, Bryan A. Stewart.** Department of Biology, University of Toronto, Mississauga, Ontario, Canada.

Evolution and Quantitative Genetics

652A
A conserved muscle differentiation complex in *Apis mellifera* and *Tribolium castaneum*. **Jessica Cande, Michael Levine.** Department of Molecular & Cell Biology, UC Berekely, Berkeley CA.

653B
Analysis of Netrin Expression in *Artemia franciscana* provides Evidence for Conserved Roles of Netrins during Arthropod Development. **Molly Duman-Scheel, Stephanie Clark, Eric Grunow, Andrew Hasley, Brandon Hill, Wendy Simanton.** Dept. of Biology, Albion College, Albion, MI.

654C
Molecular Population Genetics of a cis-regulatory network in *D. melanogaster*. **Ian Dworkin[1,2], Greg Gibson[1].** 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Department of Biology and Carolina Center for the Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

655A
Rapid evolution at some, but not all, proteins involved in the control of Drosophila germline stem cell differentiation. **Heather A. Flores[1], Vanessa L. Bauer DuMont[1], Aalya Fatoo[1], Mohammed A. Hijji[2], Diana Hubbard[3], Danial A. Barbash[1], Charles F. Aquadro[1].** 1) Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY; 2) Cornell Medical College in Qatar, Doha, Qatar; 3) University of Chicago, Chicago, IL.

656B
Genotype x environment interaction in response to novel stress combinations. **Clayton Hallman, James Thompson.** Zoology Dept, Univ Oklahoma, Norman, OK.

657C
Understanding the evolution of cis-regulatory sequences determining patterns of gene expression in Drosophilids. **Karolina M. Jastrzebowska, Pavel Tomancak.** MPI-CBG, Dresden, Germany.

658A
Evolution of Ubx Transcription Activation Domains. **Ying Liu, Kathleen Matthews, Sarah Bondos.** Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005.

659B
Genetic analysis of segmentation patterns in Drosophila: gap and pair-rule gene expression in relation to embryo size within and between species. **Susan E. Lott[1], Michael Z. Ludwig[2], Arnar Palsson[2], Martin Kreitman[1,2].** 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

660C
Do host plant toxins protect Drosophila larvae from wasp parasitism? **Neil Milan, Todd Schlenke.** Department of Biology, Emory University, Atlanta, GA.

661A
Do innate behaviors change with inbreeding? **Steven Nilsen, Camayd Cristina, Nelson Dylan, Kravitz Edward.** Dept Neurobiology, Harvard Medical Sch, Boston, MA.

662B
Shifting sands of heterochromatin? A rapidly evolving heterochromatin protein, Su(var)3-7. **Joshua J. Bayes[1,2], Harmit S. Malik[2].** 1) Molecular & Cellular Biology Program, University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

663C
Molecular Evolution of a Sperm Specific Gene Family. **Bruce Bryan[1], Timothy Karr[2], David Rand[1].** 1) Dept of Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Dept of Biology and Biochemistry, University of Bath, Bath, UK.

664A
Genome decay during the evolution of host specialization in *D. sechellia*. **Ian Dworkin[1,2], Corbin Jones[2].** 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Department of Biology and Carolina Center for the Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

665B
Genome-wide patterns of evolution of the metabolic network in the Drosophila clade. **Anthony Greenberg, Andrew Clark.** Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

666C
Rapid divergence and genome rearrangements during *swallow* gene evolution. **Mary Ann Knox, Edwin Stephenson.** Dept of Biological Sciences, University of Alabama, Tuscaloosa, AL.

667A
Evolution of the Y-chromosome: changes in gene content in 8 Drosophila species. **Leonardo Koerich[1], Andrew Clark[2], A. Bernardo Carvalho[1].** 1) Department of Genetics, Federal Univ. of Rio de Janeiro, Brazil; 2) Molecular Biology and Genetics, Cornell University.

668B
Structure of the Dras1 gene control region in a set of Drosophila species. **Alex Kulikov[1], Anna Chekunova[1], Oleg Lazebny[1], Irina Lazebnaya[2], Vladimir Mitrofanov[1].** 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow; 2) Dept Animal Genetics, Vavilov Inst General Genetics, Moscow.

669C
Wolbachia influences mating preferences in *D. melanogaster*. **Alex Kulikov[1], Alexander Markov[2], Irina Goryacheva[3], Oleg Lazebny[1], Maxim Antipin[3].** 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow; 2) Institute of Paleontology, Moscow; 3) Vavilov Inst General Genetics, Moscow.

670A
Rapid evolution of the *mu2* gene. **James Mason, Raghuvar Dronamraju.** Lab Molec Genetics, NIH/NIEHS, Research Triangle Park, NC.

671B
Phylogenetic incongruence arising from chromosomal inversions. **Bryant McAllister, Amy Evans, Paulina Mena.** Biological Sci, Univ Iowa, Iowa City, IA.

672C
Microarray analysis of interspecific interactions affecting gene expression. **Colin Meiklejohn[1], Yasuhiro Go[2], David Rand[1], Daniel Hartl[2].** 1) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

673A
On the origin and evolution of segmentally duplicated genes in the *D. pseudoobscura* genome. **Richard P. Meisel, Nadia Hasan, Ben B. Hilldorfer, Robin P. Le Gros, Rachel L. Zindren, Stephen W. Schaeffer.** The Pennsylvania State University, University Park, PA.

674B
The base composition evolution of Drosophila genome. **Yu-Ping Poh[1], Chau-Ti Ting[1,2], Charles H. Langley[3].** 1) Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan; 2) Department of Life Science, National Taiwan University, Taipei, Taiwan; 3) The Center for Population Biology & The Section of Evolution and Ecology, UC Davis, Davis, CA.

675C
Evolutionary analysis of the *D. melanogaster* betaNACtes gene family. **Lev Usakin, Oxana Olenkina, Vladimir Gvozdev.** Animal Molecular Genetics, Institute Molecular Genetics, Moscow, Russia.

676A
Transposable element estimate on selection constraint in the Drosophila genome. **Jun Wang[1], Hsin-Chien Cheng[2], Pei-San Li[2], Daniel Barbash[1], Hsiao-Pei Yang[1,2].** 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853; 2) Faculty of Life Sciences and Institute of Genomic Sciences, National Yang-Ming University, Taipei, Taiwan, R. O. C.

677B
Rapid increase in viability due to new beneficial mutations in *D. melanogaster*. **Ronny Woodruff[1], Priti Azad[1,2].** 1) Dept Biol Sci, Bowling Green State Univ, Bowling Green, OH; 2) Dept of Neurology, College of Medicine, UTHSC, Memphis, TN.

678C
Searching for the autonomous transposable elements responsible for the transpositional burst of DINE-1s in Drosophila genomes. **Hsiao-Pei Yang, Sherry Lin.** Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

Poster board is in bold above the title. See page 14 for presentation schedule. The first author is the presenter. Abstracts begin on page 77.

679A
The rate of unequal crossing over in the PIGSFEAST repeat array in the Drosophila *dumpy* gene. **Amber Carmon, Matthew Larson, Ross MacIntyre.** Dept Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

680B
Asymmetrical reproductive isolation between *D. albomicans* and *D. nasuta*. **Hwei-yu Chang, Yu-ta Tai.** Dept. Entomology, National Taiwan University, Taipei, Taiwan.

681C
Genetic changes on a non-recombining chromosome of Drosophila. **Ting-yi Gong, Hwei-yu Chang.** Dep.of Entomology, National Taiwan University, Taipei, Taiwan.

682A
An experimental test of the X-inactivation hypothesis. **Winfried K. Hense, John F. Baines, John Parsch.** Department of Biology II, Section of Evolutionary Biology, University of Munich (LMU), Munich, Germany.

683B
The evolution of and divergent expression in closely linked members of a gene family. **Diana L. E. Johnson, Paaqua Grant.** Dept Biological Sci, George Washington Univ, Washington, DC.

684C
Adaptive radiation of digestive proteases in Drosophila female reproductive tracts. **Erin Kelleher[1], Willie Swanson[2], Therese Markow[1].** 1) Deparment of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Department of Genome Science, University of Washington, Seattle, WA.

685A
The evolution of mitochondrial physiology and intergenomic epistases across the Drosophila phylogeny. **Kristi Montooth, Colin Meiklejohn, Dawn Abt, David Rand.** Dept Ecol & Evol, Brown Univ, Providence, RI.

686B
Horizontal transmission of male-killing *Wolbachia* in Drosophila. **Sara Sheeley, Bryant McAllister.** University of Iowa, Iowa City, IA.

687C
The projectin protein and the evolution of asynchronous physiology in insect flight muscles. **Richard Southgate, Catherine Kramp, Agnes Ayme-Southgate.** Dept Biol, Col Charleston, Charleston, SC.

688A
Phylogenetic analysis suggests a functional relationship between *kayak* the Drosophila Fos homolog and *fig* a predicted PP2C phosphatase rested within a *kayak* intron. **Stephanie Hudson, Elliott Goldstein, Stuart Newfeld.** Sch Life Sci, Arizona State Univ, Tempe, AZ.

689B
Phylogenetic analysis of *D. virilis* species group by two different mtDNA approaches. **Svetlana Sorokina.** Dept Genetics, Koltsov Inst Dev Biology, Moscow, Russia.

690C
Functional evolution of *X*-linked odorant binding protein genes in *D. melanogaster*. **Gunjan Arya[1,3], Ping Wang[2,3], Richard Lyman[2,3], Trudy Mackay[2,3], Robert Anholt[1,2,3].** 1) Department of Zoology, North Carolina State Univ., Raleigh, NC; 2) Department of Genetics, North Carolina State Univ., Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State Univ., Raleigh, NC.

691A
Identification of loci contributing to mating success and multicomponent sexual signals in female *D. melanogaster*. **Brad R. Foley[1], Steve F. Chenoweth[2], Sergey V. Nuzhdin[1], Mark W. Blows[2].** 1) Dept of Evolution and Ecology, UC Davis, Davis, CA; 2) Dept of Integrative Biology, the university of Queensland, Brisbane Australia.

692B
First evidence for natural genetic variation in cuticular hydrocarbon expression in male and female *D. melanogaster*. **Brad R. Foley[1], Steve F. Chenoweth[2], Mark W. Blows[2], Sergey V. Nuzhdin[1].** 1) Dept of Evolution and Ecology, UC Davis, Davis California, CA; 2) Department of Integrative Biology, the University of Queensland, Brisbane Australia.

693C
Evolution of water balance and gene expression in desiccation-selected *D. melanogaster*. **Allen G. Gibbs, Cheryl H. Vanier.** School of Life Sciences, University of Nevada, Las Vegas, NV.

694A
Divergence population genetics of the *D. simulans* species complex. **Richard Kliman, Shannon McDermott.** Dept Biological Sci, Cedar Crest Col, Allentown, PA.

695B
Linkage disequilibrium analyses of synonymous and replacement polymorphisms in Drosophila chemoreceptor genes. **Rumi Kondo[1], Miki Oshima[1], Yukako Yoshifuji[1], Nobuyuki Inomata[2], Masanobu Itoh[3], Toshiyuki Takano-Shimizu[4].** 1) Dept Biology, Ochanomizu University, Tokyo, Japan; 2) Dept Biology, Graduate School of Sci, Kyushu University, Fukuoka, Japan; 3) Dept Applied Biology, Kyoto Institute of Technology, Kyoto, Japan; 4) Dept Population Genetics, National Institute of Genetics, Shizuoka, Japan.

696C
Evidence of spatially varying selection acting on the chromatin remodeling gene, chameau. **Mia Levine, David Begun.** Section of Evolution & Ecology, Univ California, Davis, Davis, CA.

697A
Evolution at two levels revisited: The role of transcriptional and functional variation in host adaptation. **Luciano Matzkin, Therese Markow.** Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ.

698B
Chromosomal Polymorphisms and Associated DNA variation in *D. americana*. **Paulina Mena, Bryant McAllister.** University of Iowa, Iowa City, IA.

699C
Abundant genetic variation in transcript level during early Drosophila development. **Sergey Nuzhdin[1], Danielle Tufts[1], Mathew Hahn[2].** 1) Dept Evolution & Ecology, Univ California, Davis, Davis, CA; 2) Department of Biology and School of Informatics, Indiana University, Bloomington, IN 47405.

700A
Maintenance of a gene arrangement polymorphism in natural populations of *D. pseudoobscura*. **Stephen Schaeffer.** Dept Biol, Pennsylvania State Univ, University Park, PA.

701B
Biparental inheritage of mtDNA in *D. melanogaster*. **Christian Schloetterer, Daniela Nunes.** Inst Tierzucht, VMU Wien, Wien, Austria.

702C
Variations in the chromosomal locations of the rRNA genes and pairing ability during male meiosis in the *D. ananassae* complex. **Mami Shibusawa[1], Yoshiko Tobari[2], Muneo Matsuda[1].** 1) Biology, Kyorin University, Mitaka, Tokyo, Japan; 2) Institute of Evolutionary Biology, Setagaya, Tokyo, Japan.

703A
Associations of SNPs in Odorant Binding Protein Genes with Olfactory Behavior in *D. melanogaster*. **Ping Wang[1,2], Richard F. Lyman[2], Svetlana Shabalina[3], Theodore J. Morgan[1,2], Trudy F. C. Mackay[1,2], Robert R. H. Anholt[1,2,4].** 1) W. M. Keck Center for Behavioral Biology, NC State Univ, Raleigh, NC; 2) Dept. Genetics, NC State Univ, Raleigh, NC; 3) NCBI, NIH, Bethesda, MD; 4) Dept. Zoology, NC State Univ, Raleigh, NC.

704B
Quantitative trait loci affecting plasticity and allometry of ovariole number and body size. **Alan Bergland[1], Anne Genissel[2], Sergey Nuzhdin[2], Marc Tatar[1].** 1) Dept Ecology & Evolution, Brown Univ, Providence, RI; 2) Section of Evolution and Ecology, University of California Davis, Davis, CA.

705C
A microarray approach to understanding the genetic basis of variation in age-specific immune response in *D. melanogaster*. **T. M. Felix[1], J. M. Drnevich[2], K. A. Hughes[3], J. W. Leips[1].** 1) Dept. of Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD; 2) W.M. Keck Center for Comparative and Functional Genomics, University of Illinois, Urbana IL; 3) School of Integrative Biology and Institute for Genomic Biology, University of Illinois, Urbana IL.

706A
A novel method for measuring total fitness of outbred genotypes. **James D. Fry.** Dept Biol, Univ Rochester, Rochester, NY.

707B
Are the genes which contribute to species differences in sex comb tooth number also associated with tooth number variation in natural populations of *D. simulans*? **Rita M. Graze[1], Elena Naderi[2], Sergey V. Nuzhdin[2].** 1) Genetics Graduate Group, University of California, Davis, CA 95616; 2) Center for Population Biology, Section of Evolution and Ecology, University of California, Davis, CA 95616.

708C
Genetic variation in the plastic response of life history traits, energy metabolism, and age-specific immunity to different diets. **Mary F. Kaminski[1], Michelle Moses[2], Maria DeLuca[2], Jeff Leips[1].** 1) Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD; 2) Department of Nutrition Sciences, University of Alabama at Birmingham.

709A
Quantitative Genetics of Antibacterial Immunity in Drosophila. **Brian Lazzaro.** Dept Entomology, Cornell Univ, Ithaca, NY.

710B
Quantitative trait loci analysis of cryptic female choice between *D. mauritiana* and *D. simulans*. **Cheng-Lin Li[1], Chen-Hung Kao[2], Chau-Ti Ting[1,3].** 1) Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan, ROC; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan, ROC; 3) Department of Life Science, National Taiwan University, Taipei, Taiwan, ROC.

711C
Naturally segregating polymorphisms responsible for quantitative variation in gene expression and wing vein position in *D. melanogaster*. **James Lorigan, Fangfei Ye, Jason Mezey.** Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

712A
Genetic Analysis of Intraspecific and Interspecific Sexually Dimorphic Trait Differences in the *Drosophila bipectinata* Species Complex. **Chen Siang Ng, Andrew Hamilton, Artyom Kopp.** Section of Evolution and Ecology, University of California, Davis, CA 95616.

713B
The genetic basis of eye size and shape differences between two closely related species of Drosophila. **Maria Margarita Ramos[1], David Stern[1], Peter Grant[1], Andrew I. Hanna[2], Enrico Coen[3].** 1) Ecology & Evolutionary Biol, Princeton Univ, Princeton, NJ; 2) Signal and Image Processing Research Group, Royal Society Wolfson Bioinformatics Laboratory, University of East Anglia, Norwich,UK; 3) Cell and Developmental Biology Department, John Innes Centre, Norwich,UK.

714C
Transcriptional profiles of high and low selection lines for the temperature knock down phenotype in *D. melanogaster*. **David Rand[1], Colin Meiklejohn[1], Donna Folk[2], George Gilchrist[2].** 1) Ecology & Evolutionary Biol, Brown Univ, Providence, RI; 2) Dept. of Biology, College of William & Mary, Williamsburg, VA.

715A
Genetics of alcohol sensitivity in *D. melanogaster*. **Reba M. Royster[1,3], Robert R. H. Anholt[1,2,3], Trudy F. C. Mackay[1,3].** 1) Genetics, NC State University, Raleigh, NC; 2) Zoology, NC State University, Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, NC State University, Raleigh, NC.

716B
Dynamic Genetic Interactions Determine Odor-Guided Behavior in *D. melanogaster*. **Deepa Sambandan[1,2], Trudy F. C. Mackay[1,2], Robert R. H. Anholt[1,2,3].** 1) Department of Genetics; 2) W.M. Keck Center for Behavioral Biology; 3) Department of Zoology, North Carolina State University, Raleigh, NC.

717C
Natural genetic variation on the 3rd chromosome influencing a host of quantitative traits and their interactions in *D. melanogaster*. **Adrienne Starks, Jeff Leips.** Dept Biological Sciences, UMBC, Baltimore, MD.

718A
Investigating interactions between HP1 and the hybrid incompatibility protein LHR. **Nicholas J. Brideau, Xu Wang, Daniel A. Barbash.** Molecular Biology and Genetics, Cornell University, Ithaca, NY.

719B
Adaptive evolution underlies genetic divergence contributing to hybrid incompatibilities in Drosophila. **Corbin Jones[1], Alisha Holloway[2].** 1) Department of Biology & Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC; 2) Section of Evolution and Ecology & Center for Population Biology, UC-Davis, CA.

720C
Population genetics of a recently divergent group of Hawaiian Drosophila. **Richard Lapoint, Patrick O'Grady.** ESPM, UC, Berkeley, Berkeley, CA.

721A
An investigation into the molecular function of the hybrid incompatibility gene, *Lhr*. **Shamoni Maheshwari, Daniel A. Barbash.** Molecular Biology and Genetics, Cornell University, Ithaca, NY.

722B
The genetic basis of segregation distortion and male sterility in the F_1 hybrids between *D. pseudoobscura* USA and Bogotá sub-species. **Nitin Phadnis, H. Allen Orr.** Department of Biology, University of Rochester, Rochester, NY.

Immune System and Cell Death

723C
A new type of apoptosis-induced compensatory proliferation requires activity of effector caspases. **Yun Fan, Andreas Bergmann.** Department of Biochemistry & Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

724A
The caspases Strica and Dronc function redundantly during programmed cell death in oogenesis. **Kim McCall[1], Jason S. Baum[1], B. Paige Bass[1], Jeanne S. Peterson[1], Antony Rodriguez[2], John M. Abrams[2].** 1) Department of Biology, Boston University, Boston, MA; 2) Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

725B
Expression of a caspase-resistant nuclear lamin disrupts cell death and morphogenesis in oogenesis. **Elizabeth A. Tanner, Margaret Barkett, Kimberly McCall.** Department of Biology, Boston University, Boston, MA.

726C
Ionizing radiation induces caspase-dependent but Chk2- and p53-independent cell death. **Anita Wichmann[1], Burnley Jaklevic[2], Tin Tin Su[1].** 1) Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO; 2) Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, CA.

727A
C-type lectin, Furrowed, aborts development of malaria parasite, *Plasmodium berghei* in tropical vector, *Anopheles gambiae*. **Hiroka Aonuma[1,2], Stephanie Brandt[3], Shinya Fukumoto[1], Tokiyasu Teramoto[1], Masayuki Miura[4], Takeshi Yagi[2], Hirotaka Kanuka[1], David Schneider[3].** 1) NRCPD, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan; 2) Graduate School of Frontier Bioscience, Osaka University, Suita, Osaka, Japan; 3) Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA; 4) Graduate School of Pharmaceutical Sciences, University of Tokyo, Bunkyo, Tokyo, Japan.

728B
Functional analysis of a major phagocytic pattern recognition receptor in Drosophila. **Ju Hyun Cho, Christine Kocks.** Department of Pediatrics, Harvard Medical School, Developmental Immunology, Massachusetts General Hospital, Boston, MA.

729C
Undertaker, a new Drosophila mutant with defects in phagocytosis of apoptotic cells. **Nathalie C. Franc, Leigh Cuttell, Emeline Van Goethem, Claire Escaron, Christina Bakatselou, Mark Lavine, Magali Quirin.** MRC LMCB & CBU, University College London, London, UK.

730A
Rel signaling guides immune homeostasis in Drosophila. **Nina Matova, Kathryn V. Anderson.** Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

731B
Study of a new phagocytosis of apoptotic cells-defective mutant in Drosophila. **Emeline Van Goethem, Nathalie C. Franc.** MRC-LMCB/CBU, University College London, London, UK.

732C
Control of larval hematopoiesis by the Posterior Signaling Center. **Alain Vincent[1], Joanna Krzemien[1], Rami Makki[1], Laurence Dubois[1], Marie Meister[2], Michele Crozatier[1].** 1) Developmental Biology, UMR 5547 CNRS/UPS, Toulouse, France; 2) UPR 9022 CNRS, Strasbourg, France.

733A
An isoform specific requirement for lola during programmed cell death in oogenesis. **B. Paige Bass, Kim McCall.** Dept Biol, Boston Univ, Boston, MA.

734B
The RING-finger protein elfless: at the crossroads of spermatogenesis and apoptosis. **Jason Caldwell, Daniel Eberl.** Dept Biological Sci, Univ Iowa, Iowa City, IA.

735C
Identification of recessive suppressors and enhancers of Hid-induced cell death. **Hans-Martin Herz[1,2], Zhihong Chen[1], Andreas Bergmann[1].** 1) Dept Biochem & Molecular Biol, MD Anderson Cancer Ctr, 1515 Holcombe Blvd, Houston, TX 77030; 2) Center for Molecular Biology (ZMBH), University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

736A
The antiapoptotic effect of overexpressing the Drosophila homolog of the putative Phosphatidylserine receptor is mediated through modulation of the N-JNK pathway. **Ronald Krieser, Douglas Dresnek, Brett Pellock, Kristin White.** Cutaneous Biology Research Center, Massachusetts General Hospital 149 13th Street Charlestown, MA 02129.

737B
Genetic analysis of steroid-triggered cell death during Drosophila metamorphosis. **Lei Wang, Arash Bashirullah, Carl Thummel.** Dept. of Human Genetics, University of Utah School of Medicine, 15 N 2030 E, Room 2100, Salt Lake City, UT 84112-5330.

738C
Identification and characterization of Cbl function in cell death and cell differentiation in the Drosophila eye. **Yuan Wang, Zhihong Chen, Dongbin Xu, Andreas Bergmann.** Biochemistry & Molecular Biol, M D Anderson Cancer Ctr, Houston, TX.

739A
Isolation of Mutations of Apoptotic Genes in 3rd Chromosome in Drosophila. **Dongbin Xu, Andreas Bergmann.** Dept Biochemistry & Molec Biol, Univ Texas MD Anderson CA Ctr, GSBS, Houston, TX.

740B
Analysis of Drosophila TAB2 mutants reveals that IKK, but not JNK pathway activation, is essential in the host defense against *Escherichia coli* infections. **Dominique Ferrandon[1], Alain Jung[1], Vanessa Gobert[1], Rui Zhou[2], Nicholas Paquette[3], Sophie Rutschmann[1], Marie-Claire Criqui[1], Marie-Céline Lafarge[1], Matthew Singer[4], David Ruddy[4], Tom Maniatis[2], Jules Hoffmann[1], Neal Silverman[3].** 1) IBMC, CNRS UPR 9022, Strasbourg, France; 2) Dept. of Molecular and Cellular Biology, Harvard Univ., Cambridge, MA; 3) Div. of Infectious Disease, Department of Medicine, Univ. of Massachusetts Medical School, Worcester, MA; 4) Exelixis, Inc., South San Francisco, CA.

741C
Infection of *D. melanogaster* with *Providencia* species, natural bacterial pathogens. **Madeline R. Galac[1], Brian P. Lazzaro[1,2].** 1) Field of Genetics and Development, Cornell University, Ithaca, NY; 2) Department of Entomology, Cornell University, Ithaca, NY.

742A
A Comparative Dissection of Innate Immune Pathways in *D. melanogaster* using RNA Interference. **David Kuttenkeuler, Michael Boutros.** Functional Genomics, German Cancer Research Center, Heidelberg, Heidelberg, Germany.

743B
Identification of Novel Genes Affecting the *D. melanogaster* Immune Response to Drosophila X Virus. **Anne M. Macgregor, Louisa P. Wu.** Center for Biosystems Research, UMBI, College Park, MD.

744C
A tolerance factor dissected from host resistance system in Drosophila. **Naoaki Shinzawa[1,2], Hiroka Aonuma[1], Masayuki Miura[2], Hirotaka Kanuka[1].** 1) NRCPD, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan; 2) Graduate School of Pharmaceutical Science, University of Tokyo, Bunkyo, Tokyo, Japan.

745A
Drosophila STAT (STAT92E) is anti-apoptotic by directly activating diap1 transcription. **Aurel Betz[1], Hyung Don Ryoo[2], Hermann Steller[2], James E. Darnell, Jr.[1].** 1) Laboratory of Molecular Cell Biology, The Rockefeller University, NYC, NY; 2) HHMI, Laboratory of Apoptosis and Cancer Biology, The Rockefeller University, NYC, NY.

746B
In vivo imaging of DIAP1 degradation during sensory organ development. **Akiko Koto, Erina Kuranaga, Masayuki Miura.** Dept. Genetics, Grad. Sch. Pharm., Univ. Tokyo, Tokyo, Japan.

747C
The Drosophila SUMO conjugase Lesswright regulates apoptosis and cell survival during larval hematopoiesis. **Jinu Abraham[1], Liang Huang[1,2], Soichi Tanda[1].** 1) Department of Biological Sciences and MCB Program, Ohio University, Athens, OH; 2) Present Address: National Institute of Health, Bethesda, Maryland.

748A
Crystal cell rupture in Drosophila after injury requires Eiger, JNK and small GTPases. **Gawa Bidla[1], Mitchell Dushay[2], Ulrich Theopold[1].** 1) Department of Molecular Biology and Functional Genomics, Stockholm University, Arrheniuslab F425, 10691 Stockholm, Sweden; 2) Department of Comparative Physiology, Uppsala University, Norbyvägen 18A, 75236 Uppsala, Sweden.

749B
Cricket Paralysis Virus infection of *Drosophila* reveals that the Imd pathway is involved in antiviral immune responses. **Alexandre Costa, Eric Jan, Peter Sarnow, David Schneider.** Dept Microbiology & Immunology, Stanford University, Stanford, CA.

750C
What can Drosophila teach us about tuberculosis? **Marc Dionne[1,2], David Schneider[1].** 1) Dept Microbiol & Immunology, Stanford Univ, Stanford, CA; 2) Dept Craniofacial Development, Guy's Campus, King's College London, London, UK.

751A
Developmentally-regulated cell death of Drosophila salivary glands utilizes ER stress-linked apoptosis. **Robert Farkas[1], Lucia Medvedova-Mentelova[1,2], Peter Low[3], Gabor Juhasz[3], Miklos Sass[3].** 1) Inst Experimental Endocrinol, Slovak Academy Science, Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia; 3) Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Pazmány Sétány 1/C, H-1117 Budapest, Hungary.

752B
Drosophila S2 cells as a model to study E. chaffeensis infections. **Alison L. Fedrow[1], Tonia Von Ohlen[1], Roman Ganta[2], Stephen Chapes[1].** 1) Biology, Kansas State University, Manhattan, KS; 2) Diagnostic Medicine & Pathobiology, Kansas State University, Manhattan, KS.

753C
SCF ubiquitin ligase complex mediates phagocytosis through the novel F-box domain protein, Pallbearer. **Nathalie Franc, Connie Au-Yeung, Emeline van Goethem, Elizabeth Silva.** MRC LMCB & CBU, Univ Col London, London, UK.

754A
Shaggy is required for ethanol-induced olfactory receptor neuron apoptosis. **Rachael French, Ulrike Heberlein.** Department of Anatomy, University of California, San Francisco, CA, 94158-2324.

755B
Infection of *D. melanogaster* with West Nile virus induces a protective RNAi response. **Robert L. Glaser[1,2], Heather L. Chotkowski[1], Alexander T. Ciota[1], Jennifer L. Longacker[1], Laura D. Kramer[1,2].** 1) Wadsworth Ctr, New York State Dept Health, Albany, NY; 2) Dept Biomedical Sciences, University at Albany, State University of New York.

756C
Drosophila homologue of APP-BP1 (dAPP-BP1) interacts antagonistically with APPL during Drosophila development. **Hyung-Jun Kim[1], Song-Hee Kim[1], Sang-Ohk Shim[1], Eungsik Park[1], Changsoo Kim[2], Kiyoung Kim[1], Mark Tanouye[3], Jeongbin Yim[1].** 1) School of Biological Sciences, Seoul National University, Seoul, Korea; 2) School of Biological Sciences and Technology, Chonnam National University, Gwangju; 3) Department of Molecular and Cell Biology, Division of Neurobiology, University of California, Berkeley.

757A
Characterizing genetic elements regulating neuroblast apoptosis in Drosophila. **Megumu Mabuchi[1], Wei Tang[1], Susan St. Pierre[2], Reena Patel[1], Kristin White[1].** 1) CBRC, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA; 2) The Biological Laboratories, Harvard University, Cambridge, MA.

758B
Impact of the *Pseudomonas aeruginosa* type III secretion system on virulence and patterns of gene expression during the Drosophila immune response. **Kurt McKean[1], Todd Schlenke[2], Andrew Clark[1].** 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biology, Emory University, Atlanta, GA.

759C
Characterization of the *D. Myeloid Leukaemia Factor*. **Anne Plessis[1], Severine Martin-Lanneree[2], Christelle Lasbleiz[1], Matthieu Sanial[1], Herve Tricoire[1].** 1) Inst Jacques Monod, CNRS-Paris 7-Paris 6, Paris, Frrance; 2) Inst Cochin, U.567 Inserm/UMR8104 CNRS/UMR-S8104, Paris, France.

760A
Possible interactions between the JAK/STAT and the Toll pathway in Drosophila hematopoiesis. **Ying Shen[1], Soichi Tanda[1,2].** 1) Dept Biological Sci, Ohio Univ, Athens, OH; 2) Molecular and Cellular Biology program, Ohio Univ, Athens, OH.

761B
Innate immunity and circadian rhythm. **Michele Shirasu-Hiza, Marc Dionne, Linh Pham, Janelle Lamberton, David Schneider.** Microbiology & Immunology, Stanford Univ, Stanford, CA.

762C
RNAi analysis of serine protease inhibitors of the serpin family in Drosophila. **Huaping Tang[1], Zakaria Kambris[2], Bruno Lemaitre[2], Carl Hashimoto[3].** 1) Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520; 2) Centre de Génétique Moléculaire, CNRS, 91198, Gif-sur-Yvette, France; 3) Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06520.

763A
Fork Head Controls the Tissue Selectivity of Steroid-Induced Developmental Cell Death. **Michael Lehmann, Chike Cao, Yanling Liu.** Department of Biological Sciences, University of Arkansas, Fayetteville, AR.

Techniques and Genomics

764B
Evolution and developmental dynamics of Drosophila DNA-binding transcription factors. **Boris Adryan, Derek Wilson, Sarah A. Teichmann.** Structural Studies Division, MRC LMB, Cambridge, UK.

765C
Prediction of non-coding RNAs using a Drosophila whole-genome alignment. **Yuri R. Bendana, Ian H. Holmes.** Department of Bioengineering, UC Berkeley, Berkeley, CA.

766A
Large-scale analysis of transcriptional *cis*-regulatory modules: common features, distinct subclasses, and implications for regulatory module discovery. **Marc S. Halfon[1,2,3], Long Li[1], Qianqian Zhu[1], Xin He[4], Saurabh Sinha[4].** 1) Dept. of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics and the Life Sciences, Buffalo, NY; 3) Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY; 4) Dept. of Computer Science, University of Illinois Urbana-Champaign, Urbana, IL.

767B
EDGI: new algorithm for discovery of transcriptional regulatory regions in Drosophila genome by a non-alignment method for phylogenetic footprinting. **Alona Sosinsky[1,2], Barry Honig[1,2], Richard Mann[2], Andrea Califano[3].** 1) Howard Hughes Medical Institute; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 3) Department of Biomedical Informatics, Columbia University, New York, NY.

768C
FlyExpress: a growing developmental bioinformatics platform for analysis of spatial expression patterns in Drosophila embryogenesis. **Bernard Van Emden[1], Hector Ramos[1], Sethuraman Paunchanathan[2], Thomas Brody[4], Stuart Newfeld[3], Sudhir Kumar[1,3].** 1) Biodesign Inst, Arizona State Univ, Tempe, AZ; 2) School of Computing and Informatics Arizona State Univ, Tempe, AZ; 3) School of Life Sciences, Arizona State Univ, Tempe AZ; 4) Neurogenetics Unit, LNC, NINDS, Natl Inst of Health, Bethesda, MD.

769A
Gene Targeting with zinc finger nucleases in a single generation. **Kelly Beumer, Jon Trautman, Dana Carroll.** Dept Biochemistry, Univ Utah, Salt Lake City, UT.

770B
Manipulating large regulatory elements within the context of the bithorax complex. **Carole Iampietro, Fabienne Cléard, Annick Mutero, Robert Maeda, François Karch.** University of Geneva, Geneva, Switzerland.

771C
Genome-wide mapping and characterization of protein expression and interaction in *D. melanogaster* using a hybrid PiggyBac/P-element YFP gene trap system with tandem affinity tags. **Ed Ryder[1], Helen Spriggs[1], John Roote[1], Emma Drummond[1], Jenny Drummond[1], Jane Webster[1], Glynnis Johnson[1], Nick Lowe[2], Kathryn Lilley[3], Svenja Hester[3], Julie Howard[3], Johanna Rees[3], Steve Russell[1], Daniel St. Johnston[2].** 1) Dept Genetics, Cambridge Univ, Cambridge, UK; 2) Gurdon Institute, Dept Genetics, Cambridge Univ, Cambridge, UK; 3) Dept Biochemistry, Cambridge Univ, Cambridge, UK.

772A
Chromosomal deletion screens at the Bloomington Stock Center. **Kevin R. Cook, Stacey J. Christensen, Megan E. Deal, Jill M. Gresens, Thomas C. Kaufman.** Dept Biol, Indiana Univ, Bloomington, IN.

773B
Characterization and functional analysis of mRNA-like non-coding RNAs in Drosophila. **Sachi Inagaki[1], Yuji Fukuda[1], Takefumi Kondo[1], Yoshiko Hashimoto[1], Yuji Kageyama[1,2].** 1) Grad Sch Biol Sci, Nara Inst Science Technology, Ikoma, Nara, Japan; 2) PREST, Japan Science and Technology Agency.

774C
spineless: central role in building the retinal mosaic required for color vision in Drosophila. **Preet Lidder, Claude Desplan.** Dept Biol, New York Univ, New York, NY.

775A
Sex-specific expression of alternative transcripts in Drosophila. **Lauren McIntyre[1], Lisa Bono[2], Anne Genissel[3], Marina Telonis-Scott[1], Larry Harshman[4], Marta Wayne[1], Artyom Kopp[2], Sergey Nuzhdin[2].** 1) University of Florida, Gainesville, FL; 2) The Ohio University; 3) UC Davis; 4) University of Nebraska.

776B
Identification of genes involved in color vision using Affymetrix GeneChips. **Tamara Mikeladze-Dvali, Preet Lidder, Claude Desplan.** Dept Biol, New York Univ, New York, NY.

777C
Variation in transcript abundance of chemoreceptors in adult and larval *D. melanogaster* assessed by cDNA expression microarrays. **Shanshan Zhou[1,4], Christina Grozinger[3,4], Trudy Mackay[2,4], Robert Anholt[1,2,4].** 1) Zoology, NC State University, Raleigh, NC; 2) Genetics, NC State University, Raleigh, NC; 3) Entomology, NC State University, Raleigh, NC; 4) W. M. Keck Center for Behavioral Biology, NC State University, Raleigh, NC.

778A
Genome-wide analysis of embryonic mRNA localization pathways. **Eric Lecuyer[1], Hideki Yoshida[1], Neela Parthasarathy[1], Christina Alm[1], Pavel Tomancak[2], Tomas Babak[1], Timothy R. Hughes[1], J. Timothy Westwood[3], Henry M. Krause[1].** 1) Donnelly CCBR, University of Toronto, Toronto, ON, Canada; 2) Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 3) Canadian Drosophila Microarray Centre, University of Toronto, Mississauga, ON, Canada.

779B
FISH based method for automated quantification of nascent and cytoplasmic mRNA transcript numbers in fixed Drosophila embryonic cells. **Adam C. Pare[1], Derek Lemons[1], David Kosman[1], William Beaver[2], Yoav Freund[2], William McGinnis[1].** 1) Cell and Developmental Biology, UC San Diego, La Jolla, CA; 2) Computer Science, UC San Diego La Jolla, CA.

780C
Correlation of microsatellites of DNA with enzyme variation at the MDH locus in *D. melanogaster*. **Domingo A. Montano.** Genetica, Univ Antonio Narino, Bogota, Columbia.

781A
The Tucson Drosophila Species Stock Center: Resources for the Drosophila Community. **Stacy Mazzalupo[1], Sergio J. Castrezana[1], Therese A. Markow[1,2].** 1) Arizona Research Labs, Univ Arizona, Tucson, AZ; 2) Ecology & Evolutionary Biology, Univ Arizona, Tucson, AZ.

782B
A new family of Drosophila balancer Chromosomes with a w-dfd-GMR YFP marker. **Gregory Beitel[1], Tien Le[1], Zhiguo Liang[2], Heeren Patel[1], Marcus Yu[1], Gitanjali Sivasubramaniam[1], Matthew Slovitt[1], Guy Tanentzapf[3], Nihar Mohanty[1], Sarah Paul[1], Victoria Wu[1].** 1) BMBCB, Northwestern Univ, Evanston, IL; 2) Department of Microbiology and Immunology MC790, University of Illinois-Chicago, Chicago IL 60612-7344; 3) Dept. of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada M5S 3G5.

783C
CaSpeR5, a family of Drosophila transgenesis and shuttle vectors with improved multiple cloning sites. **Gregory Beitel, Tien Le, Marcus Yu, Brandon Williams, Sagar Goel.** Dept BMBCB, Northwestern Univ, Evanston, IL.

784A
The Transcriptional Landscape. **Susan Celniker, Joseph Carlson, Mark Stapleton, Bhaveen Kapadia, Soo Park, Kenneth Wan, Richard Weiszmann, Charles Yu, Ann Hammonds.** Berkeley Dros Genome Ctr, Lawrence Berkeley National Lab, Berkeley, CA.

785B
Textpresso for Fly: a Literature Search Engine for Researchers and Curators. **Hans-Michael Muller[1], Beverley Matthews[2], Susan Russo[2], Eimear Kenny[3], Arun Rangarajan[1], William Gelbart[2], Paul Sternberg[1].** 1) California Institute of Technology, Pasadena, CA; 2) Harvard University, Cambridge, MA; 3) Rockefeller University, New York, NY.

786C
Genome Sequence and Analysis of *Tribolium castaneum*, the Red Flour Beetle. **Stephen Richards, Yue Liu, Kim C. Worley, Erica Sodergren, Steven E. Scherer, Catherine M. Rives, Donna M. Muzny, George Weinstock, Richard A. Gibbs, The Tribolium Genome Consortium.** Human Genome Sequencing Center, Baylor Col Medicine, 1 Baylor Plaza, Houston, TX.

787A
A Genome-Wide RNA Interference Screen to Identify New Components of the RAS/MAPK Pathway. **Dariel Ashton-Beaucage, Marc Therrien.** Institut de Recherche en Immunologie et Cancerologie, Université de Montréal, Montreal, Quebec, Canada.

788B
Using viral suppressors of RNA silencing to explore the diversity and functions of small RNAs in Drosophila. **Bassam Berry[1], Delphine Fagegaltier[1], Ronald van Riji[2], Raul Andino[2], Jean-Luc Imler[3], Olivier Voinnet[4], Christophe Antoniewski[1].** 1) Developmental Biology, CNRS / Institut Pasteur, Paris, France; 2) University of California, San Francisco, CA 94143-2280; 3) CNRS-UPR9022, IBMC, 15 rue René Descartes, 67084 Strasbourg Cedex, France; 4) CNRS - IBMPC, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France.

789C
HDAC inhibitors and Drosophila: a fruitful team. **Marc Hild, Haidi Yang, Dan Garza.** Novartis Institutes for BioMedical Research (NIBR), Cambridge, MA.

790A
Design of a second-generation RNAi library for Drosophila. **Thomas Horn[1], Jeff Reid[2], Wolfgang Huber[3], Amy Kiger[2], Michael Boutros[1].** 1) German Cancer Research Center, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany; 2) Department of Cell and Developmental Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093; 3) EMBL - European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge CB10 1SD, UK.

791B
Rapid construction of RNAi transgenes using pRISE, a transformation vector exploiting Gateway Technology. **Yuji Kageyama[1,2], Takefumi Kondo[1], Sachi Inagaki[1].** 1) Grad Sch Biol Sci, Nara Inst Sci Tech, Ikoma, Nara 630-0192, Japan; 2) PREST, Japan Science and Technology Agency.

Drosophila Models of Human Diseases

792C
Genetic dissection of the rhabdomyosarcoma oncoprotein PAX-FKHR in a Drosophila model. **Tiana Endicott[1], Rene Galindo[1,2].** 1) Molecular Biology, UT Southwestern Med Center, Dallas, TX; 2) Pathology, UT Southwestern Med Center, Dallas, TX.

793A
Drosophila as a model of the childhood malignancy rhabdomyosarcoma. **Rene Galindo[1,2], Jay Allport[2], Eric Olson[2].** 1) Dept Pathology, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept Molecular Biology, Univ Texas SW Medical Ctr, Dallas, TX.

794B
aPKCζ and Lgl play a key role in Drosophila and human epithelial architecture. **Daniela Grifoni[1,2], Flavio Garoia[1], Paola Bellosta[4], Federica Parisi[2,4], Dario De Biase[3], Dennis Strand[5], Sandro Cavicchi[1], Annalisa Pession[2,3].** 1) Biology Dept. Alma Mater Studiorum, Bologna, Italy; 2) Pathology Dept. Alma Mater Studiorum, Bologna, Italy; 3) Oncology Dept., Pathology Section, Bellaria Hospital, Bologna, Italy; 4) Biology Dept. CUNY City College, NY; 5) First Dept. Internal Medicine, Johannes Gutenberg University, Mainz, Germany.

795C
Ras, Raf, and PI3-Kinase activities in peripheral glia regulate perineurial glial cell number in larval peripheral nerves. **William Lavery, Michael Stern.** Dept Biochemistry & Cell Biol, Rice University, Houston, TX.

796A
PDCD2/Zfrp8 in Drosophila and human hematopoiesis. **Svetlana Minakhina, Ruth Steward.** Dept Molec Biol & Biochemistry, Waksman Inst, Rutgers Univ, Piscataway, NJ.

797B
A structure-function analysis for the Drosophila fragile X protein. **Paromita Banerjee, Thomas C. Dockendorff.** Zoology, Miami University, Oxford, OH.

798C
Determining a molecular role for *Drosophila fragile X related gene (dfmr1)* in metabotropic glutamate receptor (mGluR) signaling. **Balpreet Bhogal, Thomas Jongens.** Department of Genetics, University of Pennsylvania, Philadelphia, PA.

799A
Regulation and function of Myotubularin phosphoinositide phosphatase in development. **Jared Dennis, Amy Kiger.** Dept Biological Sci, Univ California, San Diego, La Jolla, CA.

800B
Studying the Molecular Bases of *O*-mannosylation in Drosophila Model System. **Dmitry Lyalin, Naosuke Nakamura, Haiwen Li, Vladislav Panin.** Dept Biochemistry & Biophysics, Texas A&M University, College Station, TX.

801C
Drosophila Dystroglycan as a potential target of O-mannosylation by two protein O-mannosyltransferases, RT and TW. **Naosuke Nakamura, Dmitry Lyalin, Michiko Nakamura, Olga Lavrova, Haiwen Li, Vladislav Panin.** Dept. Biochem. & Biophysics, Texas A&M University, College Station, TX.

802A
Indirect flight muscles of Drosophila as a model system to study abnormal protein aggregate myopathies. **Upendra Nongthomba[1], Shital Salvi[1], Divesh Thimmaiya[1], John Sparrow[2].** 1) MRDG, Indian Institute of Science, Bengalooru, India; 2) Dept. of Biology, University of York, YO10 5DD, UK.

803B
Understanding the role of Drosophila Fragile X Mental Retardation Protein (FMRP) as a translational regulator. **Anita Pepper, Thomas Jongens.** Dept. of Genetics, Univ. of Pennsylvania SOM, Philadelphia, PA.

804C
detached encodes Drosophila Dystrophin, which acts with other members of the Dystrophin Associated Protein Complex to regulate cell signalling in developing wing veins. **Robert Ray, Christina Christoforou, Claire Greer, Benjamin Challoner, Dimitris Charitzanos.** School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG United Kingdom.

805A
The Drosophila ortholog of the mouse autoimmunity gene *roquin* is an essential gene. **Peter Smibert, Vicki Athanasopoulos, Robert Saint.** CMGD, Australian National University, Canberra, ACT, Australia.

806B
Characterization of the *torp4a* gene, a Drosophila homolog of human *DYT1* (Torsin A) associated with early-onset dystonia. **Noriko Wakabayashi-Ito, Nicole Smith, Jo-Chen Chou, Vijaya Ramesh, James Gusella, Naoto Ito.** Center for Human Genetic Res., Massachusetts General Hosp, Boston, MA.

807C
Studying the circadian defect observed in the Drosophila model of Fragile X Syndrome. **Yan Wang[1], Amita Sehgal[2], Thomas A. Jongens[1].** 1) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Neuroscience, HHMI, University of Pennsylvania School of Medicine, Philadelphia, PA.

808A
Genetic analysis of Rothmund-Thomson syndrome homolog in Drosophila. **Jianhong Wu, Carrie Marean-Reardon, Christopher Capp, Tao-shih Hsieh.** Biochemistry Department, Duke University Medical Center, Durham, NC 27710.

809B
Development of a *D. melanogaster* model for drug screening in Parkinson's disease. **Lori A. Hrdlicka[1], Joost Schulte[2], Hsin-Pei Shih[1], Christopher J. Cummings[1], James K. T. Wang[1].** 1) EnVivo Pharmaceuticals, Inc., Watertown, MA; 2) The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA.

810C
Putting the toolbox to work: A role for HDACs in disease. **Ranjani Padmanabhan, Jian Chen, Florian Gmeiner, Marc Hild, Dan Garza.** Drosophila Genetics Unit, Novartis Institutes for Biomedical Research (NIBR), Cambridge, MA.

811A
The use of a Drosophila model of Spinal Muscular Atrophy for small molecule high-throughput drug discovery. **Natasha Thomas[1], Paul Overton[1], Marcel van den Heuvel[2], Jon Tinsley[1], Emmanuel Dequier[1].** 1) VASTox plc, 91 Milton Park, Abingdon, OX14 4RY, UK; 2) MRC FGU, OCGF, University of Oxford, Oxford, OX1 3QX, UK.

812B
Copper Homeostasis and tau-mediated Neurodegeneration in Drosophila. **Kirsten E. Allan[1], James Camakaris[1], Richard Burke[2].** 1) Department of Genetics, University of Melbourne, Melbourne, Australia; 2) School of Biological Sciences, Monash University, Melbourne, Australia.

813C
Toxicity caused by Alzheimer Abeta peptides is associated with abnormal regulation of cell cycle genes. **Weihuan Cao, Tina Gangi, Mary Konsolaki.** Department of Genetics, Rutgers University, Piscataway, NJ.

814A
The *Drosophila Palmitoyl Protein Thioesterase 1 (Ppt1)*, a homolog of the Batten Disease PPT1 gene, is required for normal embryonic neural development. **Quynh Chu-LaGraff, Erika Selli, Cassandra Denefrio.** Dept Biology, Union College, Schenectady, NY.

815B
Functional dissection of Orthodenticle in the Drosophila eye: a model to understand human cone-rod dystrophy and Leber congenital amaurosis. **Pierre Fichelson, Franck Pichaud.** Cell Biology, MRC LMCB, London, UK.

816C
Genetic Modifiers of Prion Disease in Drosophila. **Brendan Gavin[1], James Geoghegan[2], Nathan Deleault[2], Maria Dolph[1], Vikram Khurana[3], Mel Feany[3], Surachai Supattapone[2], Patrick Dolph[1].** 1) Dept Biol, Dartmouth Col, Hanover, NH; 2) Department of Biochemistry, Dartmouth College, Hanover, NH; 3) Department of Pathology, Division of Neuropathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

817A
Drosophila X11 regulates the amyloid precursor protein and plays an essential role during neurodevelopment. **Garrett Gross[1], Renny Feldman[1], Volker Hartenstein[2], Ming Guo[1].** 1) Neurology, University of California, Los Angeles, CA; 2) Molecular, Cellular and Developmental Biology, University of California, Los Angeles, CA.

818B
Circadian rhythms as model systems to study the effects of transcriptional dysregulation in MJD-afflicted Drosophila. **Amy B. Hart, John M. Warrick.** Biology, University of Richmond, Richmond, VA.

819C
Mutational analysis reveals different aggregation propensity of Aβ42 is associated with distinct intraneuronal accumulation profile and pathological phenotypes in Drosophila. **Koichi Iijima[1,3], Hsueh-Cheng Chiang[3], Stephen Hearn[3], Inessa Hakker[3], Amy Leung[3], Kanae Iijima-Ando[2,3], Yi Zhong[3].** 1) Laboratory of Aging and Neuroproteinopathies,; 2) Laboratory of Neuronal Protein Misfolding, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

820A
Effects of N-terminal fragments of huntingtin harboring expanded polyglutamine stretch on CREB activity in Drosophila. **Kanae Iijima-Ando[1,3], Koichi Iijima[2,3].** 1) Laboratory of Neuronal Protein Misfolding, Farber Institute for Neurosciences,; 2) Laboratory of Alzheimer Disease and Neuroproteinopathies, Farber Institute for Neurosciences,; 3) Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA.

821B
The role of Drosophila EDEM in misfolded protein degradation. **Min-Ji Kang, Hyung Don Ryoo.** Department of Cell Biology, NYU School of Medicine, New York, NY.

822C
Reduced histone methyltransferase activity due to pathogenic and polymorphic genotypes. **Parsa Kazemi-Esfarjani[1,3,4], Zahra Fayazi[1,3,4], Jason M. Myers[2,3,4], John M. Aletta[2,3,4].** 1) Dept Physiology & Biophysics; 2) Dept Pharmacology & Toxicology; 3) Center for Neuroscience; 4) School of Medicine & Biomedical Sciences, Univ Buffalo, Buffalo, NY.

823A
Mitochondrial-targeted mRNAs as a novel gene therapy for encephalomyopathies. **Nicole Kotchey[1,2], Michael Palladino[1,2].** 1) Department of Pharmacology, University of Pittsburgh SOM, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh SOM, Pittsburgh, PA.

824B
α-synuclein Mediates Dopamine Homeostasis and Oxidative Stress Susceptibility in Drosophila. **Hakeem Lawal, Faiza Ferdousy, Glen Douglas, Zhe Wang, Janis O'Donnell.** Dept Biol, Univ Alabama, Tuscaloosa, AL.

825C
Behavioral analysis on a *D. melanogaster* Alzheimer disease model. **Matt B. Mahoney[1], Devin Keefe[1], Winnie Lee[1], Emily Lund[1], Jimmy Symonds[1], Akshay Yeshokumar[2], Lori A. Hrdlicka[1], François Huet[1], Phil O'Neil[1], Joost Schulte[3], Hsin-Pei Shih[1], Eric Sigel[1], Carol M. Singh[1], Christopher J. Cummings[1], James K. T. Wang[1], Michael Ahlijanian[1], Gerhard Köenig[1].** 1) Discovery, EnVivo Pharmaceuticals, Inc., Watertown, MA; 2) William E. Simon Graduate School of Business Administration, University of Rochester, Rochester, NY; 3) Picower Institute for Learning and Memory, MIT, Cambridge, MA.

826A
Improving scFv Intrabody suppression of Huntington Disease Pathology in a fly model. **Julie Mclear[1], Danielle Lebrecht[1], Allison Dumas[1], Anne Messer[1,2], William J. Wolfgang[1,2].** 1) Wadsworth Center, Albany, NY; 2) Dept. of Biomedical Sciences University at Albany, Albany, NY.

827B
Over and underexpression of nejire in a Drosophila model of Machado-Joseph Disease. **Ravi J. Nagraj, Brendan J. Thelen, John M. Warrick.** Department of Biology, University of Richmond, Richmond, VA.

828C
Atlastin is a novel ER protein involved in Golgi-ER transport. **Genny Orso[1,2], Jessica Tosetto[2,3], Diana Pendin[2], Andrea Daga[2,4].** 1) Dept of Genetics, Trinity College, Dublin, Ireland; 2) E. Medea Scientific Institute, Conegliano,Italy; 3) Dept of Pharmacology, University of Padova, Italy; 4) Dulbecco Telethon Institute, Italy.

829A
Characterization of genetic modifiers of *spastin* in Drosophila models of Autosomal Dominant- Hereditary Spastic Paraplegia. **Emily F. Ozdowski, Sophia F. Gayle, Nina T. Sherwood.** IGSP, Duke University, Durham, NC.

830B
Drosophila model of human inherited TPI deficiency glycolytic enzymopathy. **Michael Palladino[1,2], Adam Frank[1,2], Jacquelyn Seigle[1,2], Alicia Celotto[1,2].** 1) Department of Pharmacology University of Pittsburgh School of Medicine Pittsburgh, PA 15261; 2) Pittsburgh Institute for Neurodegenerative Diseases University of Pittsburgh School of Medicine Pittsburgh, PA 15260.

831C
Na+/K+ ATPase Alpha Isoform Specificity and Neuropathogenesis. **Michael Palladino[1,2], Rosie Miller[1,2].** 1) Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA.

832A
New continuous cell culture from RQ2 transgenic Drosophila as in vitro model of pathogenesis of human neurodegenerative diseases. **Dmitriy Panteleev[1], Abraham Grossman[4], Konstantin Pyatkov[3], Natalia Schostak[2], Elena Zelentsova[2], Boris Andrianov[1], Michael Evgen'ev[2].** 1) Mol Genet of Eukaryotes, Vavilov Institute of Gen Genet, Moscow, Russian Federation; 2) Engelhardt Inst Mol Biol, Moscow, Russian Federation; 3) California Inst Technology, Pasadena, CA 91125; 4) Q-RNA Inc., 3960 Broadway, New York, NY 10032.

833B
Developmental functions of two acyl-CoA synthetases, Bubblegum and Double Bubble, in Drosophila. **Anna Sivatchenko, Anthea Letsou.** Human Genetics, University of Utah, Salt Lake City, UT.

834C
A protective role for *PTEN induced putative kinase 1 (PINK1)* in a Drosophila model of Parkinson's disease. **Amy M. Todd, Brian E. Staveley.** Dept Biology, Memorial University of Newfoundland, St. John's, NL, Canada.

835A
Ataxin-1, a spinocerebellar ataxia type 1 disorder protein, causes cytotoxicity by perturbing nuclear receptor signaling. **Xin Tong, Chih-Cheng Tsai.** UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

836B
A role for the Batten disease gene Cln3 in endosomal trafficking at the synapse. **Richard Tuxworth, Guy Tear.** MRC Centre for Developmental Neurobiology, Kings College London, UK.

837C
SWISS CHEESE/NTE, a Gene Involved In Neural Integrity and Organophosphate Toxicity. **Jill S. Wentzell[1], Alexandre Bettencourt da Cruz[1], Max Mühlig-Versen[1], Paul Glynn[2], Doris Kretzschmar[1].** 1) Center for Research on Occupational and Environmental Toxicology, Oregon Health & Sciences University, Portland, OR; 2) MRC Toxicology Unit, University of Leicester, Hodgkin Building, Leicester LE1 9HN, United Kingdom.

838A
Deciphering the role of protein aggregation in polyglutamine pathogenesis in Drosophila. **Alan S. L. Wong[1,2], Edwin H. Y. Chan[1,2,3].** 1) Laboratory of Drosophila Research; 2) Molecular Biotechnology Program; 3) Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, China.

839B
Differential regulation of immune related genes in *D. melanogaster* Schneider 2 sells transfected with human *OTK18*. **Kimberly Carlson, Sarah Marshall, Cole Spresser.** Biology, University of NE at Kearney, Kearney, NE.

840C
Insulators flank the latency-associated transcript (LAT) promoter in HSV-1. **Qi Chen, Lan Lin, Sheryl Smith, Jing Huang, Shelley Berger, Jumin Zhou.** Wistar Institute, Philadelphia, PA.

841A
Anthrax EF and LF toxins affect several signaling pathways in Drosophila. **Annabel Guichard, Beatriz Cruz-Moreno, Abby Cooper, Ethan Bier.** Dept Biol, Univ California, San Diego, La Jolla, CA.

842B
Modeling hypercapnia in Drosophila: physiological and molecular effects of CO_2. **Iiro Helenius[1,2,5], Thomas Krupinski[1,5], Douglas Turnbull[3], Neal Silverman[4], Eric Johnson[3], Jacob Sznajder[2], Greg Beitel[1].** 1) BMBCB Department, Northwestern University, Evanston, IL; 2) Division of Pulmonary and Critical Care Medicine, Northwestern University, Chicago, IL; 3) Institute of Molecular Biology, University of Oregon, Eugene, OR; 4) Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA; 5) Co-first authors.

843C
Altered respiration in the Drosophila Bang-sensitive paralytic mutant *easily-shocked*. **Daniel Kuebler, Brian Burke.** Department of Biology, Franciscan University of Steubenville, Steubenville, OH.

844A
Mapping the stress response in the Drosophila brain. **Wendi Neckameyer.** Dept Pharmac & Physiol Sci, St Louis Univ Medical Ctr, St Louis, MO.

845B
Obesity and regulation of energy homeostasis in *D. melanogaster*. **Tania Reis, Iswar Hariharan.** MCB, UC Berkeley, Berkeley, CA.

846C
Functional analysis of EFHC1, a gene involved in Juvenile Myoclonic Epilepsy, in Drosophila. **Maria Giovanna Rossetto[1,2,3], Genny Orso[2,4], Erica Zanarella[2,3], Stefano Casonato[3], Andrea Daga[2,3].** 1) Pharmacology, University of Padova, Padova, ItalyT; 2) E. Medea Scientific Institute, Conegliano, Italy; 3) Dulbecco Telethon Institute, Italy; 4) Department of Genetics, Trinity College, Dublin, Ireland.

847A
D. melanogaster genetic based resistance and susceptibility to Bacillus cereus. **Tiffany E. Schwasinger, Wanda Layman, Lawrence Harshman.** Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

848B
A Drosophila model of Spinal Muscular Atrophy reveals a function for SMN in striated muscle. **Rajendra Thimmappaiaha, Michael Walker, Graydon Gonsalvez, Karl Shpargel, A. Gregory Matera.** Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

Physiology and Aging

849C
Life span extension by diet restriction and insulin/IGF signaling: Observations of mutual dependence and independence. **Kyung-Jin Min, Rochele Yamamoto, Marc Tatar.** Department Ecology and Evolutionary Biology, Brown University, Providence, RI.

850A
chico rescues mitochondrial defects in Drosophila longevity extension under dietary restriction. **David Rand, Rebecca Wagaman, Jeffrey Hofmann.** Ecology & Evolutionary Biol, Brown Univ, Providence, RI.

851B
Dynamics and age-dependence of resistance to environmental stresses in diet restricted Drosophila. **Isabell J. Scherer[1], Dae-Sung Hwangbo[1], Joep M. S. Burger[1,2], Daniel E. L. Promislow[1].** 1) Department of Genetics, University of Georgia, GA; 2) Department of Biology, University of Fribourg, Fribourg, Switzerland.

852C
Functional analysis of a juvenile hormone esterase binding protein in *D. melanogaster*. **Zhiyan Liu, Narinder Pal, Russell Jurenka, Bryony Bonning.** Department of Entomology and Program in Genetics, Iowa State University, Ames, IA.

Poster board is in bold above the title. See page 14 for presentation schedule. The first author is the presenter. Abstracts begin on page 77.

853A
The regulation of lifespan by *falafel*. **Brian Sage[1], Xi Lou[2], Li Qian[3], Rolf Bodmer[3], Heinrich Jasper[2], Marc Tatar[1].** 1) Dept Ecol & Evol Biol, Brown Univ, Providence, RI; 2) Dept of Biology, Univ of Rochester, Rochester, NY; 3) Center for Neurosciences and Aging, The Burnham Institute, La Jolla, CA.

854B
The dUSP36 Ubiquitin Specific Protease is required for larval growth and moulting. **Emmanuel Taillebourg, Marie-Odile Fauvarque.** Laboratoire Transduction du Signal EMI 104 INSERM CEA, Départment de Réponse et Dynamique Cellulaires, CEA-Grenoble, France.

855C
The Regulation of Lipid Storage by Insulin in *D. melanogaster*. **Justin DiAngelo[1], Morris Birnbaum[1,2].** 1) Dept Medicine, Univ Pennsylvania, Philadelphia, PA; 2) Howard Hughes Medical Institute.

856A
Sympatric *D. simulans* flies with distinct mtDNA types show difference in mitochondrial metabolism. **Subhash D. Katewa[1,2], J. William O. Ballard[1].** 1) Ramaciotti Centre for Gene Function Analysis, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia; 2) Department of Radiation Oncology, University of Iowa, Iowa City, IA 52242.

857B
Screen for genes controlling appetite and adiposity reveals genes that potentially effect lifespan. **Sergiy Libert, Emmeline Peng, Jessica Zwiener, Danielle Skorupa, Scott Pletcher.** Dept CMB, Baylor Col Medicine, Houston, TX.

858C
Processing of SREBP in Drosophila lacking Scap. **Krista Matthews, Robert Rawson.** Molecular Genetics, Univ Texas SW Medical Ctr, Dallas, TX.

859A
Roles for the DHR96 nuclear receptor in lipid metabolism and the starvation response. **Matt Sieber, Carl Thummel.** Human Genetics, University of Utah, Salt Lake City , UT.

860B
The Impact of SIR2 and Novel Candidate Genes on Triglyceride Homeostasis. **Danielle Skorupa[1], Beverly Patuwo[2], Sergiy Libert[1], Jessica Zwiener[1], Pletcher Scott[1].** 1) Huffington Center on Aging, Baylor College of Medicine, Houston, TX; 2) Center for Educational Outreach, Baylor College of Medicine, Houston, TX.

861C
Alterations in the HIF signalling pathway in median neurosecretory cells induces a diabetic phenotype in *D. melanogaster*. **Cathy Slack[1], Jake Jacobson[1], Colin Selman[2], Dominic J. Withers[2], Linda Partridge[1].** 1) Department of Biology, University College London, London, UK; 2) Centre for Diabetes and Endocrinology, Department of Medicine, Rayne Institute, University College London, London, UK.

862A
Cloning and characterization of *lot's wife (lwf)*, a mutation that disrupts food processing and digestion. **Edward M. Blumenthal.** Dept Biol Sci, Marquette Univ, Milwaukee, WI.

863B
Rearing media as a variable in *D. melanogaster* fecundity: an activity to introduce scientific methods of inquiry to biology students. **Darby Carlson[1], Laura Wollard[1,2], Benjamin Klein[1], Kimberly Carlson[1].** 1) Biology, University of Nebraska at Kearney, Kearney, NE; 2) Winfield Middle School, Winfield, KS.

864C
Effects of the antioxidant properties of blueberries on morality rates and *INDY* gene expression in Drosophila. **Kimberly Carlson, Jenna Derr.** Biology, University of Nebraska at Kearney, Kearney, NE.

865A
The Regulation of Lifespan, Fecundity and Other Phenotypes in Drosophila by DILP-producing Median Neurosecretory Cells of the Brain. **Susan J. Broughton, Cathy Slack, Timothy Bass, Nazif Alic, Jake Jacobson, Tomoatsu Ikeya, Anna Maria Tommasi, Linda Partridge.** Department of Biology, University College London, London, UK.

866B
Expression analysis of Cytochrome P450s in *D. melanogaster*. **Henry Chung, Tamar Sztal, Lee Willoughby, Chris Lumb, Mohan Sridar, Philip Batterham, Phillip Daborn.** Department of Genetics, University of Melbourne, Melbourne, Victoria, Australia.

867C
Lifespan extension by anti- diabetic drug metformin in Drosophila. **Dae-Sung Hwangbo[1], Kyung-Jin Min[2], Ho-Jin Koh[3], Laurie J. Goodyear[3], Marc Tatar[2], Daniel Promislow[1].** 1) Department of Genetics, University of Georgia, Athens, GA; 2) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI; 3) Research Division, Joslin Diabetes Center and Department of Medicine, Harvard Medical School, Boston, MA.

868A
Drosophila life span: genetic background, sex specificity, mating and social status. **Konstantin Iliadi, Natalia Iliadi, Gabrielle Boulianne.** The Hospital for Sick Children, Toronto,Canada.

869B
Identification of Delayed Aging of Negative Geotaxis Mutants. **Melanie Jones, Michael Grotewiel.** Dept. of Human Genetics, VCU, Richmond, VA.

870C
The sensory influence on lifespan appears to be conserved in Drosophila. **Ivan Ostojic[1], Werner Boll[2], Joy Alcedo[1].** 1) Growth control, Friedrich Miescher Institute, Basel, CH; 2) Institute of Molecular Biology, University of Zurich, Zurich, Switzerland.

871A
Evaluation of *foxo* activity during nutritional stress and development. **Jennifer D. Slade, Jody-Lynn E. Rotchford, Brian E. Staveley.** Biology, Memorial University, St. John's, Newfoundland, Canada.

872B
Changes in male germline stem cell cycle activity during aging in wild type and long-lived flies. **Matthew Wallenfang[1], Renuka Nayak[2], Karina Rodriguez[1], Steve DiNardo[2].** 1) Dept of Biological Sciences, Barnard College, New York, NY; 2) Dept of Cell and Dev Biology, Univ of Pennsylvania, Philadelphila, PA.

873C
Effects of oxidative damage on male germline stem cell cycle activity. **Tarnima Ahamed, Khadeejah Bari, Christine Chang, Matthew Wallenfang.** Dept of Biological Sciences, Barnard College, New York, NY.

874A
Morphometric analysis of the loss of CNS neurons, an age-associated neuropathology in Drosophila. **Kristopher Beckwith[1], Kebreten Manaye[2], Atanu Duttaroy[3].** 1) Human Genetics, Howard University, Washington, DC; 2) Dept of Physiology and Biophysics; 3) Dept of Biology, Howard University.

875B
Maternal protection against oxidative damage is offered through mitochondrial superoxide dismutase (MnSOD). **Amy Belton, Renee Forde, Atanu Duttaroy.** Dept Biol, Howard Univ, Washington, DC.

876C
Functional Study of *D. melanogaster* Omega-class Glutathione S-Transferase (DmGSTO). **Kiyoung Kim, Jaekwang Kim, Hyunsuk Suh, Songhee Kim, Jeongbin Yim.** Laboratory of Biochemistry, School of Biological Sciences, Seoul National University, Seoul, Korea.

877A
Neuronal expression of *jafrac1*, a Drosophila homolog of *hPrxII*, extends lifespan and rescues oxidative stress induced lethality. **Kyu-Sun Lee[1], Dong-Seok Lee[2], Sung-Kyu Ju[1], Kweon Yu[1].** 1) Development/Differentitation, KRIBB, Daejeon, Korea; 2) Division of Animal Science, Kaangwon University, Chuncheon, Korea.

878B
Graded Reduction of Manganese Superoxide Dismutase Causes a Proportional Acceleration of Functional Aging. **Ian Martin[1], Michael Grotewiel[1], Atanu Duttaroy[2].** 1) Dept Human Genetics, VCU, Richmond, VA; 2) Biology Department, Howard University, Washington, DC.

879C
Modifying metabolism of lipid peroxidation in Drosophila muscle by transgenic expression of mGSTA4-4 with Mhc-Gal4 driver extends life span. **Ashis K. Mondal, Sharda P. Singh, Kumar Chandra-Kuntal, Chhanda Mondal Ghosh, John J. Thaden, Robert J. Shmookler Reis, Ludwika Zimniak, Helen Beneš, Piotr Zimniak.** Univ. of Arkansas for Med Sciences & VA Hospital., Little Rock, AR.

880A
The octopamine receptor OAMB is required in the oviduct epithelium for ovulation of *D. melanogaster*. **Hyun-Gwan Lee, Kyung-An Han.** Department of Biology and Intercollege Graduate Degree Program in Genetics, Pennsylvania State University, University Park, PA 16802.

881B
Investigation of potential tyrosine transporters in the Malpighian tubule. **William F. Mueller, Edward M. Blumenthal.** Dept. Biol. Sci., Marquette University, Milwaukee, WI.

882C
Cardiac functional decline with age is dependent upon regulation of 4EBP activity in the myocardium. **Robert Wessells[1], Michael Hayes[1], Rolf Bodmer[2].** 1) Dept Intnl Med/ Geriatrics, Univ Michigan, Ann Arbor, MI; 2) The Burnham Institute La Jolla, CA.

883A
Genome wide RNAi screen identifies genes related to the insulin pathway as regulators of the transcriptional response to hypoxia. **Andres Dekanty, Lazaro Centanin, Pablo Wappner.** Fundación Instituto Leloir, Buenos Aires, Argentina.

884B
Mating increases starvation resistance and fat reserves in *D. melanogaster* females. **Jadwiga Giebultowicz[1], Brandy Rush[1], Jessica Bruer[1], Robin Roshe[2], Michael Wells[2].** 1) Dept Zoology, Oregon State Univ, Corvallis, OR. 97331; 2) Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ 85721.

885C
Immune system efficiency is increased in long lived mutants *puc* and *chico* but is unaffected by dietary restriction. **Sergiy Libert, Jessica Zwiener, Yufang Chao, Scott Pletcher.** Dept CMB, Baylor Col Medicine, Houston, TX.

886A
NfκB is a mediator of trade-offs between longevity and pathogen resistance in *D. melanogaster*. **Sergiy Libert, Yufang Chao, Xiaowen Chu, Scott Pletcher.** CMB, MHG, Baylor College of Medicine, Houston, TX.

887B
Response to selection for Oxidative Stress using *D. melanogaster*. **Devarati Mukherjee, Yue Wang, Mei-Hui Wang, Wanda Layman, Lawrence Harshman.** School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE.

888C
Role of the coactivator MBF1 in stress and aging. **Jan Rynes[1], Marek Jindra[2].** 1) Molecular Biology, University of South Bohemia, Budweis, Czech Republic; 2) Genetics, Biology Center ASCR, South Bohemia, Budweis, Czech Republic.

889A
Deletion of the Drosophila homologue of mammalian Herp decreases fly survival in response to ER stress. **Nikolaos A. Tountas, Mark E. Fortini.** LCDB, NCI-Frederick, Frederick, MD.

ABSTRACTS
Introduction

Abstracts are divided into two sections: platform and poster presentations. In each section, the number located above the title identifies the abstract in all other listings in this book.

Platform Presentations
Abstracts for platform presentations appear first and are organized chronologically by day and presentation. These abstracts are numbered consecutively from **1 through 156.**

Topic	Abstract #s	Beginning page #
Immune System and Cell Death	1-8	79
Organogenesis	9-16	82
Neurophysiology and Behavior	17-24	85
Evolution and Quantitative Genetics	25-31	88
Regulation of Gene Expression	32-38	91
Cytoskeleton and Cell Biology	39-45	94
Evolution and Quantitative Genetics	46-52	97
Regulation of Gene Expression	53-59	100
Cytoskeleton and Cell Biology	60-66	103
Gametogenesis and Sex Determination	67-74	106
Drosophila Models of Human Diseases	75-82	109
Signal Transduction I	83-90	112
Pattern Formation I	91-97	115
Cell Division and Growth Control	98-104	118
Signal Transduction II	105-111	121
Pattern Formation II	112-118	124
Cell Division and Growth Control	119-125	127
Techniques and Genomics	126-132	130
Genome and Chromosome Structure	133-140	133
Neurogenetics and Neural Development	141-148	136
Physiology and Aging	149-156	139

Poster Presentations
All posters will be displayed from Wednesday, March 7, through Saturday, March 10, in Franklin Hall on Level Four of the Philadelphia Marriott Downtown. Authors are expected to be present at their boards according to the following schedule:

Thursday: 2:00 pm–3:00 pm: even-numbered posters
3:00 pm–4:00 pm: odd-numbered posters
8: 00 pm–9:00 pm: “A” posters
9:00 pm–10:00 pm: “B” posters
10 pm –11:00 pm: “C” posters

Friday: 8:00 pm–9:00 pm: “C” posters
9:00 pm–10:00 pm: “B” posters
10:00 pm–11:00 pm: “A” posters

Saturday: 1:30 pm–2:30 pm: odd-numbered posters
2:30 pm–3:30 pm: even-numbered posters
8:00 pm–11:00 pm: open poster viewing
(authors not required to be present)

Abstracts for poster presentations are consecutively numbered from **157A through 889A** and organized as follows:

Topic	Abstract #s	Beginning page #
Cell Division and Growth Control	157A-218B	142
Cytoskeleton and Cellular Biology	219C-281B	163
Genome and Chromosome Structure	282C-328A	184
Regulation of Gene Expression	329B-406A	200
Signal Transduction	407B-448A	226
Pattern Formation	449B-498C	240
Gametogenesis and Sex Determination	499A-538A	257
Organogenesis	539B-564C	271
Neurogenetics and Neural Development	565A-603C	280
Neural Physiology and Behavior	604A-651C	293
Evolution and Quantitative Genetics	652A-722B	309
Immune System and Cell Death	723C-763A	333
Techniques and Genomics	764B-791B	347
Drosophila Models of Human Diseases	792C-848B	357
Physiology and Aging	849C-889A	376

Note: Late abstracts will be listed in the program addendum and will be available for viewing in the rear portion of Franklin Hall.

1

Epidemiology of bacterial disease in wild *Drosophila melanogaster*. Punita Juneja, Brian P. Lazzaro. Department of Entomology, Cornell University, Ithaca, NY.

Drosophila melanogaster has successfully been used to dissect the mechanisms of innate immune response to bacterial infection. The identification and characterization of the genetic basis of the immune response also makes it a powerful tool for studying the evolution of immune response. However, relatively little is known about the bacteria that infect flies in nature. To better understand the epidemiology of diseases that are likely to shape the evolution of antibacterial defenses, I have undertaken a survey of bacterial infections in wild populations of *D. melanogaster* in New York State. The aim of this study is to understand the rates, distributions, and identities of bacterial infections in the wild. To capture both culturable and non-culturable bacterial infections, I have developed a technique for amplifying and identifying bacteria directly from the hemolymph of wild-caught flies. This technique uses a nested PCR approach utilizing universal bacterial 16S rDNA primers followed by analysis of terminal restriction fragment length polymorphism (t-RFLP) patterns. By combining culture-dependent and culture-independent methods, I hope to capture most of the important bacterial species that naturally infect flies. In two years of sampling, I have found that there is seasonal variation in the rates and identities of infections, and that many infections are from a diverse array of opportunistic pathogens. Characterization of these bacteria will help us better understand the natural infections that shape the insect antibacterial immune response.

2

The relationship between immunity and microbial community richness across natural populations of *Drosophila melanogaster*. Vanessa Corby-Harris, Daniel E. L. Promislow. Dept Genetics, Univ Georgia, Athens, GA.

Bacteria are ubiquitous in natural insect host populations. Over evolutionary time, the associations between insect hosts and their bacteria may influence the evolution of the host immune response. However, the influence that entire microbial communities have on host resistance over ecological time is poorly understood. Specifically, how does the structure and diversity of the microbial communities associated with insect hosts affect host resistance to a novel bacterial challenge? Here, we describe experiments designed to measure the relationship between resistance to bacterial infection and species richness of the bacterial community that naturally associate with populations of *Drosophila melanogaster*. These two traits were measured in ten host populations collected along the East Coast of the United States. To determine resistance, host populations were infected with either *Pseudomonas aeruginosa* or *Lactococcus lactis* and monitored for survival post-inoculation. In addition, we used a metagenomic approach to determine the number of species associating with these ten host populations in nature. Last, we determined whether there was a significant relationship between host resistance and microbial species richness. Such information furthers our understanding of the ecological determinants of natural variation in levels of resistance. In addition, our results should pave the way for more ecologically relevant studies of the forces that determine variation in host resistance.

3

The Drosophila immune response exhibits specificity and memory. Linh Pham, Marc Dionne, David Schneider. Dept Microbiology & Immunology, Stanford University, Stanford, CA.

By definition, innate immunity lacks adaptive characteristics, but we show that priming *Drosophila melanogaster* with a sublethal dose of *Streptococcus pneumoniae* protects against an otherwise-lethal second challenge of *S. pneumoniae*. This protective effect is specific for *S. pneumoniae* and exhibits memory. The mechanism underlying this effect requires phagocytes and the Toll pathway. However, activation of the Toll pathway is not sufficient for priming-induced protection. This work contradicts the convention that insect immune responses lack specific memory and will promote the search for similar responses overlooked in organisms with an adaptive immune response.

4

Using Drosophila to uncover host factors involved in viral pathogenesis. Sara Cherry, Spencer Shelly, Terri Moser, Marta White. Department of Microbiology, Penn Genomics Institute, University of Pennsylvania, Philadelphia, PA.

The identification and study of host factors involved in viral infection and replication reveals fundamental insight into cell biological processes and is critical to overcoming human viral diseases. However, the study of host-virus interactions has been hampered by the dearth of host-pathogen systems amenable to genetic screening, and lack of in vivo animal models suitable for rigorously characterizing interactions between the virus and host. To address these issues, we have developed novel, high-throughput approaches that combine functional genomics with bioinformatics in the model organism Drosophila, to define and characterize host factors that regulate pathogenesis—including both factors hijacked by the virus for replication, as well as those used by the host to combat the viral invader. Genome-wide RNAi screens coupled with forward genetics in adult flies make this model organism ideal for unbiased interrogation of cellular factors central to pathogenesis. Indeed, we have infected Drosophila cells with viruses from diverse viral families of central importance to human disease. Due to the variety of our query pathogens, our analyses are likely to uncover a broad range of replicative requirements and host counter-measures. Through these investigations, we will gain a comprehensive understanding of the interplay between host and pathogen in a complex and dynamic setting, including identification of common subversion strategies and unique adaptations of individual pathogens. We will discuss our new findings using these strategies.

5

Functional genomics of stress-induced apoptosis. Su Kit Chew, Kristi R. Pogue, John M. Abrams. Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX.

Genes encoding determinants of programmed cell death (PCD) are well conserved across diverse phyla. In the fly, initiation of apoptosis involves the apoptosome protein complex comprising Dronc and Dark - homologues of Ced3/Ced4 (*C. elegans*) and Casp9/Apaf1 (humans). In previous studies, we applied null mutations and RNAi to expose fundamental requirements for both *Dronc* and *Dark* in stress-induced apoptosis. We exploited these findings to establish a platform for discovering novel determinants of cell death. We are using cell culture models of stress-induced apoptosis together with RNAi libraries to systematically interrogate the genome for effectors of apoptosis under comparative conditions. Our strategy distinguishes functional networks engaged during global cytotoxic stress from those initiated by a direct point activation of the core apoptotic machinery. As the founding screen in this effort, we used a small molecule mimetic of the pro-apoptotic protein Reaper (Smac in humans) as a point apoptosis activator to identify targets that prevent apoptosis when silenced. Reaper/Smac are antagonists of the inhibitors of apoptosis proteins (IAPs) that directly de-repress caspases, representing a point activator of apoptosis. From this screen, we recovered expected landmark genes in the apoptotic machinery such as *Dronc*, *Dark*, *Drice* (effector caspase), as well as a small collection of novel targets. Validation of these candidate genes is underway using cell culture systems and *in vivo* models. Our strategy will achieve two objectives. First, we will improve our understanding of how stress-signalling pathways converge upon the apoptosome. Secondly, we will identify novel modulators and effectors of the apoptosis machinery.

6

Live Imaging Programmed Cell Death in the Extraembryonic Amnioserosa. Bruce Reed[1], Nilufar Mohseni[1], Howard Lipshitz[2,3]. 1) Department of Biology, University of Waterloo, 200 University Ave. W., Waterloo, Ontario N2L 3G1, Canada; 2) Program in Developmental Biology, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; 3) Department of Molecular and Medical Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.

The amnioserosa (AS) is an extraembryonic epithelium that plays a role in germ band retraction and dorsal closure. Being extraembryonic, the AS is eliminated by programmed cell death during development. Using a live imaging based approach, we have made a detailed examination of the degeneration and death of the AS. As has been previously noted, a small percentage of AS cells leave the epithelium by basal extrusion during mid to late dorsal closure stages (Kiehart et al., J. Cell Biol., Apr 2000; 149: 471 - 490.). Once extruded, we find these cells rapidly display hallmarks of apoptosis, including nuclear fragmentation, membrane blebbing, and rapid removal by either macrophage or yolk engulfment. Strikingly, we find that AS cell extrusion is virtually absent in mutant backgrounds that are unable to execute apoptosis. Following dorsal closure, the bulk of the AS dissociates and is rapidly engulfed by macrophages. This tissue dissociation, like extrusion, is also inhibited in cell death defective mutant backgrounds. We have also found that ectopic expression of activated insulin receptor in the amnioserosa delays AS degeneration, suggesting that autophagy as well as apoptosis are active in AS programmed cell death. We will present data addressing the relationship between apoptosis and autophagy in regulating programmed cell death in this tissue.

7

The leucine zipper transcription factor Bunched regulates cell survival. Xiaodong Wu[1], Megumu Mabuchi[1], Silvia Gluderer[3], Erick Morris[2], Hugo Stocker[3], Nick Dyson[2], Ernst Hafen[3], Laurel Raftery[1]. 1) Cutaneous Biology Research Center, Massachusetts General Hosp, Charlestown, MA; 2) MGH Cancer Center, Massachusetts General Hosp, Charlestown, MA; 3) Zoological Inst., Univ. Zurich, Zurich, Switzerland.

Programmed cell death plays important roles in development. Many of the factors that have functions in tissue organization or patterning also have a role in regulating cell death. However, the downstream effectors that regulate cell death are not well understood. Here we show that *bunched* (*bun*), a downstream target of EGF and *dpp* pathways, promotes cell survival in follicle cells. Three transcripts are expressed from alternative promoters in *bun*, encoding proteins in the TSC-22/DIP/Bun family of leucine zipper transcription factors. We focused on BunA, a 1212 amino acid polypeptide with similarities to the large isoform of mammalian TSC-22. *bun* mutations enhanced a small eye phenotype associated with expression of apoptosis-inducers Rpr, Hid or Grim, suggesting that *bun* promotes cell survival. In the ovary, *bunA* mutant follicle cells had an increased frequency of apoptosis compared to wild type clones. *bunA* mutant follicle cells showed variably prolonged expression of Fas3, a marker for precursor follicle cells. This suggests that *bunA* mutant cells may be dying as a result of a defect in cell fate specification. Together, our data suggest that Bun transcription factors integrate patterning and growth factor signals to promote cell survival in at least two *Drosophila* tissues.

8

Determining the role of the Drosophila Bcl-2 proteins in mediating autophagy and apoptosis. Jessica P. Monserrate, Yenyun Chen, Evgueni A. Sevrioukov, Carrie B. Brachmann. Dev Cell Biol, Univ California, Irvine, Irvine, CA.

In the mammalian system, Bcl-2 proteins are essential mediators of apoptosis. The constant input of extracellular and intracellular cues require Bcl-2 proteins to have a fine-tuned mechanism for sensing cellular stress. Depending on how cellular cues are integrated, Bcl-2 proteins can tip the balance toward cell death or to maintain cell survival. Recent evidence has revealed a functional role for mammalian Bcl-2 in the regulation of autophagy, a pro-survival cellular mechanism. Autophagy leads to the bulk degradation of cytoplasmic material within lysosomes for recycling. We have generated mutants in both Drosophila Bcl-2 homologs and have examined their autophagic response to starvation conditions. The wild-type response to starvation is a transient upregulation of autophagy. In contrast, Buffy mutant (dbuffy[H37]) larvae, demonstrate a much quicker and greater autophagic response. Ultrastructural analysis confirmed the presence of more and larger autolysosomes within the fat body of starved mutant third instars. The opposite response is observed in larval fat body over expressing Buffy, thus we conclude that Buffy acts as a potent inhibitor of starvation-induced autophagy. Co-immunoprecipitation of the Drosophila pro-autophagic homolog of Beclin, Atg6, with Buffy was also observed, consistent with the interaction observed between Bcl-2 and Beclin. To determine whether larvae demonstrate an apoptotic response to starvation, we investigated caspase activity levels in starved larvae. We find altered caspase activity in starved debcl, buffy and the debcl buffy double mutants relative to starved wild-type larvae. This supports a hypothesis that the Bcl-2 proteins mediate a delicate interplay between apoptosis and autophagy in the starved animal. By uncovering the mechanisms of how Bcl-2 proteins regulate these two very distinct cellular responses, we will learn how to coax cells into taking a desired course. This knowledge will aid in the development of alternative therapeutics for combating illnesses such as cancers and many neurodegenerative diseases.

9

Homeostatic control of germ cell numbers in the Drosophila gonad. Lilach Gilboa[1], Ruth Lehmann[1,2]. 1) Developmental Genetics, NYU Med Ctr, Skirball Inst, New York, NY; 2) Howard Hughes Medical Institute.

Regulation of the relative amounts of cells within a growing organ is important for its normal development and function. Here we describe a feedback mechanism between primordial germ cells (PGCs), which are the precursor cells for germ line stem cells (GSCs), and somatic cells in the Drosophila ovary. This feedback loop is used to sense the number of PGCs and to ensure that their number is sufficient to occupy all the stem cell niches that form during ovarian development.

Oogenesis in Drosophila depends on GSCs that reside within somatic niches. These niches form at the end of larval development. PGCs proliferate during larval development and by the end of that time enough PGCs exist to occupy all the newly formed somatic niches. We show that when fewer PGCs are incorporated into the larval ovary, they divide faster, to compensate for their initial low numbers, and by the end of larval development their numbers are sufficient to occupy all the somatic niches. We discovered that the control of PGC numbers during larval development is achieved via a feedback loop between PGCs and intermingled cells (ICs), a special somatic cell population that contacts PGCs. The feedback loop is composed of a positive and a negative signal. PGCs express the Epidermal Growth Factor (EGF) ligand Spitz, which is required for IC survival. In return, ICs inhibit PGC proliferation. When fewer PGCs are incorporated into the ovary, fewer ICs survive. Consequently, less inhibition of PGC proliferation allows for rapid PGC divisions and compensation for the initial low numbers.

The properties of this feedback loop make it ideal to coordinate the growth of different cell types within an organ and to control homeostasis.

10

Transcriptional Control of Apical Mechanics During Tube Morphogenesis. Bilal E Kerman[1,5], Alan M. Cheshire[1,2,5], Warren R. Zipfel[3], Monn Monn Myat[4], Alexander A. Spector[2], Deborah J. Andrew[1]. 1) Dept. of Cell Biology, Johns Hopkins SOM, Baltimore, MD; 2) Dept. of Biomed. Engineering, Johns Hopkins SOM, Baltimore, MD; 3) Developmental Resource for Biophysical Imaging Opto-Electronics, Cornell University, Ithaca, NY; 4) Dept. of Cell and Dev. Biology, Weill Medical College of Cornell University, New York, NY; 5) These authors contributed equally.

ribbon (*rib*), which encodes a BTB/POZ-domain transcription factor is required for normal tracheal and salivary gland formation. Here, we show that Lola-like (Lolal) is a novel partner for Rib; *lolal* mutations cause mild *rib*-like defects, genetically interact with *rib*, and result in loss of Rib nuclear localization. Rib and Lolal enhance transcription of Crumbs (Crb), a transmembrane protein with key roles in apical membrane dynamics. Crb binds Moesin (Moe), an ERM family protein that cross-links the apical actin network. In *rib* mutants, higher levels of phosphorylated (active) Moe accumulate in apical regions of cells and the expression of a phosphomimetic form of Moe mimics *rib* phenotypes. High-resolution live imaging reveals that early phases of salivary gland and trachea morphogenesis are normal but slower in *rib* mutants. *rib* mutant cells extend properly directed but smaller lamellipodia. *rib* salivary glands fail to turn posteriorly upon reaching the visceral mesoderm and, upon continued cell internalization, the lumen widens and buckles. The dorsal trunk lumen also fails to elongate although the basal surfaces contact. Our findings suggest that Rib increases apical elasticity by increasing apical membrane generation through Crb and decreasing F-actin cross-linking through Moe. A finite element model predicts that decreases in apical elasticity in *rib* mutants have little effect on lamellipodial extension but a major impact on lumenal extension. The model fits observed cell shape changes and predicts that the *rib* apical surface is at least 10 times stiffer than wild type. Altogether, we provide a link between molecular components and apical mechanics from transcription to the cell surface.

11

Communication and rearrangement among tracheal epithelial cells during branching morphogenesis. Amin Ghabrial, Mark Krasnow. Biochemistry and HHMI, Stanford University, Stanford, CA.

In response to Branchless-FGF secreted by neighboring cells, a group of 5-7 cells bud from the epithelial monolayer that lines each tracheal pit to give rise to a dorsal branch of the tracheal system. These cells organize into a tube as they migrate towards the source of the FGF signal. Whether every cell, or which cells, in the migrating branch must see the Branchless-FGF signal has not been known. Further, how individual cells determine their position within the dorsal branch has not been clear. In a series of genetic mosaic experiments, we demonstrate that there are two functionally distinct classes of cells in the dorsal branch, leading cells that need to perceive the Branchless signal, and following cells for which signal reception is dispensable. Further we show that the dorsal branch cells compete for the lead positions, and that those cells with the highest FGF receptor activity assume the lead positions. We also show that Notch-mediated lateral inhibition, previously known to act in diversifying cell fates at branch tips, plays an earlier role in the competition for lead positions.

12

The Archipelago tumor suppressor protein regulates Drosophila tracheal development via the HIF-1α homolog Trachealess. Nathan T. Mortimer, Kenneth H. Moberg. Department of Cell Biology, Emory University, Atlanta, GA.

The *archipelago* gene (*ago*) encodes the F-box substrate specificity component of a conserved SCF-type ubiquitin ligase that inhibits cell proliferation in *Drosophila melanogaster* and suppresses tumorigenesis in mammals. Ago limits mitotic activity by targeting critical cell cycle and cell growth proteins (e.g. Cyclin E and Myc) for ubiquitination and subsequent degradation, but its role in other cellular processes has not been investigated. Here we show that *ago* also functions in the post-mitotic shaping of the *Drosophila* embryonic tracheal system by targeting the Trachealess (Trh) transcription factor, a member of the HIF-1α-related family of bHLH-PAS domain transcription factors. *ago* zygotic mutant embryos accumulate elevated levels of Trh and ectopically express the Trh target gene *breathless*, which encodes a Drosophila FGF receptor homolog, leading to inappropriate FGF pathway activation. Consistent with the central role this pathway plays in tubular morphogenesis in insects and mammals, *ago* zygotic mutant embryos display defects in tracheal branching that are phenocopied by activated *breathless*/FGF signaling and suppressed by alleles of *breathless*/FGF pathway components. At a molecular level, we find that *ago* is required for the developmentally regulated elimination of Trh in response to tracheal fusion cell-specific expression of the Dysfusion protein. The physical interaction between Ago and Trh is modulated in a Dysfusion dependent manner and *ago* mutations that disrupt the *in vivo* function are defective in binding to forms of Trh found in Dysfusion-positive cells. Additional work provides evidence of strong genetic synergy between *ago* and the tracheal regulator *dVHL*, the ortholog of the vertebrate Vhl tumor suppressor. These data identify a novel function for the *ago* tumor suppressor in regulating tracheal patterning via Trh and *breathless*/FGF signaling, and place *ago* within a genetic regulatory network that fulfills a conserved role in the morphogenesis of branched, tubular organs.

13

A hematopoietic niche defined by Antennapedia expression uses Hedgehog for the maintenance of blood cell precursors in Drosophila. Lolitika Mandal, Julian Agosto- Martinez, Cory Evans, Utpal Banerjee, Volker Hartenstein. Dept MCD Biol, Univ California, Los Angeles, Los Angeles, CA.90095.

The Drosophila lymph gland is a hematopoietic organ in which pluripotent blood cell progenitors proliferate and mature into differentiated hemocytes. Previous work has defined three domains, the medullary zone (MZ), the cortical zone (CZ), and the posterior signaling center (PSC), within the developing third-instar lymph gland . The MZ is populated by a core of undifferentiated, slowly-cycling progenitor cells, while mature hemocytes comprising plasmatocytes, crystal cells, and lamellocytes are peripherally located in the CZ. The PSC comprises a third region that was first defined as a small group of cells expressing the Notch ligand Serrate. We show that the PSC is specified early in the embryo by the homeotic gene Antennapedia (Antp) and expresses the signaling molecule Hedgehog. In the absence of the PSC or the Hedgehog signal, the precursor population of the MZ is lost as cells differentiate prematurely. We conclude that the PSC functions as a hematopoietic niche that is essential for the maintenance of blood cell precursors in Drosophila. Identification of this system allows the opportunity for genetic manipulation and direct in vivo imaging of a hematopoietic niche interacting with blood precursors.

14

Daughterless dictates Twist activity in a context dependent manner during somatic myogenesis. Ming-Ching Wong[1], Mary Baylies[2]. 1) Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Somatic myogenesis relies on the reiterative activity of the basic helix-loop-helix transcriptional regulator, Twist (Twi). We are interested in the regulation of Twi activity and how Twi directs multiple cell fate decisions over the course of mesoderm development. We have shown that Twi can form heterodimers with Daughterless (Da). We used transgenic flies carrying bHLH tethered dimers, and found, depending on tissue context, that Twi-Da heterodimers can repress (in mesoderm) or activate (in ectoderm) the myogenic cell fate. Domain mapping of Da revealed a transcriptional repression domain (REP domain), which, when deleted, causes Twi-Da heterodimers (Twi-Da$^{\Delta}$) to activate muscle gene expression in both the ectoderm and the mesoderm. Further analysis of Twi-Da$^{\Delta}$ revealed that the activity of this deletion construct is dependent on context and developmental timing. We observed that the expression of Twi-Da$^{\Delta}$ throughout the mesoderm resulted in the formation of ectopic muscles, but the maintained expression of Twi-Da$^{\Delta}$ disrupted myoblast fusion and muscle morphogenesis, processes that are required for muscle differentiation. In addition, the restricted expression of Twi-Da$^{\Delta}$ in specified muscle founder cells repressed myogenic differentiation. Taken together, these observations suggest that the repressive activity of Twi/Da heterodimers at early stages of mesoderm development (e.g. mesodermal subdivision) is dependent on the REP domain. However, during the later stages, which include founder cell specification and muscle differentiation, we found that Twi/Da repressive activity is no longer mediated through the REP domain, and Twi-Da$^{\Delta}$ acts to disrupt myogenesis. Therefore, we present evidence that the repressive activity of the Twi/Da dimer is dependent on the Da REP domain, and the activity of the REP domain is sensitive to tissue context and developmental timing. We speculate that similar mechanisms control Twi activity in vertebrate myogenesis and in cancer.

15

***Perdido* encodes a component of a protein complex required for muscle guidance in Drosophila embryonic muscles.** Beatriz Estrada[1], Stephen S. Gisselbrecht[1], Alan M. Michelson[1,2]. 1) Dept Medicine/Genetics, BWH/HMS, Boston, MA; 2) National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

The molecular mechanisms underlying muscle guidance and attachment to tendon cells are poorly understood both in vertebrates and in *Drosophila*. We have identified a novel gene, expressed in a subset of embryonic founder myoblasts, that is essential for *Drosophila* muscles to find their attachment sites; thus, we have named it *perdido* ("lost" in Spanish; *perd*). It encodes a conserved single-pass transmembrane cell adhesion protein that contains laminin globular extracellular domains and a small intracellular domain with a PDZ binding consensus sequence at the C-terminus. *In vivo* visualization of muscle guidance in both *perd* mutant embryos and dsRNA-injected embryos show that some ventral muscles fail to reach their attachment sites and instead form rounded multinucleated myotubes. Genetic interaction experiments done with a newly developed RNA interference assay combined with *in vivo* visualization of muscle development suggest that *perd* may be a ligand for the laminin binding αPS1-βPS integrin heterodimer, which is expressed in the tendon cells. We also have genetic and biochemical evidence indicating that *perd* is necessary to localize the muscle guidance PDZ protein DGrip to the plasma membrane. These results suggest that Perd forms an essential protein complex for muscle guidance by engaging the myotube with the tendon cell via an extracellular interaction with the tendon integrin complex and by an intracellular interaction with DGrip. The function of *perd* in muscle pathfinding resembles the role of its vertebrate orthologs NG2/MCSP in cell migration in the nervous system.

16

Requirement of the LIM homeodomain transcription factor Tailup for normal heart and hematopoietic organ formation in Drosophila. Ye Tao, Jianbo Wang, Tsuyoshi Tokusumi, Kathleen Gajewski, Robert Schulz. Department of Biochemisry & Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Dorsal vessel morphogenesis in Drosophila serves as a superb system in which to study the genetic and cellular bases of heart tube formation. We used a cardioblast-expressed Toll-GFP transgene to screen for additional genes involved in heart development and identified tailup as a locus essential for normal dorsal vessel formation. tailup encodes a LIM homeodomain transcription factor expressed in all cardioblasts and pericardial cells of the heart tube, as well as in associated lymph gland hematopoietic organs and alary muscles that attach the dorsal vessel to the epidermis. A transcriptional enhancer regulating expression in these four cell types was identified and used as a tailup-GFP transgene with additional markers to characterize dorsal vessel defects resultant from gene mutations. Two reproducible phenotypes were observed in tailup mutant embryos: hypoplastic heart tubes with misaligned cardioblasts, and the absence of most lymph gland and pericardial cells. Significant expansion of the lymph gland and abnormal morphology of the heart were found when tailup was over-expressed in the whole mesoderm. Tailup was shown to bind to two DNA recognition sequences in the dorsal vessel enhancer of the Hand bHLH transcription factor gene, with one site proven essential for the lymph gland, pericardial cell, and Svp/Doc cardioblast expression of Hand. Together, these results establish Tailup as a critical new transcription factor in dorsal vessel morphogenesis and lymph gland formation, and place this regulator directly upstream of Hand in these developmental processes.

17

PI3K regulates neuronal excitability and axonal growth and arborization via distinct effector pathways. Eric Howlett, William Lavery, Michael Stern. Biochemistry & Cell Biology, Rice University, Houston, TX.

The phosphatidylinositol 3-kinase (PI3K)/AKT pathway controls cellular survival and growth and has been implicated as a contributor to a wide variety of cancers. In the mouse CNS, activation of this pathway through loss of function mutations in the gene encoding PTEN, the phosphatase that opposes the effects of PI3K, results in increased neuronal arborization and neuronal hypertrophy; this system has been proposed to be a model for autism. PI3K has also been shown to induce synaptogenesis in Drosophila at both the larval neuromuscular junction (NMJ) and in the adult brain (Martín-Peña et al. J. Neurosci., Oct. 4, 2006. 26(40):10199-10208). Here we show that PI3K regulates motor axon diameter in the larval peripheral nerve: activation of this pathway via transgene expression increases axon diameter, whereas suppression of this pathway confers the opposite effect. Additionally, altered PI3K activity in motor neurons causes electrophysiological defects at the larval NMJ. In particular, activation of PI3K decreases neuronal excitability and both spontaneous and evoked transmitter release, whereas inhibition of PI3K confers the opposite phenotypes. Although effects of PI3K on neuronal growth have been previously shown to be mediated by the Tor pathway, we have found that the effects of PI3K on neuronal activity appear to be mediated by the transcription factor FOXO, which negatively regulates PI3K-induced transcription, and is inhibited by Akt-dependent phosphorylation. In particular, overexpression of FOXO in motor neurons increases neuronal excitability and partially suppresses the decrease in excitability conferred by PI3K. Our results suggest that the effects of PI3K on growth and activity are mediated by distinct effector pathways. These results provide a previously uncharacterized role for PI3K in regulating the relative excitability of neurons in vivo.

18

Peripheral multi-dendritic sensory neurons are necessary for locomotive pattern generation in Drosophila larvae. Wei Song, Maika Onishi, Lily Jan, Yuh-Nung Jan. HHMI, Dept. of Physiology and Biochemistry, UCSF, San Francisco, CA.

From breathing to walking, rhythmic movements encompass important physiological processes across the entire animal kingdom. The prevailing model for the neural basis of rhythmic movement is that central pattern generators (CPG), neuronal circuits within the CNS, generate the fundamental rhythmic motor pattern, while sensory feedback plays a modulatory role in shaping CPG-generated motor outputs. In contrast to this model, here we report that peripheral sensory neurons are necessary for the generation of the rhythmic locomotion behavior in *Drosophila* larvae. By conditionally silencing various subsets of *Drosophila* larval neurons, we identified the multiple dendritic (MD) neurons, a group of peripheral sensory neurons with distinct and diverse dendrite morphologies, as the ones indispensable for the generation of peristalsis and rhythmic crawling. Elimination of MD neuron function resulted in complete arrest of larval crawling. Furthermore, removal of MD neuron sensory inputs in third instar larvae disrupted rhythmic bursts of segmental nerve activities and prevented their propagation from one segment to the next. These findings reveal that sensory input serves as a critical component of the neuronal circuitry for rhythmic larval crawling; furthermore, they might suggest a muscular proprioceptive function for MD neurons. Our study provides a starting point for genetic and cellular analyses of the neuronal basis of sensory function in the generation of rhythmic behavior.

19

Odor coding and discrimination by the entire repertoire of larval receptors. Dennis Mathew, Scott A. Kreher, John R. Carlson. MCD Biology, Yale University, New Haven, CT-06511.

We have investigated fundamental principles of odor coding and odor discrimination by studying the functional properties of *Drosophila* larval odor receptors. In the larval olfactory system, an individual receptor transmits signals via a single neuron to a single glomerulus in the larval antennal lobe in the brain. Using an *in vivo* expression system (the “empty neuron” system), the response profile of each receptor of the entire olfactory receptor repertoire (25 receptors) of the larva has been tested to a panel of 27 odorants. The receptors vary in their breadth of tuning and response kinetics. Wild type larval behavior towards each odorant has also been measured. Behavioral responses to the odors range from strong attraction to repulsion. Having defined the sensory input and the behavioral output for each odorant, we have explored the relationship between the two. An n-dimensional “odor space” has been constructed in which each dimension represents the response of an individual receptor. We have found a relationship between the distance between two odors in this space and the ability of one odor to mask the other in a behavioral paradigm. We are currently expanding the odorant panel substantially and using computational algorithms to analyze relationships between the physiological response and the behavioral response. Preliminary results have already uncovered novel ligands for several larval receptors and interesting temporal patterns of neural-spike outputs, both of which may prove critical to understand odor coding. The results should also provide insights into the evolution of the receptor repertoire.

20

Identification and characterization of novel genetic pathways underlying feeding motivation in Drosophila. Benjamin Parrott[1,2], Ping Shen[1,2]. 1) Dept. of Cellular Biology, University of Georgia, Athens, GA; 2) Biomedical and Health Science Institute, University of Georgia, Athens, GA.

Aberrant eating behavior is a significant etiological component of obesity and diabetes. However, underlying molecular and cellular basis remains largely unknown. In previous studies, we have shown that neuropeptide Y (NPY)- and insulin-like systems positively and negatively regulate hunger-driven behaviors in both insects and mammals (Wu et al., 2005; PNAS 102(6):2141-6) . In natural environs, animals must adjust to changes in food source and availability in order to survive. We have shown that NPFR1, a receptor for NPY-like neuropeptide F (NPF), promotes intake of noxious or less accessible food in food-deprived fly larvae (Wu et al., 2005 Nature neuroscience 8: 1350-5). The NPFR1 neuronal activity may be directly regulated by the fly insulin-like signaling pathway. These results provide strong validation for the use of the genetically tractable Drosophila model to dissect molecular and neural mechanisms underlying feeding motivation. Here we report our efforts to uncover novel genes and molecular pathways critical for hunger-motivated behaviors. Using a high throughput assay, we have identified a number of putative genes that impact hunger-driven feeding activity in a dosage-dependent manner. Some of the candidate genes encode highly conserved proteins implicated in key signaling pathways, while the functions of others remain unknown. We are currently characterizing the physiological roles of these genes in transgenic animals.

21

Interactions between functionally coupled circadian neurons control temperature synchronization of Drosophila behavior. Patrick Emery, Ania Busza. Neurobiology, U.. Massachusetts Med. School, Worcester, MA.

Most animals rely on circadian clocks to synchronize their physiology and behavior with the day/night cycle. Light and temperature are the major physical variables that can synchronize circadian rhythms. While the effects of light on circadian behavior have been studied in detail in Drosophila, the neuronal mechanisms underlying temperature synchronization of circadian behavior have received much less attention. We found that temperature cycles synchronize and durably affect circadian behavior in Drosophila. This synchronization depends on the well-characterized and functionally coupled circadian neurons controlling the morning and evening activity under light/dark cycles: the M cells and E cells. Surprisingly, circadian neurons distinct from the M and E cells also contribute to the control of rhythmic behavior specifically under temperature cycles. Interestingly, although temperature synchronizes circadian behavior more slowly than light, this synchronization is considerably accelerated if the M-E cell neuronal network is disrupted. Thus, individual groups of circadian neurons are very sensitive to temperature cycles, but the neural network coupling the M and E cells restricts the pace of their synchronization. We propose that a stabilizing neural network connecting highly sensitive neurons is necessary for circadian behavior to be responsive to low amplitude temperature cycles while being resistant to abrupt weather changes.

22

A new behavior paradigm to study social behavior. Anne F. Simon, Evelyn Salazar, Man-Ting Chou, David E. Krantz. Psy. and Biobehavior. Sciences, UCLA , Brain Res Inst, Los Angeles, CA.

We are interested in determining the mechanisms by which we and other animals recognize, and respond to the presence of another similar organism. We have set up a new behavior assay to study social aggregation. In this assay, around 60% of wild-type flies are closer than 0.5 mm to one another. This repartition is not random, and is observed for both males and females. Social experience modifies this pattern of aggregation. Flies kept in isolation to be less close than flies kept in a socially enriched environment (40 flies in a vial). Virgin flies also tend be less close. To determine whether vision was one of the sensory modalities required for this behavior, we first tested a mutant affecting vision. Null mutants of the *white* gene have reported vision problems, but are not blind, as confirmed in the fast phototaxis assay. The *white* mutant flies also aggregate less (only 40% of them are within 0.5 mm). Tested in dark conditions, normal flies aggregate less than with light, and *white* mutants aggregate more. These modifications are such that under dark conditions, normal flies and *white* mutant aggregation patterns are not distinguishable anymore. Together, these results imply that: 1) vision is important for normal flies to aggregate, 2) the vision problems of the *white* mutants are responsible for their reduced aggregation, 3) vision is not the only sense required for this aggregation, as the flies, despite aggregating less, still aggregate, when having a vision defect, or being in the dark. We plan to test mutants defective in smell perception, and as well as candidate genes, such as *dVMAT*mutants, influencing the monoamine pathways. These data will provide a basis for a screen to genetically identify the genes required for the social response to the presence of another animal, and to map the neuronal circuitry responsible for social behavior in flies. Since many of the pathways for normal neural function and disease processes are conserved between Drosophila and humans, these data may be relevant to the genetic basis of social deficits in humans.

23

Specific subgroups of Fru^{M} expressing neurons control the sexually dimorphic patterns of aggression in *Drosophila melanogaster*. Yick-Bun Chan, Edward A Kravitz. Dept of Neurobiology, Harvard Medical School, Boston, MA.

Male and female *Drosophila melanogaster* show innate, sexually dimorphic patterns of aggression during fights. Certain behavioral patterns seen during male and female fights are similar, while others are selectively associated with male or female fights. We are interested in how the male- and female-selective patterns of fighting behavior get established in the nervous system. Accordingly, we used the *GAL4/UAS* system to feminize or masculinize neurons in fly brains via manipulation of the *transformer (tra)* gene. Using a pan-neural *elav-GAL4* driver in crosses with *UAS-tra* or *UAS-tra^{IR}*, we observe male patterns of fighting behavior in masculinized females and female patterns of aggression in feminized males. In an attempt to unravel the neural circuitry involved, we screened 60 *GAL4* lines showing different patterns of expression in CNS neurons, and identified five lines of interest. Expression of *tra^{IR}* in all five lines led to the appearance of male courtship behavior in females, but the patterns of aggression only were switched in one of these lines. The male forms of *fruitless* proteins (Fru^{M}) recently have been shown to be both necessary and sufficient to determine both who flies court and how they fight (Vrontou et al., Nat Neurosci 2006). We examined the expression patterns of Fru^{M} in pupal and adult brains in all of our experimental lines. Several clusters of neurons that normally express Fru^{M} in wild-type males showed no or reduced Fru^{M} staining in masculinized females that fought like females. In the one line in which females fought like males, however, we found normal numbers of these Fru^{M} staining neurons. This raises the possibility that different clusters of Fru^{M} positive neurons might be associated with courtship and aggression. In summary, our results show that by manipulating the transformer gene in male and female fly brains, we can both switch the sex-specific patterns of aggression, and demonstrate that specific subgroups of Fru^{M} expressing neurons might be involved in those switches. (Supported by NIGMS).

24

Odor-evoked activities in *Drosophila* mushroom bodies before, during and after odor-electric shock pairing. Akira Mamiya, Yalin Wang, Yi Zhong. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

In the olfactory avoidance learning, flies learn to avoid an odor that was presented together with electric shocks. The *Drosophila* mushroom body, a higher-order olfactory center, has been shown to play an important role in this learning and its output is necessary for the fly to avoid the odor that was paired with the shock. As an initial step in identifying the changes in neural activities that underlie the change in fly behavior after the learning, we monitored *in vivo* the odor-evoked activity in the input and output regions of the mushroom body before, during and after the odor-electric shock pairing. Activities of neurons were monitored indirectly by expressing G-CaMP, a genetically engineered calcium sensor, in mushroom body neurons using the GAL4-UAS system and recording the changes in its fluorescence using two-photon fluorescence microscope. We will present data on how odor-evoked activities in mushroom bodies change after pairing an odor with electric shocks, and discuss the possible roles of these changes.

25

Genomic analysis of adaptive differentiation between *Drosophila melanogaster* populations. Thomas Turner, Mia Levine, David Begun. Population Biology, Univ California Davis, Davis, CA.

Drosophila melanogaster shows clinal variation for several phenotypes, allozyme variants and inversions in North American and Australian populations. These observations, and the fact that most molecular markers show relatively little clinal variation in the US or Australia, suggest that clines are due to spatially varying selection rather than demographic history. To map differentiation throughout the genome, we hybridized DNA derived from temperate and subtropical population samples collected in the US and Australia to whole-genome tiling arrays. The dense genomic sampling of variants and relatively low linkage disequilibrium in *Drosophila melanogaster* enabled identification of dozens of differentiated regions spanning only one to several genes. Some, but not all, of these regions are differentiated on both continents, providing a genomic look at parallel adaptation to novel habitats. DNA sequencing of these regions generally confirmed that unusual regions inferred from chip hybridization signal show very high levels of differentiation in Northern and Southern populations. By comparing the functions of these differentiated genes, we are working to assemble a genomic perspective on adaptation and the maintenance of variation through spatially varying selection.

26

7 Mbp of Genomic Polymorphism in *D. melanogaster*. Michael E. Zwick[1], David Begun[2], David J. Cutler[4], Pieter De Jong[3], Maxim Koriabine[3], Charis Marston[2], Shoshona Lee[1], David Okou[1], Kazutoyo Osoegawa[3], Kristian Stevens[2], Janet A. Warrington[5], Charles H. Langley[2]. 1) Department of Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Section of Evolution and Ecology, University of California at Davis; 3) Children's Hospital Oakland Research Institute, Oakland, CA 94609; 4) McKusick-Nathans Inst of Genetic Medicine, Johns Hopkins Univ School of Medicine, Baltimore, MD 21287; 5) Affymetrix, Inc., Santa Clara, CA.

The goals of population genomics, such as characterizing phenotypic effects of DNA sequence variants and providing a mechanistic understanding of the evolutionary forces acting to shape global patterns of genome variation, require a complete description of DNA sequence variation, irrespective of frequency, in multiple genomes. Obtaining the vast quantities of DNA sequence data necessary for robust conclusions has only recently become feasible with the use of next-generation resequencing technologies. As a first step to obtain the complete sequence of 50 *D. melanogaster* genomes, we resequenced 7MB of genomic sequence from each of 46 *D. melanogaster* lines in two contiguous regions, 3.0 Mbp from the X (1911660 to 5005056, Rel. 4) and 3.3 from 2L (12537331 to 15942868, Rel. 4, see http://www.dpgp.org). Fosmid libraries from the lines were screened to obtain deep coverage of the two regions. Fosmid DNA isolates were pooled, fragmented, labeled, hybridized to custom Affymetrix resequencing arrays, stained, scanned and analyzed to yield greater than 90% high quality base calls. Preliminary analysis of these data, including the patterns of polymorphisms and linkage disequilibrium analyzed in terms of genomic annotations and divergence to closely related species will be presented. Our data demonstrate that obtaining the complete genome sequence from 50 Drosophila lines is technically feasible and will provide a valuable community resource to the broader Drosophila research community.

27

Large-scale turnover of functional transcription factor binding sites in *Drosophila*. Daniel Pollard[1,2], Alan Moses[1,2], Stewart MacArthur[1], David Nix[1], Venky Iyer[1,3], Xiao-Yong Li[1], Mark Biggin[1], Michael Eisen[1,2,3]. 1) Dept Genome Sci, LBNL, Berkeley, CA; 2) Biophys Grad Group, UC Berkeley, CA; 3) Mol Cell Bio Dept, UC Berkeley, CA.

The gain and loss of functional transcription factor binding sites has been proposed as a major source of evolutionary change in *cis*-regulatory DNA and gene expression. We have developed an evolutionary model to study binding-site turnover that uses multiple sequence alignments to assess the evolutionary constraint on individual binding sites, and to map gain and loss events along a phylogenetic tree. We applied this model to study the evolutionary dynamics of binding sites for the *Drosophila melanogaster* transcription factors Bicoid, Caudal, Hunchback, Kruppel, Medea and Zeste. We used genome-wide in vivo binding data (ChIP-chip) and biochemical binding data (DNAse I footprints) to identify functional binding sites and compared the genome sequences of *D. melanogaster*, *D. simulans*, *D. sechellia*, *D. erecta*, and *D. yakuba*. We estimate that more than 6% of functional Bicoid, Kruppel and Zeste binding sites in *D. melanogaster* were gained along the *D. melanogaster* lineage or lost along one of the other lineages. We find that bound regions have a reduced rate of binding-site loss and an increased rate of binding-site gain relative to flanking sequences. Finally, we show that Kruppel and Zeste binding-site gains and losses are asymmetrically distributed in the tree with respect to *D. melanogaster*, consistent with lineage-specific acquisition and loss of Kruppel and Zeste-responsive regulatory elements. In vivo binding locations in non-*D. melanogaster* species are currently being tested. These findings will greatly assist future studies of *cis*-regulatory sequence and gene expression evolution across closely related species.

28

Evidence for canalization of embryonic pattern formation in *D. melanogaster* and its evolution among closely related species. Susan E Lott[1], Michael Z Ludwig[2], Arnar Palsson[2], Martin Kreitman[1,2]. 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

The A-P axis in the Drosophila embryo is established by diffusion of the maternal morphogen Bicoid. The Bicoid gradient has been shown to form with less precision than its downstream targets (gap and pair-rule genes) and to be more sensitive to environmental perturbation. This increase in the spatial precision of gene expression during pattern formation suggests the existence of a canalizing mechanism in development. Here we investigate whether localization of gap genes *Kruppel* and *giant* and the pair-rule gene *even-skipped* is robust to genetic variation in embryo size in four *D. melanogaster* strains and two closely related species, *D. simulans* and *D. sechellia*. We identified two wild-derived strains of *D. melanogaster*, India (In) and France (Fr), which produce embryos differing by approximately 25% in length when reared under identical conditions. Genetic analysis of embryo length between these lines indicates a large maternal contribution of polygenic and largely additive factors. These two lines, plus Canton-S and w1118, exhibit significant differences in the positions of gap and pair-rule gene expression when measured as absolute distance from the anterior pole. In absolute measurements, expression patterns of larger eggs localize posteriorly compared to those of smaller eggs. But these differences disappear when measurements are made relative to embryo length (percent embryo length), suggesting the existence of a developmental mechanism to assure precise localization of gene expression across the range of embryo sizes present within this species. *D. sechellia* produces a very large embryo compared to our *D. simulans* strain, and it also exhibits comparatively more posterior gene expression. Significant differences in gene expression patterns between these two species (and also *D. melanogaster*) do not disappear, however, when the measurements are made relative to embryo length, suggesting rapid evolution of this canalized trait.

29

War and Peace in a Fly: How to Resolve a Genetic Conflict? Yun Tao, Hailian Xiao. Department of Biology, Emory Univ, Atlanta, GA.

One consequence of the evolution of sex chromosomes in a dioecious species is the genetic isolation between the *X* and the *Y* chromosomes. Because of a lack of recombination between them, selfish genetic elements like meiotic drive distorters can easily mutate on sex chromosomes. A sex-linked distorter can exploit the meiotic machinery to skew the transmission in its own favor, causing sex ratio distortion. This is a corruption of the "fair" Mendelian segregation. Genetic conflicts ensue among genes that gain or loss when sex ratio is skewed away from a normal 1:1. This creates an opportunity for selecting suppressors. This type of genetic attack and defense can be a very important evolutionary mechanism, particular for genes related to meiosis. This could also be a means for evolving reproductive isolation among incipient new species. The above evolutionary scenario is been demonstrated here in *D. simulans.* We have molecularly characterized a *sex-ratio* meiotic drive system in this species by cloning one distorter on the *X* and one suppressor on the third chromosome. The *X*-linked distorter, *Dox*, was formed through duplication and fusion from unrelated genes. The gene product of *Dox*, possibly playing a role in sperm head condensation, disrupts the maturation of most *Y*-carrying sperm. An autosomal suppressor, which we named *Nmy*, was created through a retroposition process from *Dox. Nmy* has no coding potential but its transcription can form a hairpin structure with a double stranded stem of 340 bp. A critical region in *Dox* matches perfectly with a 42 bp sequence within the stem. An RNAi mechanism appears to be responsible for *Nmy*'s suppression of *Dox*. The *Drosophila* genome seems to have come up with a very economic way to resolve an otherwise devastating genetic conflict.

30

Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. Carolyn McBride. Center for Population Biology, University of California, Davis, CA.

Our understanding of the genetic basis of ecological specialization is limited to basic information on the number and locations of genetic factors underlying changes in conspicuous phenotypes. We know nothing about general patterns of molecular evolution that may accompany specialization but are not traceable to a single prominent phenotypic change. Here, I describe changes in the entire repertoire of 136 olfactory receptor (*Or*) and gustatory receptor (*Gr*) genes of the recently specialized fruit fly *Drosophila sechellia*. I find that *D. sechellia* is losing *Or* and *Gr* genes nearly 10 times faster than its generalist sibling *D. simulans*. Moreover, those *D. sechellia* receptors that remain intact have fixed amino acid replacement mutations at a higher rate relative to silent mutations than have their *D. simulans* orthologs. The same patterns are not observed in a control set of random genes, and are therefore likely to result from changing selection pressures associated with the altered ecological niche of this fly. The distribution of amino acid substitutions along sechellia receptor proteins with elevated *Ka*/*Ks* suggests that these changes involve increased positive selection on sechellia *Or*s and relaxed purifying selection on sechellia *Gr*s.

31

High variation in putative pheromone binding receptors Gr68a and Gr32a in D. virilis species group and its role in evolution of mate recognition. Nikolai Mugue. Institute of Developmental Bio, Moscow, RU.

Gustatory receptor Gr68a has been shown to be male-specific and expressed in a few sensitive bristles of forelegs of D. melanogaster male. Expression of this receptor is thought to be important in first steps of mating -touching and assessment of female by conspecific male (Bray, Amrein, 2003). We have sequenced complete Gr68a gene and its alternative spliceform Gr32a in all 12 species of *D. virilis* group - *D. litoralis, D. ezoana, D. lacicola, D. borealis, D. montana, D. flavomontana, D. kanekoi, D. virilis, D. a. americana, D. a. texana, D. novomexicana,* and *D. lummei*. These two proteins are products of alternative splicing . Every species of the group posses unique type of Gr32a receptor, with majority of amino acid variation concentrated in the first and second extracellular regions, corresponding with ligand-binding position. Variation in Gr68a protein concentrated in transmembrane domains and can be attributed to specificity to hydrophobic pheromone ligand. Both receptors show remarkable sign of strong positive selection (high Kn/Ks ratio) and could play an important role in speciation process in this species flock formation.

32

Posttranscriptional regulation by the Pan gu kinase in the early *Drosophila* embryo. Wael Tadros[1,2], Aaron Goldman[2], Fiona Menzies[1,2], Craig A. Smibert[3], Howard D. Lipshitz[1,2]. 1) Dept Molecular & Medical Genetics, University of Toronto, Toronto, ON, CA; 2) Dept Developmental Biol, Hosp Sick Children, TDMT, Toronto, ON, CA; 3) Dept Biochemistry, University of Toronto, Toronto, ON,.

From the start of embryogenesis at egg activation to the mid-blastula transition, transcription is essentially absent. Egg activation triggers a cascade of posttranscriptional mechanisms that are therefore crucial to the control of development during this period. We show that, in the early *Drosophila* embryo, the Pan gu (Png) kinase sits near the top of this cascade. It promotes the translation of Smaug (Smg), a multifunctional posttranscriptional regulator conserved from yeast to humans. Our gene-expression profiling shows that Smg is required for the majority of the subsequent destabilization of maternal transcripts. While Png allows for the full cytoplasmic polyadenylation of *smg* mRNA, restoration of polyadenylation in *png* mutants is not sufficient to rescue Smg translation. Thus Png regulates the translation of *smg* transcripts via mechanisms that are independent of its effects on cytoplasmic polyadenylation. We present evidence that Png functions to relieve translational repression by Pumilio and one or more additional factors, which act in parallel through the *smg* mRNA's 3' untranslated region (UTR). Through these analyses we aim to identify the pathway controlling gene expression during the earliest moments of development.

33

Unmasking transcriptional activation during the maternal to zygotic transition. Stefano De Renzis[1], Olivier Elemento[2], Saeed Tavazoie[2], Eric Wieschaus[1]. 1) Department of Molecular biology Princeton University; 2) Lewis-Sigler Institute for Integrative Genomics Princeton University.

During the maternal to zygotic transition a developing embryo integrates post-transcriptional regulation of maternal mRNAs with transcriptional activation of its own genome. By combining chromosomal ablation with microarray analysis we characterized the basis of this integration in the Drosophila embryo. We show that the expression profile for at least one third of zygotically active genes is coupled to the concomitant degradation of the corresponding maternal mRNAs. The embryo uses transcription and degradation to generate localized patterns of expression and zygotic transcription to degrade distinct classes of maternal transcripts. While degradation does not appear to involve a simple regulatory code, the activation of the zygotic genome starts from intronless genes sharing a common cis-element. We identified a single protein binding to this cis-element and show that it acts as a potent enhancer capable of timing the activity of an exogenous transactivator. We propose that this regulatory mode links morphogen gradients with temporal regulation during the maternal to zygotic transition.

34

A "bottoms-up" approach to deciphering transcriptional cis-regulatory grammar in the embryo. David Arnosti[1,3], Ahmet Ay[2], Chichia Chiu[2], Walid Fakhouri[1]. 1) Dept Biochemistry & Molecular Biology; 2) Dept. of Mathematics; 3) Program in Genetics Michigan State Univ, East Lansing, MI.

Developmental gene regulation involves complex networks of transcriptional interactions, but efforts to understand these networks at a system-wide level have been stymied by the difficulty of identifying cis regulatory information in the genome. Recent approaches by Eisen, Siggia, Sinha, and Halfon among others have focused on identifying patterns of conserved or clustered putative binding sites to locate transcriptional enhancers in genomic sequences. Our work has indicated that many, if not most, developmental enhancers may follow design principles of loosely clustered "billboard" or "information display" enhancers in which placement or spacing of individual factors is weakly constrained. To probe the nature of such regulatory elements, we have identified quantitative parameters that affect enhancers regulated by gap repressor proteins such as Knirps, Giant, and Kruppel that mediate short-range repression in the blastoderm embryo. Quantitative analysis of gene expression using confocal laser imaging enables us to develop the map of particular gene regulatory surfaces associated with particular arrays of regulatory sequences carried by transgenic lines. We use this information in the development of quantitative mathematical models that associate potential functions with subelements of the enhancer, and combine them to provide predictions of the functional output of novel transcriptional arrays. We have been able to accurately predict the effects of novel spacing changes introduced into test elements, and are extending this to accommodate more extensive modifications in factor stoichiometry, binding affinity and arrangement. This work is aimed at informing and extending the power of current models of endogenous cis-regulatory elements to develop powerful bioinformatic tools applicable to population and evolutionary studies.

35

The dCtBP corepressor attenuates DNA-binding of the Dorsal activator in the Drosophila embryo. Yutaka Nibu, Hitoshi Aihara, Mark Stern. Dept Cell & Developmental Biol, Cornell Univ/Weill Medical Col, New York, NY.

Transcriptional repression mediated by Drosophila CtBP (dCtBP) is essential for tissue specification and segmentation in the early embryo. We have shown that dCtBP interacts with DNA-binding repressors, Krüppel, Knirps, and Snail, through the PxDLS amino acid motifs. dCtBP acts as a corepressor and is required for repression mediated by these repressors, which in turn inhibit adjacent activators located within 100 bp. In the early fly embryo, the Snail/dCtBP complex is required for the establishment of mesodermal cell fate, since it represses neuroectodermal genes. To date, how dCtBP mediates short-range repression is still unclear. We are currently testing: (a) whether the Snail/dCtBP complex acts locally by preventing the binding of adjacent activators/coactivators to DNA and (b) whether dCtBP-mediated repression is associated with histone modifications. We are employing chromatin immunoprecipitation (ChIP) assays to analyze representative embryonic enhancers controlled by both the Dorsal activator and the Snail/dCtBP repressor complex. Binding of both Snail and dCtBP to the rhomboid NEE enhancer in our ChIP assays is consistent with observations of the rhomboid expression pattern at genetic levels. Our ChIP assays also show that when the NEE enhancer is active, both Dorsal and dCBP (Drosophila CREB-binding protein; Dorsal's coactivator) are recruited to the enhancer. In addition to that, when the enhancers are occupied by the Snail/dCtBP complex, the binding of Dorsal seems to be prevented. Interestingly, even though dCBP is found on the repressed enhancer, histone H4 is hypo-acetylated. These results suggest the exciting possibility that dCtBP acts by locally preventing DNA-binding of adjacent activators and that dCtBP may inhibit the histone acetyltransferase activity of dCBP. These results provide the first insight on the molecular mechanisms by which dCtBP mediates short-range repression at the chromatin level.

36

MOLECULAR LOGIC OF VENTRAL APPENDAGE FORMATION. Carlos Estella, Daniel McKay, Richard Mann. Columbia University, New York.NY.

During Drosophila leg development, two secreted morphogens, Wg and Dpp, collaborate to create the proximo-distal (PD) axis. One of the molecular readouts for PD axis formation is the Distalless (Dll) gene, which is expressed in the distal ~half of the leg disc. Although previous genetic experiments have demonstrated that both Wg and Dpp inputs are necessary for Dll expression in the leg disc, the cis-regulatory architecture in the Dll gene that integrates these inputs has not been characterized. Moreover, because Dll is activated in a large region of the disc, it is not understood how this integration can occur in cells that perceive very different ratios of Wg:Dpp. To understand its activation at the molecular level we have analyzed the cis-regulatory elements of Dll that are active in the leg imaginal discs. Unexpectedly, we found that Dll is activated in a two-step manner by at least two separable regulatory elements. The first element, the Leg Trigger (LT), initiates Dll expression. Like Dll, an LT-lacZ reporter gene requires both Wg and Dpp inputs to be activated. We have shown that these inputs are direct by mapping and mutating the binding sites for the Dpp and Wg effector transcription factors. However, unlike Dll, LT-lacZ continuously requires Wg and Dpp inputs and is only active in a small region of the distal leg disc. To fully recapitulate the Dll expression pattern, we have found that LT synergizes with a second Dll regulatory element that we refer to as the Maintenance (M) element. Thus, an LT-M-lacZ reporter gene is expressed like Dll. As with Dll, LT-M-lacZ does not require continuous input from Wg and Dpp but requires Dll for expression. Consistently, we have found that Dll binding sites in the M element are necessary for full LT-M-lacZ activity. We propose that Dll expression in leg discs is controlled by a two-step mechanism: in step 1, Wg and Dpp activate LT in young leg discs. In step 2, an activated LT element allows M to function as a Dll autoregulatory element.

37

Localized recruitment of TCF/Armadillo induces widespread chromatin remodeling. David Parker[1], Yunyun Ni[1], Zhenglong Li[2], Ken Cadigan[1]. 1) Dept MCDB, Univ Michigan, Ann Arbor, MI; 2) Biologic & Materials Sci, Ann Arbor, MI.

The canonical Wingless (Wg) signal transduction pathway acts throughout development to influence cell fate decisions and is highly conserved across the animal kingdom. Wg signaling acts to stabilize cytosolic Armadillo (Arm) protein and induces translocation of Arm to the nucleus. In the nucleus, Arm can bind to the sequence-specific DNA binding protein TCF and function as a transcriptional coactivator of Wg target genes. Using chromatin immunoprecipitation, we show that Wg signaling induces TCF and Arm recruitment to a select subset of TCF binding site clusters (Wg response elements or WREs) at the loci of the endogenous Wg target genes *naked cuticle* (*nkd*) and *wingful/notum* (*wf*). We also show that Wg signaling promotes acetylation of histones H3 and H4 at these loci. However, in contrast to localized TCF/Arm recruitment, histones are acetylated over a wide region (tens of kb) surrounding the WREs, suggesting that TCF may influence chromatin remodeling at a distance from specific TCF recruitment sites. Consistent with the preferential binding of TCF/Arm to certain WREs, a 7kb reporter gene construct shows that certain TCF clusters can act as WREs in reporter gene assays whilst others cannot. Inhibition of transcription does not significantly affect widespread histone acetylation or TCF recruitment at these loci, suggesting that widespread histone acetylation is not simply a consequence of transcription. CBP is a histone acetyl transferase previously implicated in Wg signaling. RNAi experiments show that CBP is required for Wg dependent transcriptional activation of *nkd* and *wf*. Furthermore, although CBP RNAi does not affect Wg dependent Arm stabilization and TCF recruitment to the *nkd* and *wf* WREs, CBP is required for widespread histone acetylation at these loci. As CBP can bind Arm, these data suggest a model where Wg signaling induces recruitment of TCF/Arm/CBP to WRE's, leading to widespread histone acetylation and transcriptional activation of Wg target genes.

38

Multiple microRNAs act cooperatively in the developing nervous system to regulate the temporal and spatial expression dynamics of the *Drosophila* Nerfin-1 protein. Alexander Kuzin, Mukta Kundu, Thomas Brody, Ward F. Odenwald. Neural Cell-Fate Determinants Section, NINDS/NIH, Bethesda, MD.

The zinc finer transcription factor Nerfin-1 is required for proper axon guidance during early pathfinding events in the developing *Drosophila* CNS. Unlike *nerfin-1* mRNA, which is expressed in many neural precursor cells, the encoded protein is detected only in the nuclei of those neuronal precursors that will undergo a final mitotic division to generate neurons. These neural precursors include a small subset of neuroblasts and many GMCs. Subsequently, Nerfin-1 protein is detected only transiently in nascent neurons. Thus the dynamic expression of *nerfin-1* mRNA and protein suggests that the mRNA is subject to translational regulation. The *nerfin-1* 3'UTR contains 10 different predicted microRNA (miRNA) binding sites that overlap highly conserved sequence blocks. To assess the in vivo functional significance of the multiple predicted miRNA binding sites, we have generated miRNA binding site reporter lines. In the CNS, miRNAs regulate the onset of Nerfin-1 protein expression in neural precursor cells, while in the PNS miRNAs limit the duration of Nerfin-1 expression in neurons, showing differential degrees of translational inhibition in different neurons. Removal of the entire 3' UTR releases the translational block in neuroblasts and prolongs expression in PNS neurons. Our results indicate that multiple miRNAs play a cooperative role in the embryonic post-transcriptional regulation of *nerfin-1* mRNA, and no single miRNA-binding site is sufficient to completely inhibit expression to the level found using the complete 3' UTR. Nevertheless, a *mir-9A/B/C* site had a partial effect on CNS expression, exhibiting a more rapid shutdown of the reporter at stage 11. Likewise, an adjacent *mir-279* site exhibited a more rapid shutdown of the reporter in the CNS at stage 11 and also exhibited a quantitive difference in the rate of silencing in the PNS. We conclude that multiple miRNA binding sites were found to play a cooperative role in silencing of *nerfin-1* message.

39

Visualizing changes in *bicoid* mRNA localization dynamics during oogenesis. Timothy Weil[1], Richard Parton[2], Ilan Davis[2], Elizabeth Gavis[1]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Wellcome Trust Centre for Cell Biology, School of Biological Sciences, The University of Edinburgh, Edinburgh, UK.

bicoid mRNA localization to the anterior cortex of the *Drosophila* oocyte is required for patterning of the anterior-posterior axis in the early embryo. Through fluorescent tagging of *bicoid* mRNA *in vivo* we have previously shown that the majority of *bicoid* becomes localized in late oogenesis and is maintained anteriorly by continual active transport. To further explore the dynamics of *bicoid* mRNA localization, we have adopted advanced imaging techniques that increase time resolution and signal sensitivity. We have now characterized the motility of *bicoid* mRNA at different stages of oogenesis and show that *bicoid* remains mobile through the completion of oogenesis. Furthermore, we have co-visualized *bicoid* mRNA complexes moving on microtubules and investigated the roles of Exuperantia, Swallow and Staufen in modulating *bicoid* mRNA movement.

40

Trailer Hitch is part of a RNA-protein complex that is required for efficient ER exit. James Wilhelm. Biological Sciences, UCSD, La Jolla, CA.

Translational control of localized messenger mRNAs (mRNAs) is critical for cell polarity, synaptic plasticity, and embryonic patterning. While progress has been made in identifying localization factors and translational regulators, it is unclear how broad a role they play in regulating basic cellular processes. We have identified a novel protein, Trailer hitch (Tral), that it is part of a large RNA-protein complex that includes the translation/localization factors Me31B and Cup. Furthermore, Tral co-localizes with several components of the P body, including Dcp1, consistent with its playing a role in regulating mRNA expression. Surprisingly, mutations in *tral* disrupt Gurken secretion as well as the trafficking of the vitellogenin receptor, Yolkless. This phenotype is not secondary to a defect in *gurken* mRNA localization or oocyte polarity as both are normal in *tral* mutants.

A biochemical screen for targets of Tral identified *sec13* and *sar1* mRNAs as potential targets. Since both of of these proteins are required for formation of functional ER exit sites, we hypothesized that Tral may act to target messages that encode proteins required for ER exit to the surface of the ER to facilitate assembly of ER exit sites. Consistent with this, the distribution of the ER exit site marker (and Tral target), Sar1, is disrupted in *tral* mutant egg chambers and is strikingly similar to the Grk foci that form in these mutants. Furthermore, we found that the Tral RNP complex is actively transported to subdomains of the ER that border ER exit sites arguing that Tral acts directly to regulate ER exit site function. These findings raise exciting new possibilities for how the mRNA localization machinery could interface with the classical secretory pathway to promote efficient protein trafficking in the cell.

41

Analysis of Dynamin function in cytoskeletal remodelling during *Drosophila* embryogenesis by time-lapse imaging. Richa Rikhy, Manos Mavrakis, Jennifer Lippincott-Schwartz. CBMB, NICHD, NIH, Bethesda, MD.

Dynamin is a high molecular weight GTPase with an established role in clathrin-mediated endocytosis. In addition, recent studies in mammalian cells have proposed a function for Dynamin in actin cytoskeleton remodelling. In *Drosophila* embryogenesis, during nuclear division 9-13, the nuclei align as a single layer below the membrane with incomplete cytokinesis. Extensive cytoskeleton remodelling occurs to create actin caps above each nucleus during interphase followed by actin rings at metaphase furrows during mitosis. This provides a model system to investigate molecular regulators of this process. To further investigate a possible role for dynamin in actin remodelling during these stages of development we generated fluorescently tagged wild type and temperature sensitive mutant transgenes. The temperature sensitive lethality of *Drosophila* dynamin mutant, *shibire* was reversed by the wild type dynamin-GFP showing that it is partially functional. Time-lapse imaging showed that it is highly enriched at the plasma membrane in microvilli and at the centrosome during interphase and at the spindle during metaphase. Photobleaching studies showed that it continuously exchanges between these structures and the cytoplasm. The temperature sensitive mutant, shi^{ts2} was used with a plasma membrane marker Spider-GFP to determine the restrictive temperature for development. Development was severely affected at 32°C in shi^{ts2} mutants. To further dissect the molecular pathways that were disrupted shi^{ts2} mutants were analyzed with various markers. Tubulin-GFP revealed the occurrence of tripolar spindles and phalloidin staining showed a discontinuous actin cytoskeleton at 32°C. Nuclear fall out was also observed. In addition, there was an increased accumulation of dynamin-GFP at the plasma membrane. Together these observations strongly imply that dynamin plays a significant role in stabilization of the actin ring to membranes. In conclusion our results suggest that dynamin participates in cytoskeleton remodelling in cytokinesis.

42

Translocation of RhoGEF2 to the furrow canal during cellularization. Christian Wenzl, Jörg Grosshans. ZMBH Zentrum für Molekulare Biologie Heidelberg, Heidelberg, DE.

During *Drosophila* cellularization the cortical nuclei formed by the early cleavage cycles become simultanously enclosed by the plasma membrane to form the cellular blastoderm. This process is initiated by the formation of a hairpin-loop like membranous structure the so called furrow canal. The proper morphogenesis of the furrow canal depends in part on a Rho signaling pathway that leads to a localized actin polymerization at the furrow canal and hence to a stabilization of this morphological structure. We have investigated the mechanism by which the most upstream known component of this pathway, RhoGEF2, mediates the local activation of Rho. Therefore we mapped the localization domain of RhoGEF2 and we present evidence that localization of the protein depends on intact microtubules but does not depend on it's previously reported EB1-dependent association with microtubule plus ends. By using a variety of different fluorescently labeled cytoskeletal markers and confocal spinning disc microscopy we can follow the translocation of RhoGEF2 to the furrow canal *in vivo* in wildtype and in different mutant backgrounds with high spatial and temporal resolution. To find factors that function in the translocation process and in depositing RhoGEF2 at the furrow canal we performed a yeast two hybrid screen and found several promising candidates that are currently tested in our microscopy based assay. Finally we will discuss a model that could explain how RhoGEF2 gets localized to a specific subcellular compartment where it then leads to a spatially restricted activation of Rho1.

43

Planar cell polarity and the organization of cell behavior during axis elongation. Todd Blankenship, Stephanie Backovic, Justina Sanny, Ori Weitz, Jennifer Zallen. Dept. of Developmental Biology, Memorial Sloan-Kettering, New York, NY.

The elongated body axis is an essential developmental feature of vertebrates and invertebrates and relies on the coordination of multiple processes - including polarized motility, selective adhesion, and intercellular communication - across a three-dimensional field of cells. A widely-used mechanism for tissue elongation is cell intercalation, in which oriented cell movements cause a tissue to narrow in one dimension and lengthen in a perpendicular dimension. In Drosophila, the A-P patterning system is required to establish molecularly distinct regions of the cell surface that may serve as a prerequisite for polarized cell behavior. We found that planar polarity in the Drosophila embryo is generated through a sequential enrichment of actin-myosin cables and adherens junction proteins in complementary membrane domains. F-actin accumulation at A-P interfaces represents the first break in planar symmetry and occurs independently of the proper distribution of adherens junction proteins at D-V interfaces. These polarized cytoskeletal and junctional proteins are dynamically reorganized during a novel program of cell behavior in which cells form multicellular rosette structures that assemble and resolve in a directional fashion. Contractile actin-myosin structures align across multiple pairs of cells during rosette formation and a second phase of polarized adherens junction assembly promotes rosette resolution. The A-P patterning genes that are essential for cell polarity selectively affect the frequency and directionality of rosette formation. We propose that the generation of higher-order rosette structures links local cell interactions to global tissue reorganization during morphogenesis.

44

The hh pathway induces non-muscle Myosin II-driven apical epithelial cell constriction and groove formation during organogenesis. Franck Pichaud. LMCB, MRC Cell Biology Unit, Dept Anatomy & Dev Biol, University College London, London, GB.

Modulation of epithelial cell shape, from columnar to squamous or cuboidal, and discrete instances of apical cell constriction or groove formation are crucial for organogenesis. The latter is believed to promote epithelial sheet invagination and influence cell-cell signaling during embryogenesis across phyla. However, the mechanisms and signaling pathways governing such cell shape changes are not well understood. To study this question we are using the developing fly eye. Patterning of the Drosophila eye relies upon the propagation of a morphogenetic wave (MF) characterized by a cell cycle arrest in G1 and a striking apical cell constriction. We show that the evolutionary conserved dpp and hh pathways are major regulators of epithelial cell shape. dpp and hh signaling modulate the status of the apical microtubule and cortical F-actin networks and this is coupled to the activation of diaphanous and non-muscle myosin II. In the eye, Myo II functions downstream of the hh pathway in inducing apical cell constriction in the MF while inducing groove formation in undifferentiated columnar epithelium. This represents a novel function for the hh and dpp pathways that is likely to be relevant to vertebrate development, in particular neural tube morphogenesis and in a variety of pathological contexts.

45

Moesin regulates apoptosis by blocking activation of Rho1 and JNK signaling. Amanda Neisch[1], Olga Speck[2], Richard Fehon[1]. 1) Committee on Developmental Biology and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Department of Biology, Duke University, Durham, NC.

Apical/basal polarity and epithelial integrity are crucial for normal proliferation and differentiation of epithelial tissues during development. Loss of polarity and integrity is thought to be an important contributing factor in malignant transformation and epithelial to mesenchymal transformation that allows tumor cell metastasis. Moesin, a membrane associated protein, acts to regulate the actin cytoskeleton by negatively regulating the small GTPase Rho1 (Speck et al., 2003). This regulation is crucial for the maintenance of epithelial integrity. In addition, we find that Moesin mutant cells undergo apoptosis. We have begun to explore how loss of Moesin function triggers apoptosis. In *Drosophila* there are two major apoptotic pathways, the classical pathway that involves a stimulus that regulates the expression of the pro-apoptotic genes Hid, Reaper, and Grim; and the Tumor Necrosis Factor (TNF) receptor/ligand pathway that involves Jun N-terminal kinase (JNK) activation. We have found that loss of Moesin promotes apoptosis through activation of the JNK signaling pathway leading to transcriptional activation of the proapoptotic gene Hid. Additionally, we have shown that this apoptosis occurs through activation of Rho1 but is independent of Rho kinase activation. These results suggest a previously unexplored role for the Rho small GTPase in regulating apoptosis in epithelial cells. We are currently investigating how Rho1 feeds into the JNK signaling cascade, as well as through which effectors Rho1 acts.

Speck, O., Hughes, S. C., Noren, N. K., Kulikauskas, R. M. and Fehon, R. G. (2003). Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity. Nature 421, 83-7.

46

A sperm's eye view of evolution: Lessons from the *Drosophila* sperm proteome. Timothy Karr, Steve Dorus, Ursula Gerike. Dept Biol & Biochemistry, Univ Bath, Bath, GB.

Although the ultimate function of sperm is to deliver a haploid genome to the egg during fertilization, sperm possess additional critical functions including egg activation, origination of the zygote centrosome and delivery of paternal factors. Despite this, little comprehensive knowledge exists concerning the molecular basis of sperm form and function. Whole-sperm mass spectrometry was used to identify 381 proteins of the *Drosophila melanogaster* sperm proteome (DmSP), increasing the number of characterized sperm proteins by greater than 60-fold. Major functional categories, including mitochondrial, metabolic and cytoskeletal proteins, were identified in addition to several novel functional categories. Amongst these are a recently expanded and functionally diversified family of leucyl aminopeptidases (Laps) including one whose abundance is significantly altered in mutations of another newly evolved paternal effect gene, *ms(3)K81*. *K81* sperm fertilize eggs but cannot complete zygote formation due to failure of the paternal chromosomes to properly decondense and align during the first mitosis. Intriguingly, *ms(3)K81* sperm are depleted in Lap CG32351 indicating that *K81* encodes an upstream component that regulates CG32351 levels in sperm. As such, this represents novelty at both the individual protein level and at the genetic network level between *K81* and CG32351. The establishment of a novel genetic interaction between these two recently evolved genes provides a rare example of the evolutionary construction of a new genetic network. Nonrandom gene clustering and under representation on the X chromosome was also observed in the DmSP. Evolutionary analyses identified global functional conservation indicating that positive selection has played a limited role in sperm evolution and function in this taxa. Therefore, a large number of proteins in the DmSP may serve critical, yet in many cases unknown, functions in reproduction including male-infertility factors. This study will serve as a foundation for future work on the sperm evolution in *Drosophila* and other species.

47

Widespread adaptive evolution of Drosophila genes with sex-biased expression. John Parsch, Zhi Zhang, Matthias Proeschel. Biology, University of Munich, Germany.

Many genes in higher eukaryotes show sexually dimorphic expression, and these genes tend to be among the most divergent between species. In most cases, however, it is not known whether this rapid divergence is caused by positive selection, or if it is due to a relaxation of selective constraint. To distinguish between these two possibilities, we surveyed DNA sequence polymorphism in 91 *Drosophila melanogaster* genes with male-, female-, or nonsex-biased expression and determined their divergence from the sister species *D. simulans.* Using several single- and multi-locus statistical tests, we estimated the type and strength of selection influencing the evolution of the proteins encoded by genes of each expression class. Adaptive evolution, as indicated by a relative excess of nonsynonymous divergence between species, was common among the sex-biased genes (both male and female). Male-biased genes, in particular, showed a strong and consistent signal of positive selection, while female-biased genes showed more variation in the type of selection they experience. Genes expressed equally in the two sexes, in contrast, showed no evidence for adaptive evolution between *D. melanogaster* and *D. simulans*. This suggests that sexual selection and intersexual co-evolution are the major forces driving genetic differentiation between species.

48

Gain and loss of sex-specific expression of a HOX gene is associated with rapid morphological evolution. Artyom Kopp, Olga Barmina. Dept Evolution & Ecology, University California - Davis, Davis CA 95616.

The HOX gene cluster forms the backbone around which animal body plans develop and evolve. However, it has also been suggested that HOX genes can act as "micromanagers" that control the differentiation of specific morphological structures. We show that rapid evolution of spatially modulated and sexually dimorphic expression of the HOX gene *Sex combs reduced* is associated with the origin and diversification of a novel sex-specific organ. The sex comb is a male-specific array of modified bristles that evolved recently in one of the *Drosophila* lineages, and shows remarkable morphological diversity among closely related species. We find that in species that primitively lack sex combs, *Scr* expression shows little or no spatial modulation, whereas in species that have sex combs, *Scr* is strongly upregulated in the presumptive sex comb region. The spatial extent of high *Scr* expression correlates with the position and morphology of the sex comb in each species. Moreover, in species with large sex combs, *Scr* expression is much higher in males than in females. To our knowledge, this is the first example of sexually dimorphic regulation of a HOX gene. Phylogenetic analysis shows that sex-specific expression of *Scr* has evolved independently in at least four different clades. Instances of secondary loss of the sex comb are associated with the loss of sex-specific *Scr* expression. To understand the mechanism by which *Scr* induces sex comb development, we used microarray experiments to identify the joint downstream targets of *Scr* and the sex determination pathway. Our results suggest that quantitative changes in gene expression can produce qualitatively novel phenotypes.

49

New candidate genes for sex comb divergence between *Drosophila mauritiana* and *Drosophila simulans*. Rita M. Graze[1], Olga Barmina[3], Daniel Tufts[2], Elena Naderi[2], Kristy L. Harmon[2], Maria Persianinova[2], Sergey V. Nuzhdin[2]. 1) Genetics Graduate Group, University of California, Davis, CA 95616; 2) Center for Population Biology, Section of Evolution and Ecology, University of California, Davis, CA 95616; 3) Center for Genetics and Development, University of California, Davis, CA 95616.

A large effect QTL for divergence in sex comb tooth number between *D. simulans* and *D. mauritiana* was previously mapped to 73A-84AB. Here we identify genes that are likely contributors to this divergence. We first improved the mapping resolution in the 73A-84AB region using 12 introgression lines and 62 recombinant nearly isogenic lines. To further narrow the list of candidate genes, we assayed leg specific expression and identified genes with transcript level evolution consistent with a potential role in sex comb divergence. Sex combs are formed on the prothoracic (front) legs, but not on the mesothoracic (middle) legs of *Drosophila* males. We extracted RNA from the prothoracic and mesothoracic pupal legs of two species to determine which of the genes expressed differently between leg types were also divergent for gene expression. Two good functional candidate genes, *Scr* and *dsx*, are located in one of our fine-scale QTL regions. In addition, three previously uncharacterized genes (CG15186, CG2016, and CG2791) emerged as new candidates. These genes are located in regions strongly associated with sex comb tooth number differences and are expressed differently between leg tissues and between species. Further supporting the potential involvement of these genes in sex comb divergence, we found a significant difference in sex comb tooth number between co-isogenic lines with and without a P element insertion at CG2791.

50

Genetic basis of pigmentation differences within and between *Drosophila* species. Patricia Wittkopp, Belinda Haerum, Emma Stewart, Gabriel Smith-Winberry. Ecology & Evolutionary Biol, Univ Michigan, Ann Arbor, MI.

Discovering the genetic basis of phenotypic divergence is essential for understanding evolutionary mechanisms. New mutations and standing genetic variation both provide raw material for evolutionary change, but the relative contribution of each is largely unknown. To examine the source of variation used for interspecific divergence, we are studying pigmentation differences within and between Drosophila species in the virilis group. *D. novamexicana* is a young species that evolved yellow pigmentation since its divergence from *D. americana* approximately 380,000 years ago. *D. americana* has brown pigmentation that ranges from dark to light in an east to west cline and is consistent with local adaptation. Two genes, *ebony* and *tan*, have been identified that appear to contribute to pigmentation differences between species. These genes encode enzymes that catalyze opposite directions of a reversible reaction in the pigment biosynthesis pathway. Haplotypes of *ebony* and *tan* fixed in *D. novamexicana* are segregating in *D. americana* and contribute to pigmentation differences within this species, suggesting that standing genetic variation may be an important source of phenotypic divergence.

51

Comparative genomics of innate immune pathways in *Drosophila*. Timothy Sackton[1], Brian Lazzaro[2], Todd Schlenke[3], Jay Evans[4], Dan Hultmark[5], Andrew Clark[1]. 1) Molecular Biology and Genetics, Cornell Univ, Ithaca, NY; 2) Dept of Entomology, Cornell Univ, Ithaca NY; 3) Dept of Biology, Emory Univ, Atlanta GA; 4) Bee Research Lab, USDA ARS, Beltsville MD; 5) UCMP, Umeå Univ, Umeå, Sweden.

The immune system is a hotspot for rapid evolution in a number of organisms. Understanding the patterns of adaptive diversification of immune systems can shed light on targets of host-pathogen interactions and the mechanisms of host resistance. Here we present work on the patterns of selection, diversification, and constraint in the *Drosophila* innate immune system, using data from the recently completed genomes of twelve *Drosophila* species. Rates of duplication and loss are unevenly distributed across functional classes of immunity genes. Signaling proteins are less likely to duplicate or be lost across the phylogeny, compared to recognition or effector proteins. This suggests a bow-tie structure for the innate immune pathway, with relative flexibility of gene content for the input and outputs to the system, but strong conservation of gene content for the signaling modules. By fitting standard codon substitution models, we have tested for signatures of adaptation and constraint in alignments of 200 known and putative immune system genes in *Drosophila*. Antimicrobial peptides and other effector proteins show less evidence for positive selection than recognition and signaling proteins. Together, these data suggest a model where pleiotropic constraints result in conserved signaling pathways across large evolutionary distances, which integrate signals from a diverse array of recognition proteins and lead to transcription of a wide spectrum of effector proteins. We speculate that the flexibility of inputs and outputs to the system relaxes the coevolutionary pressures on recognition and effector proteins due to functional redundancy. Signaling proteins, on the other hand, appear to be undergoing rapid evolution because with their limited functional redundancy they present an easy target for short-circuiting the immune response by pathogens.

52

Ancestral repeats in Drosophila. Anat Caspi[1], Lior Pachter[2]. 1) Joint Graduate Group Bioengineering, UC Berkeley/UCSF, Berkeley, CA; 2) Department of Mathematics, University of California, Berkeley, CA.

Whole genome alignments among the sequences of the 12 released drosophila genomes allowed for the investigation of the presence of ancestral repeats. Using a previously published method which identifies the unique pattern of conservation left by ancient insertions, we locate ancestral repeats in different sub-groups of the 12 genomes(1). The ability to align ancient repeat sequences generally decreases with increasing evolutionary distance. Using a comparative method, we identify over 40,000 ancient insertion segments among the 12 genomes, most of which are not detectable by pair-wise sequence comparisons alone. We do not use any alignment method or sequence similarity criteria in order to avoid the bias towards finding more recently inserted repeats. To test the signal in the data, we use the presence/absence of repeats as phylogenetic characters. The data comes remarkably close to a tree metric. We reconstruct a tree from our data that topologically exactly matches the accepted 12-genome tree, supporting the placement of D.yakuba as a sister group to D.erecta. To our knowledge, this is the first instance of the use of the presence/absence of ancestral repeats to build trees. Among the insertions that are not consistent with a tree, we present a number of ancestral repeats that are likely to have been lost in certain lineages and not others. The remaining discordant data could be due to missing genome sequences, or bad alignments.

Our analysis of insertions highlights the variation in genome dynamics among these species. Examining the patterns of conservation of the ancient repeats, we estimate the rate of insertions and deletions for different branches of the tree. The data show non-clocklike behavior on the tree. Some terminal branches are very long (e.g. D. willistoni, or D.persimilis/D.pseudoobscura branch)- as they must have undergone increased levels of sequence evolution via repeat insertions/deletions.

(1) Caspi & Pachter(2006). Genome Research 16:260-270.

53

The JAK/STAT pathway regulates heterochromatin formation. Song Shi, Kimberly Larson, Fan Xia, Willis X. Li. Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY.

The JAK/STAT pathway plays essential roles in multiple developmental processes, and aberrant activation of STAT transcription factors by phosphorylation is associated with multiple types of human cancers. The effects of JAK/STAT signaling have been attributed largely to STAT's direct transcriptional regulation of specific target genes that promote tumor cell proliferation or survival. Recently, however, we have demonstrated that Drosophila oncogenic JAK kinase induces hematopoietic tumors by globally disrupting heterochromatin formation, an epigenetic tumor suppressive mechanism, and that this disruption allows derepression of genes that are not direct targets of STAT (Shi et al., 2006, Nature Genetics 38, 1071-6). Here, we investigate the role of STAT in JAK-induced heterochromatin destabilization. We demonstrate that Drosophila STAT is essential for heterochromatin stability and the correct localization of heterochromatin protein 1 (HP1). Our results suggest that nonphosphorylated transcriptionally inactive STAT promotes heterochromatin formation and gene silencing by stabilizing HP1 on heterochromatin, and that STAT activation by JAK-mediated phosphorylation leads to HP1 displacement and heterochromatin destabilization. Thus, phosphorylation triggers conversion of STAT from a heterochromatin stabilizer to a transcriptional activator, controlling both access to chromatin and activity of the transcription machinery.

54

EPIGENETIC SWITCHES BETWEEN PRECURSOR CELLS AND DIFFERENTIATION CELLS DURING SPERMATOGENESIS OF DROSOPHILA. Xin Chen, Margaret Fuller. Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

The Polycomb group (PcG) machinery has been implicated in maintenance of stem and precursor cell fates, but how this repression is reversed to allow differentiation in normal cells is unclear. PcG is thought to act in two discrete but interacting protein complexes: PRC1 and PRC2. While investigating the mechanisms that turn on the terminal differentiation program in male germ cells, we have discovered that certain PRC1 components are recruited to nucleolus in primary spermatocytes, at the onset of cellular differentiation program. Such recruitment requires wild-type function of a set of tissue-specific homologs of general transcription machinery components, the TBP-associated factors (TAFs). Loss-of-function mutations in any of these testis-specific TAFs (tTAFs) result in male sterility due to decreased transcription of many target genes, resulting in defects in both meiotic cell cycle progression and terminal differentiation of spermatids. The expression of testis TAFs exactly coincides with re-localization of PRC1, which may sequester PRC1 away from target genes to help relieve repression of differentiation genes at a specific stage of spermatocyte maturation. In addition, several PRC2 complex components were expressed highly in precursor cells, including transit-amplifying spermatogonial cells, but were abruptly down regulated in tTAF-expressing spermatocytes. Developmentally programmed loss of PRC2, re-localization of PRC1 and expression of tTAFs label epigenetic switches from precursor cells to differentiation cells in male germline lineage, which may contribute to a release of repression by PcG in precursor cells and allow onset of terminal differentiation.

55

Ecdysone Receptor, homolog of FXR/RXR, functions in Drosophila as a Type 1 nuclear receptor. Danika M Johnston, Yurii Sedkov, Svetlana Petruk, Kristen M Riley, Miki Fujioka, James B Jaynes, Alexander Mazo. Biochemistry, Thomas Jefferson University, Philadelphia, PA.

The Drosophila Ecdysone Receptor triggers the onset of key events during development in response to the steroid hormone ecdysone. This receptor consists of two proteins, EcR, a homolog of the Farnesoid X Receptor, FXR, and Usp, a homolog of the Retinoid X Receptor, RXR. Similar to RXR, Ecdysone Receptor is thought to act as a Type 2 nuclear receptor (NR), occupying its DNA response elements (EcREs) regardless of the presence of hormone, and recruiting either co-repressors or co-activators depending on the presence of ligand. Surprisingly, we found that neither EcR nor USP is associated with repressed genes, while both are recruited after induction with ecdysone. Moreover, changes in ecdysone concentration lead to shuttling of these molecules between the nucleus and cytoplasm, further indicating that this heterodimer acts as a Type 1 NR. Elimination of either partner, using RNAi, leads to a drastic loss in association of the other component with all of its sites on salivary gland polytene chromosomes, suggesting that EcR and Usp act exclusively as components of the same heterodimer. We also show that a co-activator of Ecdysone Receptor, the histone H3 lysine 4 (H3-K4) methyltransferase (HMT) Trithorax-related (Trr) also shuttles between the nucleus and cytoplasm in an ecdysone-dependent fashion, and that Trr is exclusively associated with Ecdysone Receptor on their target genes. These data suggest a new, unexpected mechanism of functioning of this major Drosophila NR and suggest that a similar mechanism may exist for their mammalian orthologs.

56

Cell fate in the Drosophila embryo depends on regulation of transcriptional elongation. Xiaoling Wang, John. Peter Gergen. Dept Biochem & Cell Biol, SUNY-Stony Brook, Stony Brook, NY.

The Drosophila embryo provides an excellent model for investigating the regulatory mechanisms of cell fate specification during development. Gradients of maternally provided positional information are translated into differential programs of gene expression with a single cell level of resolution by the completion of cellularization at three hours of development. On the antero-posterior axis this initial cell fate specification is revealed by the segmentally-repeated expression of several segment-polarity genes. Transcriptional regulation is central to this process, and many of the upstream regulators in the segmentation gene hierarchy encode DNA-binding transcription factors. Here we take advantage of the simple combinatorial logic responsible for the striped expression of *sloppy-paired-1*(*slp1*) to investigate the in vivo biochemistry of developmentally regulated transcriptional repression. By the over-expression of Runt and Fushi tarazu (Ftz), we generated populations of Drosophila embryos in which all cells establishes a *slp1*-repressed state at early gastrulation. We find that the initial establishment of repression does not involve changes in chromatin re-modeling or modification, nor the assembly of an initiation complex at the *slp1* promoter. Indeed, the association of NELF (negative elongation factor) to the *slp1* promoter downstream indicates that repression involves regulation of transcriptional elongation. The finding that NELF is also associated with the promoter downstream of segment-polarity genes *engrailed* (*en*)and *wingless* (*wg*) in blastula stage suggests that the regulation of transcriptional elongation is a common mechanism for controlling gene expression during this critical stage of Drosophila embryogenesis.

57

Transcriptional elongation of non-coding bxd RNAs promoted by the Trithorax TAC1 complex represses Ubx by a transcriptional repression mechanism. Svetlana Petruk[1], Yurii Sedkov[1], Kristen Riley[1], Jacob Hodgson[2], Francois Schweisguth[3], Susumu Hirose[4], James Jaynes[1], Hugh Brock[2], Alexander Mazo[1]. 1) Department of Biochemistry, Thomas Jefferson University, Philadelphia, PA 19107, USA; 2) Department of Zoology, University of British Columbia, 6270 University Boulevard, V6T 1Z4, Vancouver, BC, Canada; 3) Ecole Normale Superieure, CNRS UMR 8542, Paris, France; 4) Department of Developmental Genetics, National Institute of Genetics, and Graduate University for Advanced Studies, Mishima, Shizuoka-ken 411-8540, Japan.

Using a novel method, we show that the Trithorax (Trx) complex TAC1 is reciprocally recruited to Ultrabithorax (Ubx) and the bxd epigenetic maintenance element (ME) in nuclei expressing or not expressing Ubx, respectively (Petruk et al., Cell in press). TAC1 acts coordinately with elongation factors to facilitate transcriptional elongation of Ubx and several bxd non-coding RNAs. Surprisingly, Ultrabithorax and several bxd non-coding RNAs are expressed in non-overlapping patterns in the embryo, suggesting that transcription of the bxd ME is associated with repression. Our data rule out siRNA or micro RNA-based mechanisms and suggest that transcription of bxd RNAs per se represses Ubx. The bxd non-coding RNAs are not present where Ubx is expressed in imaginal discs. We suggest that transcriptional elongation of non-coding bxd RNAs promoted by TAC1 represses Ubx by a transcriptional interference mechanism, and leads to mosaic expression of Ubx in embryos. We propose that this may be a key event in the heritable maintenance of Ubx pattern by trithorax during development.

58

Re-coding of mRNA transcripts in *Drosophila*: functional consequences and the identification of 27 new targets of ADAR. Mark Stapleton, Joseph W Carlson, Susan E Celniker. Genome Biology, Lawrence Berkeley Nat'l Lab, Berkeley, CA.

Adenosine deaminases that act on RNA (ADARs) catalyze the site-specific conversion of adenosine to inosine in primary mRNA transcripts. These re-coding events affect coding potential, splice-sites, and stability of mature mRNAs. *Adar* is an essential gene and studies in mouse, *C. elegans*, and *Drosophila* suggest its primary function is to modify adult behavior by altering signaling components in the nervous system. Although directed approaches to identify ADAR targets have been employed successfully, we have taken a different approach based on a systematic analysis of the Drosophila Gene Collection (DGC; http://www.fruitfly.org/DGC). The DGC contains cDNAs representing 10,398 of the predicted 13,449 genes in *D. melanogaster* (Release 4.1). Utilizing high-quality sequence data from full-insert sequences of adult head cDNA clones, we have identified and experimentally verified 27 new targets of ADAR, doubling the total to 55. The edited sites verified in our analysis are within coding regions and in the 3' UTRs of these targets. Previous studies on one of our targets (*CG31116*), a member of the CLC family of chloride channels, suggest a clear functional consequence of editing upon the gating properties of the ion channel. Our analyses lead us to identify new classes of genes whose transcripts are targets of ADAR including components of the actin cytoskeleton, and genes involved in ion homeostasis and signal transduction. Our results indicate that editing in *Drosophila* increases the diversity of the proteome, and does so in a manner that has direct functional consequences on protein function.

59

In vivo Screening for Nuclear Receptor Agonists and Antagonists in Drosophila. Aleksandar S. Necakov, Heidi M. Sampson, Henry M. Krause. The Centre for Cellular and Biomolecular Research, The University of Toronto, Toronto, Ontario, CA.

Nuclear receptors comprise a family of transcription factors whose activation/repression state can be modulated by the binding of small molecules. The identification of ligands capable of binding to particular nuclear receptors is central to understanding the mechanisms through which these receptors elicit their function. To date, 18 nuclear receptor genes have been identified in the Drosophila melanogaster genome. However, cognate ligands for 17 of the 18 receptors have not yet been identified. As part of a project to identify the remaining ligands, we have developed an in vivo screen that allows for the dynamic visualization of ligand mediated nuclear receptor activation in live Drosophila tissues. Each of the 18 fly nuclear receptor ligand binding domains has been fused to the DNA binding domain of Gal4 and introduced into flies under control of a heat-shock inducible promoter. Activity of the chimeric transcription factors requires the presence of ligand, and is visualized with the use of a GAL4-dependent GFP reporter. Nine of the 18 chimeric proteins were found to exhibit unique spatio-temporal activity patterns during embryonic and larval development. Using the Ecdysone receptor (EcR) fusion protein, we have shown that these limited activity patterns are ligand dependent and can be expanded both temporally and spatially by exogenously provided ligand. Thus, we expect that the other ligand sensor activity patterns are likely to reflect the locations of their cognate ligands and the tissues in which these receptors are normally active. Compounds and mutations that affect these response patterns can also be used to help identify the unknown ligands. In this respect, we are currently carrying out high throughput chemical screens to identify potential nuclear receptor agonists and antagonists as these compounds may lead to the development of new species-specific pesticides. Using this approach we have identified several nuclear receptor agonists which we are further investigating as bona fide nuclear receptor ligands.

60

Antagonistic functions of Par-1 kinase and protein phosphatase 2A are required for localization of Bazooka and photoreceptor morphogenesis in Drosophila. Sang-Chul Nam[1,2], Bibhash Mukhopadhyay[3], Kwang-Wook Choi[2,3,4]. 1) Department of Biology, Baylor University, Waco, TX; 2) Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Ophthalmology, Baylor College of Medicine, Houston, TX.

Apical basal cell polarity establishment and maintenance are essential for epithelial morphogenesis and have been studied extensively using the Drosophila eye as a model system. Bazooka (Baz), a component of the Par-6 complex, plays important roles in cell polarity in diverse cell types including the photoreceptor cells. In ovarian follicle cells, Localization of Baz at the apical region is regulated by Par-1 protein kinase. In contrast, Baz in photoreceptor cells is targeted to adherens junctions (AJs). To examine the regulatory pathways responsible for Baz localization in photoreceptor cells, we studied the effects of Par-1 on Baz localization in the pupal retina. Loss of Par-1 impairs the growth of AJ and apical membranes of photoreceptor cells but not the establishment of cell polarity. In contrast, overexpression of Par-1 or Baz causes severe mislocalization of junctional and apical markers, resulting in abnormal cell polarity. However, similar overexpression of kinase-inactive mutant Par-1 or unphosphorylatable mutant Baz shows relatively normal photoreceptor development. These results suggest that dephosphorylation at the Par-1 phosphorylation sites in Baz is essential for proper Baz localization. We also show that the inhibition of protein phosphatase 2A (PP2A) mimics the polarity defects caused by Par-1 overexpression. Further, Par-1 gain-of-function phenotypes are strongly enhanced by reduced PP2A function. Thus, we propose that antagonism between PP2A and Par-1 plays a key role for Baz localization at AJ in photoreceptor morphogenesis.

61

Recruitment of the cellular actin polymerization machinery is essential for myoblast fusion in Drosophila embryos. Eyal Schejter, R'ada Massarwa, Shari Carmon, Benny Shilo. Dept Molecular Genetics, Weizmann Inst Science, Rehovot 76100, ISRAEL.

Formation of syncytial muscle fibers involves repeated rounds of cell fusion between growing myotubes and neighboring myoblasts. We have established that WASp, a key nucleation promoting factor of Arp2/3-based actin polymerization, is an essential facilitator of myoblast fusion in Drosophila embryos. D-WIP, a previously uncharacterized homolog of the conserved Verprolin/WASp Interacting Protein family of WASp-binding proteins, performs an essential mediating role in this context. D-WIP, which is expressed specifically in myoblasts, associates with both the WASp-Arp2/3 system, and with the myoblast fusion receptors Dumbfounded and Sticks-and-Stones, thereby recruiting the actin polymerization machinery to sites of myoblast attachment and fusion. Our analysis demonstrates that this recruitment is required late in the fusion process, for enlargement of nascent fusion pores, and breakdown of the apposed cell membranes. These observations suggest novel cellular and developmental roles for the WASp-Arp2/3 pathway, and provide a new link between force-generating actin polymerization and cell fusion.

62

Characterization of Drosophila WIP reveals a critical function of the actin cytoskeleton in myoblast fusion. Elizabeth H Chen. Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

Myoblast fusion is an important step during myogenesis in species as diverse as fly and human. In Drosophila, somatic muscle cells are composed of two populations, founder cells (FCs) and fusion competent myoblasts (FCMs). Fusion between a FC and its surrounding FCMs leads to the formation of a multinucleated myotube. Previous studies have begun to reveal a signaling cascade that relays the fusion signal from the plasma membrane to the actin cytoskeleton. However, the regulation and function of the actin cytoskeleton during myoblast fusion remain unclear. In a genetic screen for genes involved in muscle development, we uncovered solitary (sltr) that is required for myoblast fusion. sltr encodes the Drosophila homologue of the mammalian WASP Interacting Protein (WIP). It is specifically expressed in FCMs at the time of fusion. The Sltr protein is recruited to sites of fusion by the fusion competent cell receptor Sns, mediated by the small adaptor protein Crk. Sltr promotes actin polymerization through its interaction with WASP and the monomeric G-actin in S2 cells and it is required for the localized actin polymerization at sites of fusion in FCMs in vivo. Therefore, Sltr functions as a cell type-specific adaptor protein that couples fusion signal transduction with actin cytoskeleton rearrangement. Interestingly, our EM analysis revealed abnormal accumulations of prefusion vesicles along the apposing membranes of adhering myoblasts in sltr mutant embryos, indicating a role of the actin cytoskeleton in targeting and fusion of these vesicles to proper sites on the plasma membrane. Thus vesicle-membrane fusion defines a critical step prior to plasma membrane fusion. Furthermore, we show that WIP, the mammalian homologue of Sltr, plays an important role in fusion of mouse C2C12 myoblasts, suggesting an evolutionarily conserved function of the actin cytoskeleton during myoblast fusion.

63

Fwd/PI4Kβ binds Rab11 and acts in a common pathway with Rab11 during cytokinesis. Gordon Polevoy, Ho-Chun Wei, Julie A. Brill. Developmental & Stem Cell Biol, The Hospital for Sick Children, Toronto, Ontario, CANADA.

Cytokinesis - the physical separation of daughter cells at the end of the cell cycle - relies on membrane trafficking for its completion. Among the factors regulating specificity and directionality of membrane trafficking are phosphoinositides (phosphorylated derivatives of the membrane lipid phosphatidylinositol [PI]) and small G proteins (Arfs, Rabs). For example, PI 4-phosphate (PI4P) is required for trafficking from the trans-Golgi and Rab11 regulates transport through the recycling endosome. We showed previously that the PI 4-kinase (PI4K) encoded by *fwd* is required for late stages of cytokinesis in *Drosophila* spermatocytes (Brill *et al.*, 2000), suggesting a role for Fwd in post-Golgi trafficking. Consistent with this model, we find that a functional GFP-Fwd fusion protein colocalizes with PI4P to the Golgi, PI4P is reduced in *fwd* mutant spermatocytes, and secreted proteins accumulate at the equator of dividing *fwd* mutant spermatocytes during telophase. To identify potential targets of Fwd during cytokinesis, we performed yeast two-hybrid experiments and found that Fwd binds to Rab11. Rab11 has previously been shown to regulate cytokinesis in mammalian tissue culture cells and cellularization of *Drosophila* embryos. As predicted, expression of a dominant negative Rab11 mutant transgene causes a weak cytokinesis defect in spermatocytes. More importantly, Fwd regulates Rab11 *in vivo*, as Rab11 is mislocalized in *fwd* mutant cells and activated Rab11 suppresses the cytokinesis defects observed in *fwd*. Based on our results, we hypothesize that Fwd acts via Rab11 to promote membrane trafficking required for successful completion of cytokinesis.

64

Drosophila post embryonic neuroblasts undergo a unique asynchronous centrosome maturation cycle. Nasser M. Rusan, Mark Peifer. Biology, University of North Carolina, Chapel Hill, NC.

Drosophila post-embryonic central brain neuroblasts (Nbs) are an excellent model for studying the mechanisms underlying asymmetric cell division. Recent work by Siller et al. nicely characterized mitotic spindle behavior in Nbs. Here, we examined the events that occur prior to mitotic entry; specifically testing the possibility that coarse spindle alignment is a result of anchoring the centrosome from the precious round of mitosis. The canonical view of centrosomes suggests that centrosomes separate, mature synchronously and adjacent to one another, followed by their separation to opposite sides of the nucleus in prophase and/or prometaphase. We have identified an exciting new centrosome cycle in Drosophila post-embryonic Nbs. We performed 4 and 5D spinning-disk confocal microscopy to follow centrosomes and microtubules (MTs) and noticed that during mitotic entry, the second centrosome materializes at some distance from the first, appearing out of nowhere. Our live imaging shows that mother/daughter centrioles disengage during late telophase. While one centriole remains stationary and retains PCM and a MT aster, the second centriole sheds all of its PCM and MTs, and migrates to the opposite side of the nucleus before the next round of mitosis. This unique behavior of the centrioles suggests a molecular or structural difference between the centriole(s). To address these possible differences, we stained Nbs for Centrosomin, gamma-tubulin and Polo kinase. We also imaged Polo-GFP in conjunction with a RFP-tagged centriole marker. In all cases, one centriole contained gamma-tubulin, Centrosomin and Polo, while the other was devoid of all the markers. Taken together, these data reveal a novel centrosome cycle that is unique to Nbs. This cycle differs from the canonical model and suggests a role for this asynchronous maturation in organizing the interphase cytoplasm, establishing cell polarity, orienting the mitotic spindle, and asymmetric Nb division. We are currently investigating this further.

65

CRM1 mediates regulation of oxygen-dependent HIF1/SIMA subcellular localization. Pablo Wappner[1], Maximiliano Irisarri[1], Peggy Roth[2], Christos Samakovlis[2], Nuria M. Romero[1]. 1) Fundacion Instituto Leloir, Buenos Aires, Argentina; 2) Department of Developmental Biology, Wenner-Gren Institute, Stockholm University, S-106 96 Stockholm, Sweden.

The Hypoxia Inducible Factor (HIF) has a central conserved role in oxygen homeostasis and is composed of two basic-helix-loop-helix (bHLH)-PAS protein subunits, HIF-α and HIF-β. Regulation of HIF-α by oxygen levels is mediated by several different mechanisms that include protein stability, transcriptional co-activator recruitment and subcellular localization. Previously, we have described that the Drosophila HIF-α protein, Sima, is mainly nuclear in hypoxia and accumulates in the cytoplasm in normoxia, but the molecular basis of this regulation is not understood. We show here that Sima shuttles continuously between the nucleus and the cytoplasm. Removal of the bHLH domain led to Sima nuclear accumulation, consistent with the occurrence of two nuclear export signals (NES) in this region that promote CRM1-dependent Sima nuclear export, both in cell culture and in vivo. Site directed mutagenesis of either NES provoked Sima nuclear retention and increased transcriptional activity of a LacZ reporter and of endogenous target genes, suggesting that nuclear export contributes to Sima regulation. These NES are conserved and functional in the bHLH domain of several other proteins of the bHLH-PAS family and therefore, we propose that these NES are important for rapid nuclear clearance of bHLH-PAS proteins upon cessation of the external stimulus.

66

The *Grainy head* downstream gene *Dret2* is required for efficient wound repair in *Drosophila* embryos. Shenqiu Wang[1], Vasilis Tsarouhas[1], Nikos Xylourgidis[1], Nafiseh Sabri[1], Marco Gallio[2], Katarina Tiklova[1], Christos Samakovlis[1]. 1) Department of Developmental Biology, Wenner-Gren Institute, Stockholm University, S-10691, Stockholm, Sweden; 2) Howard Hughes Medical Institute and Department of Neurobiology, University of California at San Diego, La Jolla, California 92093-0649, USA.

Efficient wound healing is a fundamental survival response. The repair of the embryonic epidermal barrier after wounding is mediated by *grainyhead (grh)* . Re-epithelialization depends on the activation of the JNK cascade, and the small GTPases rho and cdc42, which promote the dynamic cytoskeletal rearrangements crucial for wound closure . Here we report that *Dret2*, one of the two *Drosophila* homologs of the human oncogene *Ret*, is a direct target of Grh. Dret2 is an apically localized receptor tyrosine kinase expressed in most epithelial tissues. *Dret2* transcripts accumulate around epidermal wounds generated by puncture with a sterile needle. To test for a potential role of *Dret2* in the reconstruction of the cuticular barrier, we used a *ddc*GFP transcriptional reporter, which contains Grh, AP1and NfκB binding sites and is robustly activated at the site of embryonic wounds. The *ddc*GFP signal is reduced in wounded *Dret2* mutants suggesting that cuticle sclerotization is defective in *Dret2* animals. We analyzed re-epithelialization in wild type and *Dret2* embryos expressing the GFP-Moe cytoskeletal marker in the epidermis by live imaging. Aseptic puncture wounds of ~500 μm^2 become sealed within ~60 minutes in wild-type embryos. The wounds of 26% of *Dret2* embryos (n=19) did not close, although the embryos hatched and developed into larvae. In the remaining of the mutants the wounds closed in ~110 minutes, indicating that *Dret2* is required for the rapid re-epithelialization during embryonic wound healing. We propose that Dret2 promotes efficient wound healing by 1) activating the expression of genes involved in cuticular barrier repair and 2) regulating cytoskeletal rearrangements during re-epithelialization.

67

Zfh1 is required in the somatic stem cells of Drosphila testes, and can non-autonomously cause accumulation of excess early-stage germ cells. Judith Leatherman, Steve DiNardo. Dept Cell & Developmental Biol, Univ Pennsylvania, Philadelphia, PA.

Drosophila spermatogenesis is an excellent model system to study the properties of adult stem cells, particularly with regard to how stem cells interact with their niche. The germline stem cells (GSCs) are supported by two somatic niche populations: the hub and the somatic stem cells (SSCs). The hub is a group of non-dividing cells that provide a local signal, the Jak/STAT ligand Outstretched, that is required for GSC self-renewal. SSCs are a second stem cell population that produce the cyst cells, which surround each differentiating group of germ cells. While it is clear that cyst cells are required for germ cell lineage progression, the mechanisms controlling SSC behavior as a stem cell population are not well understood. We have identified a transcription factor, zfh1, which is required for SSC function. Zfh1 protein accumulates only in the hub and SSCs. It persists briefly in the daughters of the SSCs, but rapidly decays as the lineage progresses. We show by clonal analysis that zfh1 mutant SSCs do not persist in the testis. Thus, zfh1 accumulation in SSCs is required either for their self-renewal or for their survival. The rapid loss of Zfh1 protein in the cyst cell daughters of the SSCs suggested that its decay is important for proper cyst cell differentiation. Indeed, persistent zfh1 expression in cyst cells led to a dramatic over-proliferation of somatic cells. Furthermore, the zfh1-expressing somatic cells caused a striking non-autonomous effect on encysted germ cells: most did not express the differentiation marker Bam, and were organized in pairs, sharing a fusome between them, even though they were distant from the hub. These undifferentiated germ cells resemble GSC-daughter cell pairs with their long-lived cytoplasmic bridges, and are similar to what is observed with ectopic activation of the Jak/STAT pathway (Tulina and Matunis, 2001, Kiger et al, 2001). Alternatively, the excess germ cell pairs could be blocked in differentiation at the two-cell spermatogonial cyst stage. We are currently trying to distinguish between these possibilities.

68

Socs36E mediated JAK/STAT signal attenuation regulates the balance of germline and somatic stem cells in the *Drosophila* testis niche. Melanie Issigonis[1], Natalia Tulina[2], Laurel Mellinger[1], Crista Brawley[1], Erika Matunis[1]. 1) Cell Biology Department, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Genetics and Gene Regulation Program, University of Pennsylvania Medical School, Philadelphia, PA.

Stem cells are essential for generating, sustaining, and repairing tissues. Stem cells are maintained in microenvironments, or niches, that control their behavior, but little is known about the extrinsic cues and intrinsic signaling that regulate stem cell niches. In the *Drosophila* testis, germline stem cells (GSCs) and somatic stem cells (SSCs) both adhere to a cluster of non-mitotic cells called the hub, which prevents stem cell differentiation by local secretion of the JAK/STAT pathway ligand Unpaired. The JAK/STAT signaling pathway is required for GSC and SSC maintenance. Although factors required for stem cell identity have been characterized for many different niches, little is known about signal attenuation. Proteins of the mammalian Suppressor of Cytokine Signaling (SOCS) family are the best-characterized negative regulators of the JAK/STAT pathway. Like its mammalian orthologues, several studies have indicated that *Drosophila socs36E* negatively regulates JAK/STAT signaling as well. However, loss-of-function studies of *socs36E* have not been reported. We have found that *socs36E* is a STAT target in the testis and is expressed strongly in the hub as well as in SSCs. An enhancer trap line with a P-element insertion in the coding region of the *socs36E* gene, which is a loss-of-function allele designated as *socs36E[1647]*, causes testes to have a significantly lower number of GSCs. Mosaic analysis indicates that GCSs do not directly require *socs36E* in order to prevent GSC loss but rather SSCs need *socs36E* to suppress SSC accumulation in the niche and subsequent GSC loss. This work demonstrates how a single niche coordinately regulates two stem cell populations. By carefully regulating the level of signaling within each cell lineage, a proper balance of each stem cell population is maintained in the tissue.

69

Analysis of Modulo-dependent transcription factors identifies essential regulators of testes specific gene expression. Dmitry Nurminsky, Lyudmila Mikhaylova. Dept Anatomy & Cellular Biol, Tufts Univ Sch Medicine, Boston, MA.

We recennly demonstrated that Modulo, Drosophila homologue of nucleolin, binds to the testes specific promoters, interacts with testes specific components of transcriptional machinery, and positively regulates transcription of a number of testes specific genes. Some of the Modulo-dependent genes do not possess Modulo binding sites in their promoters, indicating that they may be regulated by Modulo indirectly. Such regulation may be mediated by Modulo-dependent transcription factors. We analyzed a panel of 98 transcriptional regulators with testes-biased transcription pattern, and found that ten of these regulators are severely down-regulated in Modulo mutant testes. Available mutants for these regulators were analyzed for male fertility, leading to identification of two genes that are required for spermatogenesis. These genes code for conserved proteins, one of which also has a documented function in male gametogenesis in mammals. Thus, dissection of the transcriptional cascade downstream of Modulo is fruitful for identification of essential components of the mechanism of transcriptional regulation in testes. We are currently generating tissue specific knock-down constructs in order to characterize involvement of other Modulo-dependent transcriptional regulators in testes-specific gene expression.

70

Defects in the individualization step of spermatogenesis produced by depression of Androcam expression with RNAi. Kathleen Beckingham, Robert YS. Lee, Rebecca A. Simonette, William Deery. Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

Androcam (Acam) is a testis-specific protein that shows 68% sequence identity to the universal calcium sensor protein Calmodulin. Biochemical studies of Acam indicate that, like Calmodulin, Acam is a calcium sensor. Thus Acam shows high affinity calcium binding accompanied by marked conformational change. Immunolocalization studies previously indicated that Acam has a role in the sperm individualization step. Before individualization, 64 immature spermatids are all contained within the same cytoplasm. Production of 64 individual sperm, each encased in its own plasma membrane, involves the movement of 64 specialized structures, termed the actin cones, in concert along the sperm axonemes. Excess cytoplasm is pushed ahead of the cones and plasma membranes are laid down behind them around each axoneme. Acam accumulates at the flat leading edge of the cones where we have evidence that it plays a role as a light chain for Myosin VI. In order to investigate this and other possible roles of Acam in individualization, we have generated lines that express Acam RNAi constructs in the testis. Several defects in individualization are seen in these lines. Fewer cone sets mature and become capable of movement. Further, resorption of the "waste bags" of discarded cytoplasm does not proceed normally. Most surprisingly, in some cases two sets of cones, not one, are seen moving up the sperm axonemes. These defects and their possible origins will be discussed in detail.

71

Prostaglandins mediate *Drosophila* egg maturation in vitro. Tina L. Tootle, Allan Spradling. HHMI/Carnegie Institution, Baltimore, MD.

Prostaglandins are both short-lived and short-ranged lipid signaling molecules that have been implicated in almost every aspect of biology, from roles during female reproduction to both normal and cancerous cell migrations. In mammals, prostaglandins are required for follicle maturation, ovulation, fertilization, implantation, maintenance of pregnancy, and induction of labor. While individual prostaglandins and distinct downstream receptors have been implicated in these events, the exact signaling cascade and molecular outcome of prostaglandin signaling during female reproduction remain unclear.

We have established *Drosophila* oogenesis as a model system for studying prostaglandin signaling. By taking advantage of the fact that a stage 10B egg chamber can develop in vitro to a virtually mature stage 14, we have manipulated prostaglandin signaling using both pharmacological and genetic means. Inhibitors of prostaglandin synthesis block egg chamber maturation, while the addition of prostaglandins rescues development. BLAST analyses reveal *Drosophila* Pxt is likely to be a homolog of mammalian cyclooxgenase (COX) enzyme, a.k.a. prostaglandin-endoperoxidase G/H synthase (PGHS), the rate-limiting enzyme responsible for prostaglandin synthesis. Mutations in pxt block in vitro egg maturation and are rescued by exogenous prostaglandins. Additionally, manipulation of downstream aspects of prostaglandin synthesis and signaling also inhibit egg chamber maturation. Therefore, prostaglandins and Pxt, a putative COX enzyme, mediate *Drosophila* egg chamber or follicle maturation.

This is the first genetic study revealing a role for prostaglandins in insects. *Drosophila* oogenesis is an excellent system to use to piece together the details of prostaglandin signaling and it biological outcomes.

72

The *stall* gene is an extra-ovarian regulator of follicle formation that encodes an ADAM-TS metalloprotease. Emily F. Ozdowski, Claire Cronmiller. Univ Virginia Biology, Charlottesville, VA.

Oogenesis in *Drosophila* requires somatic-germline intercommunication to organize follicle formation, and *stall* (*stl*) is an important component of this regulation. *stl* mutant females are completely sterile due to early defects in interfollicular stalk formation and failed germline cysts individualization. The *stl* mutant phenotype first appears as disorganization in the pupal ovary. At eclosion, ovariole morphology is severely defective with extensive apoptosis. Clonal analysis and ovary transplantation indicate that wildtype *stl* function within the ovary alone is not sufficient for follicle formation: Normal oogenesis requires extra-ovarian *stall* function. We used numerous genetic and molecular approaches to identify *stall* molecularly, identifying unique lesions in *CG3622* for five independent *stl* alleles. *stl* encodes a protein similar to an ADAM-TS, a disintegrin and metalloprotease with thrombospondin domains. Members of this conserved protein family are involved in extracellular matrix modifications and membrane protein domain shedding, generally in cell adhesion and intercellular signaling. How Stl protease regulates ovarian follicle formation from some distant tissue is not known, but preliminary RT-PCR data indicate that (1) *stl* is expressed in adult brain and ovary and (2) two Stl isoforms are differentially expressed in these tissues. *In situ* hybridization and immunofluorescence reveal *stl* expression in ovarian somatic cells as they first surround a germline cyst, but not in stalk cells, or more mature follicular epithelia. Based on these observations and computer predictions of isoform-specific intracellular localization, we present a model for extrinsic Stl function, which includes separate roles for brain versus ovary Stl isoforms. Ongoing studies are aimed at the identification of the tissue source of extra-ovarian Stl and the targets of Stl's regulatory protease activity.

73

Ecdysone Receptor Activity Regulates Cell Migration in the Ovary. Jennifer F. Hackney, Leonard Dobens, Benjamin D. Levine, Angela Truesdale. Molecular Biology and Biochemistry, University of Missouri-Kansas City, 5007 Rockhill Rd, Kansas City, MO.

Ecdysone Receptor (EcR) mediates effects of the steroid hormone ecdysone during larval molts, pupal metamorphosis and oogenesis in the female. In the adult female ovary, egg chamber maturation requires interactions between the somatic follicle cell (FC) epithelium and the germ line nurse cell/oocyte cyst. Previous work has demonstrated a role for EcR in the germ line for vitellogenesis, and here we examine EcR requirements for FC differentiation. EcR protein is ubiquitous in the FC but its activity is restricted visualized by expression of an *EcRE-lacZ* target gene and the 'ligand sensor' GAL4-EcR ligand binding domain fusion. EcR activity at late stage 10 was modulated by both opposing ecdysone and ras-MAPK signals. To determine the significance of restricted sites of EcR activity and target gene expression in the FC, we used targeted misexpression of a dominant negative EcR (DNEcR) molecule. In the nurse cell FC, DNEcR lowered *EcRE-lacZ* expression, indicating that DNEcR effectively blocks ecdysone responses in this tissue. At stage 10, DNEcR expression interfered with proper FC migrations, including (1) a block of centripetal migration resulting in cup-shaped eggs and (2) abnormal dorsal appendage tube formation resulting in shortened, branched dorsal appendages. DNEcR effects on FC migrations were associated with aberrant increases in DE-cadherin expression indicating that EcR modulates cell adhesion during FC migrations. At later stages, DNEcR caused thin eggshell phenotypes that correlated with reduced levels of chorion gene expression and reduction of amplification of the chorion gene loci. Our results indicate that tissue-specific modulation of EcR activity in part by the ras signaling pathway mediates temporal ecdysone signals that regulate FC differentiation and migration.

74

Intersexuality in the gonadal soma provides surprising insights into *transformer(tra)* and *doublesex(dsx)* function. Scott Siera, Thomas W. Cline. Department of Molecular Cell Biology, University of California Berkeley, Berkeley, CA.

Our studies of intersexuality in the gonadal soma have revealed that control of sexual differentiation in this tissue differs from that in other somatic tissues. The gonadal soma is unusually sensitive to perturbations affecting the *Sex-lethal(Sxl)* positive autoregulatory loop, which cell-autonomously establishes and maintains the female developmental fate. We identified a combination of hypomorphic *Sxl* alleles that interfere with engagement of the *Sxl* autoregulatory loop in the female gonadal soma without greatly compromising female viability or appearing to alter the sexual differentiation of other tissues. Although the ovaries of these females exhibit wild-type morphology through the larval stage, sexual differentiation of the gonadal soma becomes grossly abnormal during metamorphosis, resulting in small, disorganized adult gonads that express markers indicating intersexuality. We observed that *dsx* is misregulated in the larval gonads of these mutants, but *dsx* misregulation does not seem to be the cause of this gross disruption of gonad differentiation. The gonads of *dsx* null females were intersexual, but well organized and fully differentiated as expected, while the gonads of *Sxl dsx* double mutants displayed the disrupted phenotype characteristic of the *Sxl* single mutants. In contrast, when we made the *Sxl*-mutant soma uniformly male by eliminating *tra*—the target of *Sxl* and direct regulator of *dsx*—gonad organization was rescued. Rescue was also observed when we made the soma uniformly female by constitutively expressing tra^F, the female form of *tra*. These females were fertile. Together, these results argue for the existence of a *dsx*-independent target of *tra* necessary for the growth and organization of the gonadal soma. Surprisingly, overexpressing tra^F in the maternal germline also rescued the *Sxl* mutant daughters, even though such expression had no apparent effect on sons. Although tra^F is not required for the differentiation and function of female germ cells, we found tra^F in wild-type unfertilized eggs, suggesting there may be a subtle role for tra^F in the female germline.

75

The tumor suppressor Lgl controls the distribution of Fragile X protein in developing neurons. Daniela Zarnescu, Marianna Pinter, Patty Estes, Subha Srinivasan, Adeel Yang. Dept Mol & Cell Biol, Univ Arizona, Tucson, AZ.

Fragile X protein (FMRP) is an RNA binding protein thought to function in neural development and plasticity by controlling the transport and translation of target mRNAs. Fmrp deficiency leads to defects in neural morphology and function and causes Fragile X syndrome, the most inherited form of mental retardation. Recently, we took a forward genetic approach to identify novel functional interactors of FMRP in *Drosophila* and identified *lethal giant larvae* (*lgl*), a tumor suppressor involved in the establishment and maintenance of cell polarity. Through a combined genetic, biochemical and cell biological approach, we have shown that Lgl as well as the PAR cell polarity complex are novel functional partners of FMRP. Furthermore, we have identified a subset of mRNAs associated with the FMRP/Lgl complex. Taken together, our previous work suggests that Lgl functions with Fmrp and a subset of target mRNAs during synaptic development and/or function. To elucidate the molecular mechanism by which Lgl regulates FMRP/mRNA complexes during neural development we have developed an imaging system for Lgl/FMRP/mRNA in *Drosophila* neurons. Primary neurons derived from the larval central nervous system exhibit well-developed and polarized neurites, which contain motile FMRP/Lgl particles. Furthermore, FMRP colocalizes with target mRNAs within developing neurites. We examined the distribution FMRP granules in an *lgl* mutant background and found that most FMRP is distributed in the cell soma and excluded from neurites. Interestingly, overexpression of a phospho-mutant form of Lgl (Lgl3A) specifically abolishes the localization of FMRP in the axon but not in the dendrites. Taken together, our data suggest that we have uncovered a novel mechanism for the polarized delivery of FMRP and associated target mRNAs in neurons, which may provide new insights into the molecular mechanisms of neuronal development and Fragile X syndrome.

76

Minocycline blocks oxidative stress and confers neuroprotection against paraquat induced Parkinson's model. Arati Inamdar, Anathbandhu Chaudhuri, Amellia Cannon, J. Barne, Janis O'Donnell. Dept Biological Sci, Univ Alabama, Tuscaloosa, AL.

Parkinson's Disease (PD) is a common neurodegenerative disease pathologically characterized by the loss of dopaminergic (DA) neurons in substantia nigra. Genetic and environmental factors are proposed to play role in pathogenesis of PD. Paraquat (PQ), a common herbicide,has been known as a potential neurotoxin associated with PD in epidemiological studies. Using paraquat, we have successfully generated a Parkinson's Disease *Drosophila* model, which recapitulates characteristic symptoms of PD with modulation of DA pools and marked reduction in specific clusters of the DA neurons in a dose-dependent manner. Recently minocycline, a semi-synthetic, second generation tetracycline derivative, has shown promising ameliorative effects for neurodegenerative diseases including PD. While the exact mechanism of action of minocycline is not clear, it has both anti-inflammatory and anti-apoptotic properties. We investigated whether minocycline has protective properties in our PD model, reasoning that a conservation of its protective effects could contribute to an understanding of cellular mechanisms of minocycline action and identification of necessary genetic components. First, we tested the effects of minocycline by feeding it alone to wild-type adults and found no apparent effects on their behavior or survival. We found that co-feeding of paraquat and minocycline prolonged survival duration and rescued the PQ-induced mobility defect that is typical in paraquat exposure. Minocycline blocks the PQ induced changes in the dopamine biosynthesis pathway indicative of oxidative stress. We further found that minocycline also scavenges the reactive oxygen species as evident by catalase and lipid peroxidase assays. We also examined the neuroprotective role of minocycline on DA neurons in the brains of paraquat-exposed adults and found that neurons typically at risk from PQ induced oxidative stress survived longer.We will further report the studies of minocycline effects on DA-regulatory mutants, *Punch* and *Catsup* that are important in the identification of components necessary for minocycline action.

77

Hsp70 protects Drosophila brain neurons against Prion-dependent neurodegeneration. Pedro Fernandez-Funez, Sergio Casas-Tinto, Ana Cepeda-Nieto, Claudio Soto, Diego Rincon-Limas. Dept Neurology, Univ Texas Medical Branch, Galveston, TX.

Prion diseases or Transmissible Spongiform Encephalopathies (TSE) are a group of lethal neurodegenerative disorders affecting humans and animals for which no therapeutic treatments exist. These maladies have genetic, sporadic, and infectious origins; however, prion transmissibility is the most studied feature because of the public health concerns. In TSE, the normal cellular protein (PrPC) is converted into a misfolded and pathogenic isoform (PrPSc), which subsequently accumulates in the brain, leading to rapid spongiform neurodegeneration and death. Despite the progress made in understanding prion biology, a major gap exists in the knowledge of how the conformational conversion of PrP ultimately kills neurons. Recent in vitro studies suggest that protein quality control and cellular stress may be critical factors mediating neuronal dysfunction. We have generated transgenic flies expressing wild type PrP from hamster (HaPrP) and mutant form of HaPrP carrying a mutation transmitted in genetic prion disorders. HaPrP accumulates in membranous structures associated to the Golgi and the secretory machinery, and in the cellular membrane. HaPrP exhibits progressive insolubility and leads to neurodegeneration in flies. Thus, HaPrP processing, cellular distribution, conformational alterations and neurodegenerative effects in flies are consistent with features described in animal models and in human patients of TSE. Our new models of sporadic and genetic prion neurotoxicity can be used for uncovering new mechanisms implicated in PrP-dependent neurodegeneration. We show for the first time that Hsp70 contributes to PrP turnover and protects against PrP-dependent neurodegeneration in flies. We propose that Hsp70 and other molecular chaperones could be relevant to PrP neurotoxicity in humans.

78

pink1 and parkin function in the same pathway to regulate mitochondrial function. Ming Guo, Mark Dodson, Ira Clark, Changan Jiang, Renny Feldman, Joseph Cao. Dept Neurology, Univ California, Los Angeles, Los Angeles, CA.

Mutations in PTEN-induced kinase 1 (pink1, PARK6) and parkin (PARK2) cause familial and sporadic forms of Parkinson's disease (PD). pink1 encodes a serine/threonine kinase with a mitochondrial targeting sequence. We show that removal of Drosophila pink1 results in male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology and increased sensitivity to oxidative stress. Expression of human pink1 in Drosophila restores mitochondrial function in pink1 mutants, demonstrating functional conservation. Loss of Drosophila parkin shows phenotypes similar to loss of pink1. Genetic tests show that pink1 and parkin function in the same genetic pathway, with pink1 functioning upstream of parkin to regulate mitochondrial function. Biochemical studies suggest that Pink1 is localized to mitochondria, and that Pink1 binds to Parkin. Though pink1-mediated PD is thought to be largely recessive, several mutations have been found in heterozygous patients, raising the possibility that these mutations may have gain-of-function, haplo-insufficient (loss-of-function) or dominant negative effects. Expression of human disease version of pink1 (G309D and Q456Stop) at physiologic (endogenous) levels in wildtype flies has no phenotype, whereas expression in the pink1 null background results in partial function. These results suggest that the human mutations G309D and Q456Stop exert their disease-causing effects as a result of partial loss of pink1 function.

79

Mitochondrial encephalomyopathy in Drosophila: Pathogenic mechanisms and therapeutic approaches. Michael Palladino[1,2], Alicia Celotto[1,2], Nicole Kotchey[1,2], Adam Frank[1,2]. 1) Department of Pharmacology, Univ Pittsburgh SOM, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, Univ Pittsburgh, Pittsburgh PA.

Mitochondrial encephalomyopathies are a diverse and complex class of diseases that result from defects in mitochondrial respiratory chain function. These disorders present with several overlapping symptoms that include neuromuscular degeneration, cognitive decline, visual impairment, lactic acidosis, and reduced longevity. Model systems in which to study encephalomyopathies are limited at best. Using the fruit fly *Drosophila*, we have isolated a missense mutation in the *ATP6* gene, which represents the first *in vivo* genetic model of an endogenous mitochondrial genome mutation. The *ATP6* gene encodes an essential subunit of the ATP synthase (Complex V). The human diseases neuropathy, ataxia, retinitis pigmentosa (NARP), maternally inherited Leigh Syndrome (MILS), and familial bilateral striatal necrosis (FBSN) all result from missense mutations within this genetic locus. Strikingly, the phenotypes of *ATP6[1]* missense mutants - shortened lifespan, muscle degeneration, mitochondrial dysfunction, stress sensitivity, and progressive neural dysfunction - mirror the symptoms of these human conditions. Our data demonstrate profound ultrastructural impairment of the mitochondria and severely reduced mitochondrial ATP synthase activity. Surprisingly, respiration is not altered despite the marked reduction in mitochondrial ATP production. We are utilizing our model to explore the underlying mechanisms of disease pathogenesis and to test the efficacy of allotopic expression as a potential gene therapy for mitochondrial disease.

80

Ataxin-2 is a critical modulator of neurodegeneration induced by the pathogenic polyglutamine protein Ataxin-3. Derek Lessing, Nancy Bonini. Department of Biology & Howard Hughes Medical Insititute, University of Pennsylvania, Philadelphia, PA.

Many human movement disorders are the result of neural degeneration in the cerebellum and other regions of the CNS. Six of these diseases, called spinal cerebellar ataxias, are each caused by a dominant mutation in the respective *SCA* gene, an expansion of a CAG repeat that results in 40 or more glutamine residues. The SCA proteins, known as ataxins, have a wide diversity of functions and no common domains outside of the polyglutamine repeat. The pathogenic ataxins are thought to adopt a misfolded conformation upon polyglutamine expansion, usually resulting in accumulation into inclusions. There is intriguing but minimal clinical evidence of interactions between *SCA2* and *SCA3* in the promotion of neurodegeneration, despite the common type of mutation and the common symptoms of these two SCA diseases. Previously we have modeled SCA3 by expressing the human pathogenic Atx3 protein with an expanded polyglutamine domain in the fly eye. Here, we show that *atx2* is a potent modifier of this SCA3-induced degeneration. Drosophila *atx2* is the ortholog of human *SCA2*, which causes the eponymous human ataxia when mutated. We show that overexpression of wild-type *atx2*, encoding a normal-length polyglutamine domain, can synergistically enhance the degeneration caused by both truncated and full-length forms of pathogenic human SCA3 in flies. Overexpression of normal *atx2* by itself is sufficient to cause neurodegeneration. Strikingly, loss of endogenous *atx2* function suppresses SCA3-induced neurodegeneration. Atx2 hastens the accumulation of SCA3 protein into inclusions and shows limited colocalization with SCA3 in these inclusions. The conserved PAM2 domain of Atx2 is required for Atx2-mediated degeneration and to enhance SCA3 degeneration, and we are investigating the potential role of Atx2 effectors that bind the PAM2 domain. Finally, we show that Atx2 interacts selectively with other polyglutamine disease models. Our work suggests that the SCA genes are likely to interact in humans in influencing the pathogenesis and progression of neurodegeneration.

81

Induction of autophagy and impairment of the proteasome in Drosophila model of spinal and bulbar muscular atrophy. Udai Pandey[1], Zhiping Nie[1], Yakup Batlevi[2], Stephanie Schwartz[1], Deborah Berry[2], Oren Schuldiner[3], Eric Baehrecke[2], J. Paul Taylor[1]. 1) Dept Neurology,233 Stemmler Hl, Univ Pennsylvania, Philadelphia, PA; 2) Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742 USA; 3) Department of Biological Sciences, Stanford University, Stanford CA 94305.

Spinobulbar muscular atrophy (SBMA, also known as Kennedy's disease) is an inherited neurodegenerative disease that is caused by trinucleotide repeat expansion in the androgen receptor (AR) gene. The ubiquitin-proteasome system (UPS) and macroautophagy (autophagy) are distinct pathways that carry out regulated protein degradation. The UPS is responsible for tightly-regulated proteolysis of short-lived proteins and rapid elimination of abnormal proteins, whereas autophagy is responsible for bulk degradation of cytoplasmic components through a lysosome-dependent pathway. Here we demonstrate that a Drosophila model of a spinal and bulbar muscular atrophy (prototypical conformational disease) shows impairment of the UPS and induction of autophagy. We also show that impairment of the 20S proteasome by mutations in the β2 subunit results in the induction of autophagy in Drosophila. Silencing the autophagy genes atg6 or atg12 enhances these degenerative phenotypes, whereas treatment with the specific TOR inhibitor rapamycin suppresses degeneration. Together, these results indicate that induction of autophagy is cytoprotective when the UPS is impaired. These observations provide evidence of UPS impairment in spinal and bulbar muscular atrophy in vivo, reveal a complementary relationship between the UPS and autophagy, and have important implications regarding both the pathogenesis of neurodegenerative diseases and strategies of therapeutic intervention.

82

Genetic modifiers of *Drosophila* palmitoyl-protein thioesterase 1 induced degeneration. Christopher Korey, Haley Buff, Alexis Smith. Department of Biology, College of Charleston, Charleston, SC.

Infantile Neuronal Ceroid Lipofuscinosis (INCL) is a pediatric neurodegenerative disease caused by mutations in the human *cln1* gene. *Cln1*encodes palmitoyl-protein thioesterase 1 (Ppt1) suggesting an important role for the regulation of palmitoylation in normal neuronal function. Loss of Ppt1 function in patients produces autoflourescent lysosomal inclusions in all cells, but neurons are primarily affected leading to massive neurodegeneration. *Ppt1*$^{-}$ flies are viable although with a reduced life span; show a CNS-specific accumulation of autoflourescent storage material and cytoplasmic inclusions suggesting an evolutionary conservation of function in Drosophila that will make the fly a powerful model for understanding the molecular etiology of human NCL phenotypes. To further elucidate Ppt1 function, we performed a gain-of-function modifier screen using a collection of enhancer promoter (EP) transgenic overexpression lines to suppress or enhance the degeneration produced by overexpression of Ppt1 in the adult visual system. Modifier genes identified in our screen tie Ppt1 function to synaptic vesicle cycling (*endophilin A, synaptotagmin 1, stoned A*), endo-lysosomal trafficking (*blue cheese*), and several genes known to be important for neuronal development and function (*saxophone, myospheroid, misshapen, fasciclin II*). Furthermore, several of the modifying genes, such as synaptotagmin 1, are known to be regulated by palmitoylation in other systems and may be in vivo substrates for Ppt1. The pathways and processes implicated by our modifier loci shed light on the neuronal dysfunction underlying the disease and will be valuable points of entry in the future development of therapeutics that aim to ameliorate the early symptoms of INCL and thus avoid the neurodegeneration typical of later stages of the disease.

83

Actin related protein-3(Arp3) is required for Delta trafficking during Notch signaling. Akhila Rajan[1,4], An-Chi Tien[2,4], Karen L. Schulze[3], Hugo J. Bellen[1,2,3]. 1) Dept of Molecular and Human Genetics; 2) Program in Developmental Biology; 3) HHMI,Baylor College of Medicine; 4) Equal Contribution.

Bristles present on the thorax of the adult fly are external sensory organs (ESOs) that are a part of the peripheral nervous system (PNS) of *Drosphila* . Their specification is controlled by the Notch signaling pathway. Therefore, we performed a forward genetic screen to isolate mutations affecting ESO development. We isolated one group with bristle loss in thorax clones. Phenotypic analysis revealed that all the ESOs in the clone had undergone a cell fate transformation to form only neurons, similar to a *Notch* loss-of-function phenotype. Mapping identified lesions in *Arp3*, a component of the evolutionarily conserved Arp2/3 protein complex, which is required for actin branching. Mutations in *Arpc1*, another component of the Arp2/3 complex, shows a similar cell-fate transformation phenotype,but the mechanism underlying this phenotype is unknown. Work in yeast has shown that the Arp2/3 complex is required for endocytosis. It is known that the ligand Delta(Dl) needs to be endocytosed in the signal-sending cell in order to activate Notch in the receiving cells. Based on these observations our hypothesis was that Arp2/3 is required for a crucial step in endocytosis of Dl. We performed endocytosis assays to assess the ability of *Arp3* and *Arpc1* mutant cells to endocytose Dl. Surprisingly we find that Dl can be endocytosed normally in *Arp3* and *Arpc1* mutant cells. It has been shown that the internalized Dl needs to traffic through the recycling endosomal compartment in order to signal properly. When we investigated if the endocytosed Dl traffics correctly we find that in *Arp3* and *Arpc1* mutant cells the endocytosed Dl does not traffic to the recycling endosome. This suggests that incorrect trafficking of Dl is the defect underlying the phenotype. Therefore,we have identified that the cytoskeletal complex Arp2/3 is required for Notch signaling, and it functions in a specific trafficking event required for Dl activation during the process of cell fate specification.

84

The *O*-fucosyltransferase *O-fut1* is an extracellular component that is essential for the constitutive endocytic trafficking of Notch in *Drosophila*. Kenji Matsuno, Takeshi Sasamura, Hiroyuki Ishikawa, Nobuo Sasaki, Tomonori Ayukawa, Kenta Yamada, Tomoko Yamakawa. Dept. Biol. Sci./Tec., Tokyo University of Science, Noda, Chiba, JP.

Notch is a transmembrane receptor that mediates the cell-cell interactions necessary for many cell-fate decisions. Endocytic trafficking of Notch plays important roles in the activation and down-regulation of this receptor. A *Drosophila O*-FucT-1 homolog, encoded by *O*-fut1, catalyzes the *O*-fucosylation of Notch, a modification essential for Notch signaling and ligand binding. It was recently proposed that *O*-fut1 acts as a chaperon for Notch in the endoplasmic reticulum and is required for Notch to exit the endoplasmic reticulum. Here, we report that *O*-fut1 has additional functions in the endocytic transportation of Notch. *O-fut1* was indispensable for the constitutive transportation of Notch from the plasma membrane to the early endosome, which we show was independent of *O*-fut1's *O*-fucosyltransferase activity. We also found that *O-fut1* promoted the turnover of Notch, which consequently down-regulated Notch signaling. *O*-fut1 formed a stable complex with the extracellular domain of Notch. In addition, *O*-fut1 protein that was added to conditioned medium and endocytosed was sufficient to rescue normal Notch transportation to the early endosome in *O-fut1* knockdown cells. Thus, an extracellular interaction between Notch and *O*-fut1 is essential for the normal endocytic transportation of Notch. We propose that *O*-fut1 is the first example, except for ligands, of a molecule that is required extracellularly for receptor transportation by endocytosis.

85

Lethal giant discs, a novel C2-domain protein, restricts Notch activation during endocytosis. Jennifer Childress[1,2], Melih Acar[3], Chunyao Tao[1], Georg Halder[1,2,3]. 1) Dept Biochem & Molecular Biol, Univ Texas, Houston, Houston, TX; 2) Program in Genes and Development, Univ Texas, MD Anderson Cancer Center, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX.

The Notch signaling pathway plays a central role in animal growth and patterning, and its deregulation leads to many human diseases, including cancer. Mutations in the tumor suppressor *lethal giant discs* (*lgd*) induce strong Notch activation and hyperplastic overgrowth of *Drosophila* imaginal discs. However, the gene that encodes Lgd and its function in the Notch pathway have not yet been identified. Here, we report that Lgd is a novel, conserved C2-domain protein that regulates Notch receptor trafficking. Notch accumulates on early endosomes in *lgd* mutant cells and signals in a ligand-independent manner. This phenotype is similar to that seen when cells lose endosomal-pathway components such as Erupted and Vps25. Interestingly, Notch activation in *lgd* mutant cells requires the early endosomal component Hrs, indicating that Hrs is epistatic to Lgd. These data suggest that Lgd affects Notch trafficking between the actions of Hrs and the late endosomal component Vps25. Taken together, our data identify Lgd as a novel tumor-suppressor protein that regulates Notch signaling by targeting Notch for degradation or recycling.

86

Analysis of the function of Presenilin during the development of Drosophila. Gunter Merdes[1,2], Denise Stempfle[1,2], Alexandra Wojtalla[1,2], Abil Saj[1,2], Renato Paro[1,2]. 1) BSSE, ETH Zuerich, Basel, CH; 2) ZMBH, University of Heidelberg, Heidelberg, D.

The Presenilin (PS) complex cleaves proteins within their transmembrane domain. Mutations in PS have been linked to Alzheimer's Disease. Besides the Amyloid Precursor Protein (APP) multiple transmembrane proteins have been identified undergoing PS-mediated regulated intramembrane proteolysis (RIP). Particularly, PS is essential for the RIP of the Notch receptor. Consequently, lof alleles of PS result in Notch lof phenotypes. However, despite extensive analyses little is known how PS exactly executes its function, how PS activity is regulated, and which signaling pathways besides Notch depend on PS activity in vivo. To address these questions, we have established a reporter system in Drosophila allowing us to analyse the PS-dependent processing of APP and Notch during development. Using this system, we have demonstrated spatial and temporal differences between APP and Notch processing. Furthermore, our results indicate that, in contrast to current models, the release of the intracellular domain of APP and two other PS substrates can be regulated in different cell types by modulated PS activity. Currently, we are extending our reporter system to other potential endogenous PS substrates. Several groups have identified additional PS substrates besides APP and Notch in mammalian cell culture systems, suggesting that PS may be involved in multiple signaling pathways. However, in most of the cases the in vivo relevance of these findings has not been established, and it is unclear whether, like in the case of Notch, the released ICDs directly participate in nuclear signaling events. Therefore, we have modified our reporter system to study processing and nuclear signaling of Drosophila homologs of potential PS substrates simultaniously in vivo during development (dual reporter system). We will present data about the cleavage and signaling capacity of numerous transmembrane proteins analysed in different tissues and during different timepoints of development.

87

The roles of the D-cbl long and short isoforms in the regulation of MAPK. Hannah Robertson, Gary Hime. Department of Anatomy & Cell Biololgoy, University of Melbourne, Melbourne, VIC , AU.

A great deal is known about the biochemical functions and interactions of the cbl family of proteins as E3 ubiquitin protein ligases that link activated cell surface receptors to the endocytic pathway, but the precise impact of this negative regulation on specific downstream signalling pathways remains unclear. The current model is that c-Cbl and CblB regulate the length of time receptor complexes are able to signal, thus regulating signal duration. We have used the *Drosophila* eye to investigate how the two D-cbl proteins, D-cblL and D-cblS, impact on MAPK activation. Here we show that 1) ectopic D-cblL is able to reduce MAPK activation to a functional level of zero; 2) D-cblS has both postive and negative effects on MAPK activation, 3) neither isoform is required for MAPK activation and 4) D-cbl is required for timely attenuation of MAPK activation. Our data suggest that D-cblL and, by inference, c-Cbl and CblB, prevent signalling in response to low levels of ligand while D-cblS ensures that, once activated, signalling can be terminated. Thus we propose a model in which D-cblL acts first to elimate the effects of low levels of receptor dimerisation at the far edge of a ligand gradient, while D-cblS reduces the duration of signalling, as previously proposed for the D-cblL analogues c-Cbl and CblB. Our observations differ significantly from studies of mammalian c-Cbl and CblB and we present evidence that this is due to differences in experimental systems and approaches.

88

The exon junction complex component Mago-nashi is required for EGF receptor signaling. Jean-Yves Roignant[1], Florence Janody[2], Jessica treisman[1]. 1) Dept Developmental Genetics, Skirball Inst, New York, NY; 2) Instituto Gulbenkian de Ciência Rua da Quinta Grande, 6 P-2780-156, Oeiras, Portugal.

In a mosaic genetic screen for genes that affect photoreceptor differentiation, we isolated three alleles of *mago-nashi* (*mago*). Mago is a highly conserved component of the exon junction complex, which associates with spliced mRNAs in the nucleus and remains associated at the exon junctions after nuclear export. The exon junction complex is required for nonsense-mediated decay and to enhance translation of vertebrate mRNAs, but its only proven role in *drosophila* is to regulate *oskar* RNA localization during oogenesis. In *mago* mutant clones, the R8 photoreceptor can differentiate normally, but it fails to recruit R1-7, a process that requires signaling through the epidermal growth factor receptor (EGFR) pathway. We showed that expression of *argos* and *pointed P1*, two positively regulated targets of the EGFR pathway, is absent in *mago* mutant clones while expression of *cyclin B*, which is normally repressed by the EGFR pathway posterior to the morphogenetic furrow, is increased in *mago* mutant clones. We also detected increased cell death in the absence of *mago*, consistent with the anti-apoptotic role of EGFR signaling. *mago* is also required in the wing disc to promote *argos* expression, suggesting that it may be a general component of the EGFR pathway. Epistasis experiments indicate that Mago acts downstream of Raf activity but upstream of MAPK. We are investigating whether other core components of the exon junction complex, including Y14 and Barentsz, have similar effects on EGFR signaling. If so, it will suggest that the exon junction complex regulates a component of the EGFR pathway by altering the localization or translation of its mRNA.

89

Evidence for intrinsic signaling differences between EGF and FGF receptor tyrosine kinases. Marc S. Halfon[1,2,3], John Leatherbarrow[1]. 1) Dept. of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics and the Life Sciences, Buffalo, NY; 3) Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY.

We are investigating the mechanisms underlying signaling specificity by receptor tyrosine kinases (RTKs), particularly *Egfr* and the *htl* FGF-receptor. RTK signaling plays a major role in development and disease, with mutations in RTK pathways a leading cause of birth defects and cancer. Although the various RTKs signal via a complex network of interconnected downstream factors, the most striking aspect of their signal transduction is that these downstream pathways are common to all of the receptors. For example, all of the RTKs are able to activate ERK Map Kinase (MAPK) through the Ras/Raf/MAPK signaling cassette. Although this has led some researchers to conclude that RTKs generate a "generic signal" rather than each inducing a specific response, other evidence suggests that RTKs have intrinsic signaling differences.

We are testing the idea that RTKs have intrinsic signaling differences, and at the same time identifying downstream branchpoints in the pathway, by using a high-throughput in situ hybridization screen to find genes that are differentially regulated by *Egfr, htl, Ras1,* and *pointed* (a downstream transcriptional effector). We express constitutively active versions of these factors in the embryonic mesoderm and look for changes in pattern or abundance of gene expression of putative target genes at embryonic stages 10-12, a time during which RTK signaling is known to be important for mesoderm development. In the first one hundred genes screened we have already discovered at least four that show clear differential regulation by Egfr and Htl and have confirmed these results using quantitative RT-PCR. Another eight genes are differentially regulated in at least one of the four genotypes covered by our screen. Our data demonstrate that different RTKs have intrinsic differences in signaling capability and provide a starting point for mechanistic studies into the basis of these differences.

90

Ninjurin A Signaling Regulates Cell Adhesion. Andrea Page-McCaw, Shuning Zhang, Bernadette Glasheen, Caitlin Piette, Nicholas Simms, Aashish Kabra. Dept Biol, Rensselaer Polytechnical Inst, Troy, NY.

We have identified a new signaling molecule, the ectodomain of the transmembrane protein Ninjurin A (NijA). The Ninjurin family is highly conserved but relatively obscure, with two members in mammals and three in Drosophila. Cells overexpressing *NijA* release adhesion from their substrate. Media conditioned by *NijA*-expressing cells is able to induce wild-type cells to release adhesion; and conversely washing *NijA*-expressing cells in fresh media restores their ability to adhere. These experiments demonstrate that *NijA* acts non-cell-autonomously, secreting an adhesion-releasing activity into the media. This signal is generated by cleavage of NijA from the cell surface by the extracellular protease Mmp1, as inhibition of Mmp1 function by three different methods inhibits adhesion loss. When the ectodomain of NijA is secreted directly into the media, however, Mmp1 is no longer required for the production of signaling activity, indicating that Mmp1's function is to liberate the ectodomain from the transmembrane NijA. Interestingly, despite the previous characterization of Ninjurins as homotypic adhesion molecules, *NijA* itself is not required to maintain adhesion.

Mmp1 is required for the release of tracheal epithelium from cuticle (an apical extracellular matrix) at the larval molts. NijA and Mmp1 are co-expressed along the cell borders of larval tracheal epithelia, in a developmentally regulated manner. This co-localization and *Mmp1* phenotype suggest that Mmp1 may be cleaving NijA to regulate adhesion during tracheal molting.

91

BICAUDAL-C regulates *nos* expression during oogenesis. Chiara Gamberi, Paul Lasko. Department of Biology, McGill University, Montreal, PQ, CA.

Bicaudal-C (BIC-C) is a KH domain protein with RNA binding activity, which functions during oogenesis to establish anterior to posterior polarity in the *Drosophila* oocyte. Homozygous *Bic-C* mutant females arrest oogenesis at stage 10. Heterozygous *Bic-C* mutant females produce embryos with a range of patterning defects, including bicaudal embryos. We are characterizing the RNA binding properties of BIC-C. We showed that BIC-C can selectively bind to the *nos* 3' UTR. We mapped the BIC-C binding site to an evolutionarily conserved region of about 20 nucleotides that can potentially fold into a stem-loop structure. This is within a large regulatory module for the *nos* mRNA that was identified genetically and that contains multiple elements for mediating its localization and translational control. *nos* translational control is crucial during early development and it is tightly regulated both spatially and temporally. Immunostaining of *Bic-C* homozygous mutant ovaries reveals higher levels of the NOS protein. Furthermore, embryos produced by *Bic-C/+* heterozygote mothers exhibit anterior ectopic expression of the NOS protein. This suggests that NOS de-repression may underlie the observed bicaudal phenotype. Together, these evidences imply that BIC-C is a key regulator of the expression of posterior-group genes *in vivo*.

92

Regulation of *nanos* mRNA by *Drosophila* hnRNP M. Roshan A. Jain, Elizabeth R. Gavis. Dept of Molecular Biology, Princeton University, Princeton, NJ.

In early *Drosophila* embryos, *nanos* (*nos*) activity is restricted to the posterior through translation of a subset of *nos* mRNA that is localized to the posterior pole, together with translational silencing of unlocalized *nos* mRNA throughout the bulk of the embryo. Thus the coupling of *nos* localization with translational activation is critical for the asymmetric distribution of Nos protein required for proper anterior-posterior axis development. We have used a RNA affinity purification strategy to isolate a candidate *nos* localization factor, a homolog of the human hnRNP M, and have demonstrated that hnRNP M exists in an *in vivo* RNP complex with *nos* mRNA in ovaries. This protein interacts specifically with a region of the *nos* 3'UTR involved in regulating both posterior localization and translation. Point mutations in this regulatory region that disrupt *in vitro* interaction with hnRNP M also disrupt RNA localization *in vivo*. Moreover, hnRNP M mutants interact genetically with *nos* and disrupt the regulation of *nos* mRNA. Intriguingly, hnRNP M interacts directly with both ovarian Glorund and embryonic Smaug *in vivo*, the two known translational regulators of *nos*, consistent with a role in coordinating the localization and translation of *nos*.

93

Precision and reproducibility of biological patterning. Thomas Gregor[1,2,3,4], William Bialek[1,2], David W. Tank[1,2,3], Eric F. Wieschaus[3,4]. 1) Lewis-Sigler Institute for Integrative Genomics; 2) Joseph Henry Laboratories of Physics; 3) Department of Molecular Biology; 4) Howard Hughes Medical Institute, Princeton University, Princeton, NJ.

During embryonic development, information about spatial location is represented by the concentration of various morphogen molecules. The reproducibility and precision of biological pattern formation thus is limited by the accuracy with which these concentration profiles can be established and "read out" by their target pathways.

We consider four measures of precision for the Bicoid morphogen in the *Drosophila* embryo: The concentration differences that distinguish neighboring cells, the limits set by the random arrival of Bcd molecules at their targets (which depends on the absolute concentration), the noise in readout of Bcd by the activation of Hunchback, and the reproducibility of Bcd concentration at corresponding positions in multiple embryos. We show, through a combination of different experiments, that all of these quantities are ~10%.

This agreement among different measures of accuracy, which depend on very different molecular mechanisms, indicates that the embryo is *not* faced with sloppy input signals and noisy readout mechanisms; rather we have to understand how the embryo exerts precise control over absolute concentrations and responds reliably to small changes in these concentrations, down to the limits set by basic physical principles.

94

Quantitative study of the terminal system in drosophila embryo. Matthieu Coppey, Alistair N. Boettiger, Stanislav Y. Shvartsman. Carl Ichan Lab, Princeton Univ, Princeton, NJ.

We will present a quantitative experimental analysis and biophysical modeling of the terminal patterning system in the Drosophila embryo. The patterning of the terminal regions of the embryo relies on the spatially restricted activation of the torso/Ras/MAPK signaling pathway. torso receptors are uniformly expressed on the surface embryo and their localized activation has been shown to depend on the localized processing and trapping of the trunk ligand, which binds to Torso and activates the MAPK signaling. Graded levels of MAPK activation then control the two downstream targets tailless (tll) and huckebein (hkb) by de-repressing capicua (cic). We have analyzed the physical mechanisms dictating the gradient formation. We stained dp-ERK in the whole embryo, and used it as readout of the graded activation of tor receptors. We quantified the gradient with the help of image processing routines. Using the divisions of nuclei as an internal clock, we showed that the gradient of dp-ERK exhibits a dynamical evolution during the transient time course of tor activation. To test the hypothesis according to which ligand trapping shapes the gradient, we used a reaction-diffusion model to predict the changes that would happen in fly mutants. Then, we imaged and quantified the corresponding mutants to validate these predictions. In addition we have begun to explore the mechanisms of MAPK gradient interpretation by blastoderm nuclei. Starting at cell cycle 13, nuclei express tll and hkb within sharp domains, reflecting high levels of tor activity. To understand the transduction of spatial information, we modeled the dp-ERK dependent shuttling of cic between nuclear and cytoplamic compartments. We have validated the results of our models by experimentally determining the quantitative the input-output map between nuclear/cytoplasmic ratio cic and the level of MAPK phosphorylation.

95

Anterior patterning independent of the Bicoid gradient. Ulrike Lohr[1], Ho-Ryun Chung[2], Mathias Beller[1], Herbert Jäckle[1]. 1) Molecular Developmental Biol., MPI for biophysical Chemistry, Goettingen, DE; 2) Computational Molecular Biol., MPI for molecular Genetics, Berlin, DE.

The anterior-posterior axis of the *Drosophila* embryo is first established by maternal factors, which are deposited in the egg as mRNA. The mRNA of the anterior determinant *bicoid* (*bcd*) is localized to the anterior pole of the embryo where it is translated resulting in an anterior-posterior Bcd gradient. Bcd is a homeodomain containing transcription factor, which has been proposed to possess all the characteristics of a morphogen: (1) localized source of cytoplasmic activity, which (2) forms a concentration gradient from the source and (3) concentration-dependent activity that determines positional information within the gradient. This means that the enhancers of Bcd target genes should be sensitive to a particular amount of Bcd along the anterior posterior axis. Here we have used the GAL4/UASp system to ectopically express *bcd* in the female germline without a localizing 3'UTR. If Bcd indeed functions as a morphogen, it was expected that the anterior would be drastically expanded to encompass the entire embryo. To test this we observed the effect on diverse target genes. We found that the direct targets *hunchback* and *orthodenticle* were expressed throughout the embryo, while Caudal, a target of translational repression through Bcd, was no longer detected. Interestingly we found that the expression domains of other anterior genes such as the Hox genes *deformed* and *lab* were duplicated in the posterior half of the embryo. This indicates, that instead of causing anterior expansions, uniform levels of Bcd cause mirror image duplications of the anterior. As no posterior-anterior gradient of Bcd is provided in these embryos, we conclude that a gradient of Bcd is not necessary to impart positional information. Instead our results indicate that the gradient of Bcd activity is functionally redundant to an activity gradient produced by the terminal system. This leads to the conclusion that Bcd may not strictly function as a morphogen during *Drosophila* development.

96

Oriented cell divisions contribute to germ band extension in Drosophila embryos. Sara Morais da Silva, Jean-Paul Vincent. Developmental Neurobiology, NIMR, London, GB.

Detailed observations by others of the anterior part of the germ band have shown that elongation follows from an orderly process of cell intercalation driven by local junctional remodelling. We have followed cell behaviour at the posterior end, the leading edge, of the extending germ band. We find that, in this region of the extending germ band, cell divisions are mostly oriented along the anterior-posterior axis, parallel to the direction of elongation. In the absence of cell division - in *string* mutants, germ band extension is slower in rate and reduced in extent as compared to the situation in wild type embryos. We suggest that, as in zebrafish embryos, cell intercalation and oriented cell divisions together contribute to tissue elongation. Like polarised cell intercalation at the anterior of the germ band, oriented cell divisions at the posterior require segmental patterning by the pair-rule gene *even-skipped*, suggesting that a common polarising cue may be used.

97

The Role of D-Ets4 in Primordial Germ Cell Migration. Anita Hsouna, Dennis Watson, Tien Hsu. Hollings Cancer Ctr, Medical Univ South Carolina, Charleston, SC.

The *Drosophila* D-ets4 is a member of the ETS family of transcription factors. D-ets4 is involved in the migration and maturation of Primordial Germ Cells (PGCs). The process of PGC migration during development, interestingly, resembles the process of tumor metastasis. Furthermore, PDEF, the mammalian homolog of D-ets4, has been reported to down-regulate the tumor metastatic potential in breast cancer. In *Drosophila*, during embryonic development, PGCs go through a pre-migratory, migratory, and a post-migratory stage. D-ets4 protein is detected only in pre-migratory PGCs. Loss of function mutant embryos of D-ets4 show improper PGC migration during all stages, including decreased E-cadherin expression in PGCs, a phenotype that can be rescued by overexpression of E-cadherin in PGCs. Interestingly, overexpression of D-ets4 in S2 cells also affects E-cadherin expression and localization. The effect of overexpressing D-ets4 in embryos is currently being investigated. In addition, embryos heterozygous for *snail*, a transcriptional repressor of E-cadherin, show abnormally clumped PGCs at various stages of development, accompanied by increased levels of E-cadherin. Loss of one copy of *snail* in D-ets4 mutant embryos is capable of rescuing the D-ets4 PGC migration defects and restores E-cadherin expression in PGCs. On the other hand, overexpression of Snail in PGCs in D-ets4 mutant background enhances the PGC migration defects. To confirm down-regulation of Snail by D-ets4 in embryos, the levels of *snail* RNA are examined. Thus, D-ets4/PDEF may function as a negative regulator of cell migration by down-regulating *snail*, which is itself a putative metastasis-promoting gene that down-regulates E-cadherin.

98

The *lines* gene controls growth by repression of *bowl* and *wingless* in the Drosophila wing. Victor Hatini, David Nusinow. Dept Anatomy & Cellular Biol, Tufts Univ, Boston, MA.

Growth control is critical for an organism's proper development, and improper growth control is a hallmark of many diseases. Despite its importance, our current understanding of growth control is fragmented. We have previously shown that the genes *drm*, *lines*, and *bowl* operate in a linear pathway to pattern a variety of ectodermal structures during development. We have extended the analysis to the developing imaginal discs, and now report a role for this pathway in controlling cell proliferation. Removal of *lines* in clones led to the activation of Bowl, and through Bowl to ectopic epithelial growth. Expression of the *lines* inhibitor *drumstick* (*drm*) led to similar phenotypes. By quantifying clonal growth, we found that Bowl-expressing cells had an increased cell size and division rate. We then explored the cellular and molecular underpinning of these phenotypes. We found that Bowl promoted epithelial cell proliferation both cell autonomously and non-autonomously, and prevented the timely cell cycle exit in the margin (ZNC). To determine if Bowl promoted neoplastic growth, we examined epithelial organization in clones using markers for apical-basal polarity. We observed proper localization of these markers and normal epithelial organization, indicating that the growth was not neoplastic. We also analyzed a variety of patterning markers in clones. We found that the subdivision of the wing epithelium into compartments was normal. However, the expression of several *wingless* (*wg*) and *decapentaplegic* (*dpp*) target genes was repressed. The secreted ligand Wg was produced ectopically, and the removal of *wg* suppressed the overproliferation phenotype. Taken together, our data demonstrates that *lines* acts as a tumor suppressor gene, while *drm* and *bowl* act as oncogenes in the developing wing. We provide evidence that inappropriate activation of *bowl* affects wing patterning and the expression of survival/mitogenic signals. We propose that as a result of these changes, wing progenitors fail to respond to growth inhibitory signals while becoming self sufficient for growth promoting signals.

99

Regulation of spindle stability and chromosome segregation by dephosphorylation of the Microtubule-Associated Protein dTACC. Shengjiang Tan, Ekaterina Lyulcheva, Jon Dean, Daimark Bennett. Department of Zoology, University of Oxford, South Parks Road, Oxofrd, UK.

The formation and stability of spindles is essential for the proper segregation of chromosomes during mitosis and meiosis. It is now recognized that Microtubule Associated Proteins (MAPs) play an essential role in these processes by controlling the stability of spindle microtubules. However, in many cases the regulation of MAP function is poorly understood. Recent studies suggest that the Drosophila Transforming Acidic Coiled-Coil protein (dTACC) promotes microtubule assembly by recruiting the microtubule-stabilising protein Minispindles (Msps) to the centrosome. Phosphorylation of dTACC on Ser863 by Aurora A kinase appears to allow dTACC-Msps complexes to stabilise the minus ends of centrosome-associated microtubules. Although dTACC is found on both the centrosome and mitotic spindle, phosphorylated-dTACC (p-dTACC) is tightly localized to the centrosomes, suggesting either that p-dTACC is rapidly dephosphorylated on the spindle or that p-dTACC cannot exchange with the pool of dephosphorylated dTACC. However, the importance of generating p-dTACC only at the centrosome had not been established. Here we report the identification of a protein phosphatase, consisting of the catalytic subunit of protein phosphatase 1 (PP1) and a putative targeting subunit, which is required to dephosphorylate dTACC on mitotic spindles in Drosophila embryos. Loss-of-function mutants in the targeting subunit or disruption of PP1-binding resulted in elevated levels of p-dTACC on spindles. This was accompanied by a loss of spindle microtubules and centrosome detachment, leading to a failure in chromosome segregation. Mutants in the targeting subunit were completely rescued by a mutant form of dTACC that cannot be phosphorylated on Ser863. Taken together, our data indicate that the dephosphorylation of dTACC on spindles, mediated by a PP1 holoenzyme, is essential for normal spindle stabilisation and mitotic progression.

100

Activated ERM control cortex dynamics and cell shape throughout mitosis. Sebastien Carreno[1], Ilektra Kouranti[2], Edith Szafer Glusman[3], Margareth Fuller[3], Arnaud Echard[2], Payre Francois[1]. 1) CBD - CNRS UMR5547, Toulouse, FRANCE; 2) Institut CURIE - CNRS UMR144, Paris, FRANCE; 3) Stanford University School of Medicine, Stanford, USA.

Mitosis in animal cells involves a stereotyped sequence of changes in cell morphology regulated by localized acto-myosin contractions of the cortex. In order to secure the equal transmission of cellular and genetic materials to each daughter cell, these acto-myosin contractions must be precisely coordinated, spatially and temporally, with chromosome separation operated by the microtubule spindle. However, an important unresolved question is to understand how these cortical contractions are dynamically regulated and coupled to the plasma membrane in order to direct the characteristic mitotic cell shape transformations. Combining in vivo approaches in flies and live analysis ex vivo, we show here that dMoesin, the sole ERM member in flies, is essential for mitotic cell shape and cell division, as it is required to control the dynamics and the contractibility of the cellular cortex throughout the successive steps of mitosis. Moreover, we provide evidence that the dMoesin-dependent organization of the actin network is necessary for a productive interaction between microtubules and the cortex to center the mitotic spindle and to maintain its orientation throughout mitosis. Finally, we demonstrate that the dMoesin mitotic activation through the phosphorylation of a conserved threonine residue is essential for cell division and we found that it depends on the dPlkk/Slik threonine kinase. This study provides the first evidence for the critical role of an ERM protein during cell division and reveals that this family of proteins is essential for regulating cell shape throughout mitosis.

101

dp53 coordinates tissue repair through a novel, DNA-damage independent pathway. Brent Wells, Laura Johnston. Dept Genetics & Development, Columbia Univ, New York, NY.

Control of organ size during development requires several processes, including cellular growth, cell proliferation, and cell death. Under optimal developmental conditions little cell death occurs in the wing imaginal disc; however when all cell death is prevented wing size asymmetry occurs with high frequency within individual flies. This observation suggests that the precise control of wing size requires feedback between the process of cell death and regulation of wing growth, such as occurs during regeneration. During disc regeneration extra cell proliferation occurs to compensate for lost tissue and to reconstruct missing cell fates. This process requires the activity of cell cycle, growth, and patterning genes, but what initiates and controls their activity is unknown. By creating a persistent state of apoptotic signaling in the wing disc to induce tissue damage and regeneration, we find that the wing disc responds with a temporal program of cell-autonomous and non-cell autonomous cell cycle and growth regulation that requires the function of the tumor suppressor, *dp53*. Interestingly, the damage-response kinases Atm and Chk2 are not required, suggesting that the activity of *dp53* is not stimulated by a DNA damage response. Instead, *dp53* is transcriptionally induced in cells subject to persistent apoptotic signals, in a manner that requires the initiator caspase, *dronc*. Finally, we demonstrate that in a model of disc regeneration, the formation of a blastema, a step necessary for compensatory proliferation during regeneration, is impaired in *dronc* and *dp53* mutants. Our results suggest a novel role for *dp53* in response to tissue damage, which does not involve the canonical DNA damage pathway. Additionally, we have found that the PI3 kinase, Dp110 is activated and required for compensatory proliferation. Epistasis experiments suggest that this requirement is *dp53*-dependent. We will discuss these and other experiments addressing how *dp53* coordinates the response to tissue damage, leading to compensatory proliferation and regeneration.

102

Fat Cadherin Modulates Organ Size in Drosophila via the Salvador/Warts/Hippo Signaling Pathway. Kieran F Harvey, F Christian Bennett. Cancer Cell Biology, Peter MacCallum Cancer Centre, East Melbourne, Victoria, AU.

The atypical Fat cadherin has long been known to control cell proliferation and organ size in Drosophila, however the mechanism by which Fat controls these processes has remained elusive. We demonstrate that Fat limits organ size by modulating activity of the Salvador/Warts/Hippo pathway. *fat* interacts genetically with positive and negative regulators of this pathway, and tissue lacking *fat* closely phenocopies tissue deficient for genes that normally promote Salvador/Warts/Hippo pathway activity. Cells lacking *fat* grow and proliferate faster than their wild-type counterparts, and exhibit delayed cell cycle exit due to elevated expression of Cyclin E. Intriguingly, *fat* does not appear to be required for developmental apoptosis, despite the fact that *fat* mutant cells express increased levels of the anti-apoptotic DIAP1 protein. Collectively, these defects lead to increased organ size and organism lethality in *fat* mutant animals. Fat modulates Salvador/Warts/Hippo pathway activity by promoting abundance and localization of Expanded protein at the apical membrane of epithelial tissues. These studies aid our understanding of developmental organ size control, and have implications for human hyperproliferative disorders, such as cancers.

103

Drosophila ALS regulates animal growth rate and metabolism through direct and functional interaction with Drosophila insulins. Nathalie Arquier[1], Charles Géminard[1], Marc Bourouis[1], Gisèle Jarretou[1], Basil Honneger[2], Alexandre Paix[1], Pierre Leopold[1]. 1) ISBDC, CNRS UMR 6543, Nice, France; 2) Zoologisches Institut, Universitat Zurich, Zurich, Switzerland.

Drosophila has a single insulin/IGF signaling system (IIS) functionally analogous to the dual mammalian IIS. Seven distinct genes code for Drosophila insulin-like peptides (Dilps), activate a unique insulin receptor, and carry both the metabolic functions of vertebrate insulins (carbohydrate homeostasis) and the growth functions of mammalian IGFs. We explored the possibility that Dilp-binding proteins might participate in the control of Dip biological activity, specially in response to nutritional input. We show that dALS, a gene encoding the fly ortholog of the vertebrate IGF-Binding Protein Acid Labile Subunit (ALS), negatively regulates animal growth rate through a physical and functional interaction with Dilp2 and a third partner encoded by the Imp-L2 gene, functionally related to a mammalian IGFBP. Interestingly, dALS also modulates the action of the fly insulins on energy balance and is required for starvation-induced growth inhibition during larval development. We propose that dALS is a key regulator of Dilp function, participating in the adaptation of animal growth rate and metabolism to ever changing nutritional cues.

104

The cytohesin Steppke is essential for insulin signalling in *Drosophila*. Ingo Zinke, Bernhard Fuβ, Thomas Becker, Michael Hoch. University Bonn, LIMES (Life and Medical Sciences), Program Unit Development & Genetics, Bonn, Germany.

In metazoans, the insulin signalling pathway plays a key role in regulating energy metabolism and organismal growth. Its activation stimulates a highly conserved downstream kinase cascade that includes phosphoinositide 3-kinase (PI3K) and the serine-threonine protein kinase Akt. This study identifies a novel component of insulin signalling in *Drosophila*, the *steppke* gene (*step*). *step* encodes a member of the cytohesin family of guanine nucleotide exchange factors (GEFs) which have been characterized as activators for ADP-ribosylation factor (ARF) GTPases. In *step* mutant animals both cell size and cell number are reduced, resulting in decreased body size and body weight in larvae, pupae and adults. *step* acts upstream of PI3K and is required for proper regulation of Akt and the transcription factor FOXO. Temporally controlled interference with the GEF activity of the Step protein by feeding the chemical inhibitor SecinH3 causes a block of insulin signalling and a phenocopy of the step mutant growth defect. Step represses its own expression and the synthesis of growth inhibitors such as the translational repressor 4E-BP. Our findings indicate a novel and crucial role of an ARF-GEF in insulin signalling which has implications for understanding of insulin related disorders, such as diabetes and obesity.

105

Characterization of a novel regulatory interaction in the Hedgehog pathway. Anne Plessis[1], Sandra Claret[1], Matthieu Sanial[1], Sebastien Malpel[1], Tristan Piolot[2], Amira Brigui[1], Laurent Daviet[3]. 1) Génétique du Développement et Evolution , Institut Jacques Monod, UMR 7592, CNRS/Universités Paris6- Paris7, 2 Place Jussieu 75251 Paris cedex 05, France; 2) Imageries des Processus Dynamiques en Biologie Cellulaire et Biologie du Développement , IFR 117 Biologie Systémique -Institut Jacques Monod, 2 Place Jussieu 75251 Paris cedex 05, France; 3) Hybrigenics, 3/5 impasse Reille, 75014 Paris, France.

The Hedgehog (HH) signalling pathway is crucial for the development of many organisms and its inappropriate activation is involved in numerous cancers. HH signal controls the traffic and activity of the seven-pass trans-membrane protein Smoothened (SMO), leading to the transcriptional regulation of HH responsive genes. The intracellular transduction events following SMO activation depend on a mulproteic complex that include the Fused (FU) protein kinase. Here, we show that the regulatory domain of FU physically interacts with the 52 last amino-acids of SMO and that the two proteins co-localize in vivo within vesicles. The deletion of the region of SMO that interacts with FU leads to a constitutive activation of SMO, indicating that this region normally plays a negative role in the control of SMO activity. We also demonstrate that in response to HH, SMO is able to directly recruit FU to the plasma membrane. The forced localisation of FU to the plasma membrane (FU-GAP fusion) leads to a SMO dependent constitutive activation of the pathway. We show that FU can act on the sub-cellular localisation, stability and phosphorylation of SMO and that it renders SMO insensitive to PTC inhibitory effects. We propose a model which includes novel feedback loops in which FU can act on SMO both to further inhibit it in the absence of HH and to further activate it in response to HH.

106

Lipids and Lipoproteins as carriers for Hedgehog spreading and reception. Ainhoa Callejo, Joaquím Culi, Isabel Guerrero. Centro de Biología Molecular (CSIC), Universidad Autónoma de Madrid, Madrid, ES.

Hh is synthesized as a precursor that undergoes autoproteolytic cleavage, and is doubly modified by addition of a cholesterol moiety at its C-terminal and by palmitoylation at its N-terminal. Lipid modifications on Hh are essential for Hh interaction with the heparan sulfate proteoglycans (HSPGs) and with Shifted (Shf), a new component of the extracellular matrix that collaborates with the HSPGs for Hh stabilization and spreading. Lipid modifications on Hh are also required for the optimal interaction of Hh with Dispatched for secretion and with Patched (Ptc) for reception. These two proteins have a Sterol Sensing Domain (SSD), like some proteins involved in lipid and cholesterol metabolism. Recently, lipoprotein particles have been proposed to carry lipid-modified ligands from the cell surface, acting as vehicles for long-range transport (Panakova et al., 2005). To test the importance of lipids in the Hh signaling, we have genetically manipulated the amount of lipid production in Drosophila. It is known that SREBP is a transcription factor that controls multiple lipid metabolism genes, such as the HMGCoA reductase, which is the key enzyme in sterol biosynthesis. This enzyme has been recently suggested to regulate Hh gradient (Despande and Schell, 2005). Here, we have analyzed the effect on secretion, spreading and reception of Hh in loss and gain of function conditions of these two genes. We have found that over-expression of SREBP or HMGCoA reductase rescued the Shf mutant phenotype by stabilizing Hh in the extracellular environment. Also, phenotypes caused by increase in Hh production in the wing were rescued by lowering the dosage of these gene products. These data evidence the importance of lipids in Hh stabilization and spreading in the extracellular matrix. In addition, we have tested the implication of the LDL receptors in Hh signaling, and found that none of them are directly involved in Hh reception. However, we observed that Ptc actively internalized Lipophorins, acting as a Lipoprotein receptor and also regulating the intracellular lipid metabolism.

107

Non-canonical *dpp* signalling mediates communication between R7 and R8 in the *Drosophila* eye. Daniela Pistillo, Claude Desplan. Dept Biol, New York Univ, New York, NY.

The *Drosophila* eye is composed by ~800 ommatidia, each consisting of eight photoreceptor cells (PR). The six outer PRs, R1-R6, are involved in motion detection and image formation, while the two inner PRs, R7 and R8 are involved in color vision. Ommatidia are classified into two sub-types depending on the type of the color-sensitive photopigment Rhodopsin (Rh) expressed in R7 and R8: they either co-ordinately express UV-sensitive Rh3 in R7 and blue-sensitive Rh5 in R8 (**pale** ommatidia, **p**) or UV-Rh4 in R7 and green-Rh6 in R8 (**yellow** ommatidia, *y*) with a conserved ratio of 30% pale and 70% yellow in the eye. The decision on **pale** versus **yellow** fate is made in R7, and then an instructive signal from pR7 to the underlying R8 induces Rh5 expression. This signal is then reinforced in R8 by a bi-stable loop between the Drosophila large tumor suppressor gene *warts* (*wts*) and the cell growth regulator *melted* (*melt*). We have shown that the instructive signal from R7 to R8 is mediated by a "non-canonical" TGF-β signal. The TGF-β super-family can be subdivided into Activin and BMP families. In flies the two Activin homologues, dActivin and Activin like protein (Alp) signal trough the receptor Baboon to induce phosphorilation and activation of the transcription factor DSmad2, while the three BMP homologues Dpp, Gbb and Scw signal through the receptors Thickvein (Tkv) and Saxophone (Sax) to activate the transcription factor Mad. Once phosphorilated, both DSmad2 and Mad bind to the Co-Smad Medea to be translocated to the nucleus and activate transcription of downstream genes. Little evidence has been found so far of cross-talk between these two branches. Our experiments demonstrate that Dpp signals through the Activin receptor Babo to induce Rh5 expression. The signal is transduced by DSmad2 and regulates the activity of the *wts*/*melt* bi-stable loop at the transcriptional level, thus revealing an important crosstalk between these two pathways. We also showed that a further level of regulation of the activity of this pathway is provided by the balance between the levels of P-Mad and P-DSmad2.

108

dSno facilitates Baboon signaling in the Drosophila brain by switching the affinity of Medea away from Mad and toward dSmad2. Stuart Newfeld[1], Cathy Hyman-Walsh[2], Ying Ye[3], Robert Wisotzkey[1], Michael Stinchfield[1], Michael O'Connor[3], David Wotton[2], Norma Takaesu[1]. 1) Sch Life Sci, Arizona State Univ, Tempe, AZ; 2) Dept of Biochemistry and Molecular Genetics, Univ of Virginia, Charlottesville, VA; 3) Dept of Genetics, Cell Biology and Development and HHMI, Univ of Minnesota, Minneapolis, MN.

A screen for modifiers of Dpp adult phenotypes led to the identification of the Drosophila homolog of the Sno oncogene (dSno). The dSno locus is large, transcriptionally complex and contains a recent retrotransposon insertion that may be essential for dSno function, an intriguing possibility from the perspective of developmental evolution. dSno is highly transcribed in the embryonic central nervous system and transcripts are most abundant in third instar larvae. dSno mutant larvae have proliferation defects in the optic lobe of the brain very similar to those seen in baboon (Activin Type I receptor) and dSmad2 mutants. This suggests that dSno is a mediator of Baboon signaling. dSno binds to Medea and Medea/dSno complexes have enhanced affinity for dSmad2. Alternatively, Medea/dSno complexes have reduced affinity for Mad such that in the presence of dSno Dpp signaling is antagonized. We propose that dSno functions as a switch in optic lobe development - shunting Medea from the Dpp pathway to the Activin pathway to insure proper proliferation. Pathway switching in target cells is a previously unreported mechanism for regulating TGFb signaling and a novel function for Sno/Ski family proteins.

109

Drosophila miR-315 regulates Wingless signaling. Eric Lai[1], Serena Silver[2], Joshua Hagen[1], Norbert Perrimon[2]. 1) Sloan Kettering Institute, Developmental Biology, New York, NY; 2) Harvard Medical School, Genetics Department, Boston MA.

Fine control of cell signaling is essential to generate precise biological outputs. Therefore, we suspect that components of the fundamental signaling pathways may prove to be especially sensitive to manipulation of miRNAs. To test this idea, we are analyzing miRNA function with respect to several key signaling cascades.

In the first approach, we performed quantitative reporter assays of Wingless, Notch and Hedgehog signaling in cultured cells and examined how they are perturbed by ectopic miRNAs. We observe that a handful of miRNAs significantly activate or repress these pathways. In the second approach, we generated a library of transgenic flies carrying UAS-miRNA transgenes. Interestingly, we found that several miRNAs induce mutant phenotypes that are characteristic of manipulating these same signaling pathways.

Here, we present some specific observations regarding miR-315. In cultured cells, ectopic miR-315 specifically and strongly induces a Wingless transcriptional response in unstimulated cells; i.e., in the absence of ligand. Misexpression of miR-315 in vivo also potently activates Wingless signaling in the wing imaginal disc, where it recapitulates the ability of ectopic Wingless to respecify the notum of the fly as a second set of wings. Using both cell-based reporters and in vivo sensors, we identified two negatively acting components of the Wingless pathway-Axin and Notum-as strong targets of miR-315.

Endogenous miR-315 is expressed in a subset of the developing central nervous system and brain, and we are now working to understand how it might normally influence Wingless signaling and/or the activity of other target genes. An important lesson of the gain-of-function work, though, is that inappropriate miRNA activity can profoundly affect the activity of basic signaling pathways, a fact that undoubtedly has strong functional implications for human disease.

110

Canonical Wnt signaling depends on Evi/Wls, a conserved transmembrane protein required for Wnt secretion. Kerstin Bartscherer, Nadège Pelte, Dierk Ingelfinger, Michael Boutros. Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

Wnt signaling pathways are important for various biological processes during development and disease. They are activated by Wnts, secreted lipid-modified glycoproteins that induce target gene expression in both a short- and long-range manner. While processes in the signal-receiving cell are well understood, little is known about how Wnts are released from producing cells, and how they can travel over long distances despite their hydrophobic properties. We have identified a conserved transmembrane protein, Evenness interrupted (Evi/Wls), through an RNAi survey for proteins involved in *Drosophila* Wingless (Wg) signaling. During development, *evi/wls* mutants have patterning defects that phenocopy Wg loss-of-function phenotypes, and fail to express Wg target genes. *evi/wls*'s function is evolutionary conserved, as depletion of its human homolog disrupts Wnt signaling in human cells. Epistasis experiments and clonal analysis place *evi/wls* upstream of the receptor complex, and in the Wg producing cell. In the absence of *evi/wls*, Wg is retained in cells that produce it. Our results suggest that *evi/wls* is the founding member of a gene family specifically required for Wg/Wnt secretion. Elucidation of the role of *evi/wls* in Wnt secretion might be the key to an understanding of how Wnts are released, and thus of how they can spread across target tissues.

111

The extracellular domain of the Frizzled receptor functions as a ligand by binding to Van Gogh/Strabismus during planar cell polarity signaling in Drosophila. Jun Wu, Marek Mlodzik. Department of Molecular, Cellular and Developmental Biology, Mount Sinai School of Medicine, New York, NY.

The Frizzled (Fz) receptor is required in both Wnt/?-catenin and planar cell polarity (PCP) signaling, and acts through the downstream effector Dishevelled (Dsh) in both cases. In addition, Fz acts non-autonomously on neighboring cells during PCP establishment, which is reflected by the alteration in polarity of wild-type cells surrounding fz- or Fz overexpressing patches. The molecular mechanism(s) of non-autonomous Fz-signaling are unknown. Our in vivo studies of different Fz isoforms identify the extracellular domain (ECD) of Fz as necessary and sufficient for all non-autonomous Fz-PCP activity, suggesting that it acts as a ligand. We demonstrate biochemical and physical interactions between the FzECD and Van Gogh/Strabismus (Vang/Stbm). Thus, Vang/Stbm appears to act as a FzECD receptor, allowing cells to sense Fz levels of their neighbors. This interaction determines cell polarity orienting cells from higher towards lower levels of Fz.

112

Function of the Rho-GEF Pebble in FGF-dependent mesoderm migration. Andreas van Impel, Sabine Schumacher, Arno Müller. Division of Cell and Developmental Biology, College of Life Sciences, University of Dundee, Dundee, Scotland, GB.

pebble (*pbl*) encodes a Rho-specific GEF that is known for its conserved function in cytokinesis. Like its mammalian homolog, the proto-oncogene *ect2*, Pbl locally activates RhoA at the cell cortex, leading to formation of the contractile actin-myosin ring during cell division. In contrast to cytokinesis, the mechanism of the transforming potential of *ect2* is not well understood. We have shown that *pbl* acts as an essential player during fibroblast growth factor - triggered cell migration and that it is required for formation of cellular protrusions. Our structure function analysis of Pbl suggests that during mesoderm migration Pbl functions through Rac GTPases rather than RhoA. We show that the DH-PH domains of Pbl genetically interact not only with RhoA but also with Rac1 and Rac2. The DH domain of Pbl not only promotes nucleotide exchange activity for RhoA-GDP, but also exhibits exchange activity for Rac1-GDP and Rac2-GDP in vitro. In addition we found that embryos with strongly reduced levels of all three *Drosophila* Rac GTPases exhibit strong mesoderm migration defects. Misexpression experiments indicate that the carboxy-terminus of Pbl specifies its interaction with RhoA - dependent processes. This implies an important function for this conserved region in the regulation of the substrate specificity. Another problem of Pbl function in cell migration is that in interphase cells the protein has thus far only been reported to localize to the nucleus. Here we show that functional Pbl is also localized at the cortex of migrating cells. These data suggest that regulation of subcellular localization and substrate specificity of Pbl - potentially through FGF-signaling - might be essential for its function in cell migration and might help to shed light on the transforming activity of *ect2*.

113

The JAK/STAT pathway regulates proximal-distal patterning in *Drosophila*. Aidee Ayala[1], Laura Ekas[1], Maria Sol Flaherty[1], Gyeong-Hun Baeg[2], Erika Bach[1]. 1) Department of Pharmacology, New York University School of Medicine, New York, NY; 2) Children's Cancer Research Laboratory, New York Medical College, Valhalla, NY.

JAK/STAT signaling has been thought to function primarily to control cell proliferation. Here we show that Stat92E and other components of the JAK/STAT pathway are also required to pattern Drosophila appendages. The JAK/STAT pathway ligand Unpaired is expressed in two central domains in the leg and antennal disc, leading to expression of a reporter for JAK/STAT pathway activity in a domain complementary to the *wingless* expression domain. Loss of Stat92E function results in mis-expression of *wingless* in leg and antennal discs, while activation of the JAK kinase Hopscotch can autonomously repress *wingless*. Ectopic *wingless* expressed in *stat92E* mutant clones ventralizes the leg and leads to duplications of the proximal-distal axis in the leg and antenna. The lack of any autonomous effect of JAK or Stat92E on genes controlling the proximal-distal axis supports the hypothesis that *wingless* mis-expression is the primary cause of these duplications. Lastly, we identify a Stat92E-responsive enhancer of the *wingless* gene; this enhancer contains no canonical Stat92E binding sites, suggesting that its regulation by Stat92E may be indirect. The interaction we describe between the JAK/STAT and Wingless pathways indicates that JAK/STAT signaling can be used to specify positional information in addition to stimulating cell proliferation. Moreover, we show that one of the main functions of the JAK/STAT pathway in appendage development is to promote the formation of a single proximal-distal axis per disc. Finally, our work raises the possibility that a similar interaction exists in higher organisms.

114

Interaction between the nuclear import adapter importin-α3 and the Wnt antagonist Naked cuticle. Chih-Chiang Chan[1,3], Raphaël Rousset[2], Keith Wharton, Jr.[3]. 1) Genetics and Development Graduate Program, UT Southwestern, Dallas, TX; 2) Institute of Signaling, Developmental Biology and Cancer, Centre de Biochimie, University of Nice, France; 3) Departments of Pathology and Molecular Biology, UT Southwestern, Dallas, TX.

Segmentation of the *Drosophila* embryo requires graded Wnt/β-catenin signaling across each segmental anlage. *naked cuticle (nkd)* encodes a novel, conserved protein that acts via a negative feedback mechanism to limit accumulation of β-catenin and expression of Wnt target genes. Our previous work revealed Nkd sequences that target the cytoplasmic Wnt signal transducer Dishevelled (Dsh) as well as an unconventional nuclear localization sequence (NLS). A recent report suggests that nuclear entry is required for vertebrate Dsh activity in gain-of-function assays. How Dsh transmits Wnt signals into the nucleus, as well as how Nkd inhibits Wnt signaling and Dsh action, remains unclear. We hypothesize that Nkd performs a crucial function to antagonize Wnt signaling in the nucleus, and predict that Nkd should engage the nuclear transport apparatus. In the canonical nuclear import paradigm, importin-α links NLS-containing cargo proteins to the nuclear transport factor importin-β. Animals contain three paralogous groups of importin-α proteins, α1, 2, and 3. Using Y2H and GST-pulldown assays, we have found that Nkd specifically binds to fly importin-α3. Nkd's importin-α3-binding motif is conserved between *D. melanogaster* and *D. pseudoobscura*, and is similar to the NLS of fly heat shock transcription factor (dHSF), a known importin-α3 binding partner. Point mutation of Nkd residues homologous to those of dHSF that are critical for nuclear entry disrupts the Nkd/importin-α3 interaction, suggesting a similar mode of binding between dHSF or Nkd and importin-α3. The results of experiments that investigate the requirement of *importin-α3* for *nkd* function *in vivo* will be presented. Our finding that Nkd binds to a component of the nuclear import machinery supports the hypothesis that Nkd functions in the nucleus to restrain Wnt signaling.

115

Hedgehog restricts its expression domain in the Drosophila wing. Fernando Bejarano[1], Lidia Perez[1], Yiorgos Apidianakis[2], Christos Delidakis[2], Marco Milan[1]. 1) Parc Cientific de Barcelona, Institut de Recerca Biomédica, Barcelona, ES; 2) Institute of Molecular Biology and Biotechnology, Fo.R.T.H., and Department of Biology, University of Crete, 71110 Heraklion, Greece.

Stable subdivision of *Drosophila* limbs into an anterior (A) and a posterior (P) compartment is a consequence of asymmetric signaling by Hedgehog (Hh) from P to A cells. The activity of the homeodomain protein Engrailed in P cells helps to generate this asymmetry by inducing expression of Hh in the P compartment and at the same time repressing the expression of the essential downstream component of the Hh pathway Cubitus interruptus (Ci). Ci is a transcription factor which in the absence of Hh signaling is converted to a repressor form (Ci^{rep}). Ci^{rep} represses *hh* In A cells. The transcriptional corepressor Groucho (Gro) is also known to repress *hh* expression in A cells, thus helping to maintain the aforementioned asymmetry. Gro is ubiquitously expressed but it is required only in A cells that receive the Hh signal. Here we present evidence that Gro exerts this action by binding to the product of *master of thickveins* (*mtv*), a target of Hh activity encoding a nuclear Zinc Finger protein. Two different mechanims are then used to repress *hh* expression in A cells. The first one is based on Ci^{rep} acting mainly in those cells not receiving the Hh signal. The second one is based on Hh restricting its own expression domain through the activity of its target gene*mtv*. We provide evidence that these two mechanisms are independent.

116

Identification of genes expressed in specific leg segments using microarray. Reiko Tajiri[1], Tetsuya Kojima[1,2], Kaoru Saigo[1]. 1) Dept Biophys Biochemistry, Univ Tokyo Grad Sch Sci, Tokyo, JP; 2) Dept. Integrated Biosci., Grad. Sch. Frontier Sci., Univ. Tokyo.

In the initial phase of organ development, graded morphogen activity subdivides the corresponding developing field through instruction of region-specific expression of genes encoding transcription factors. However, we have little information about genes downstream of these transcription factors.

The adult leg of *Drosophila* is segmented along the proximodistal axis. Several transcription factors that are expressed in specific leg segments have been implicated in their specification. Since their downtream target genes are expected to be expressed also in segment-specific manners, we searched for such genes using cDNA microarray. The distal legs (pretarsus and tarsal segments) were cut into fragments at segment boundaries and probes were prepared using mRNA extracted from each segment as templates. Using these probes, RNA expression profiles of individual segments were directly compared on cDNA microarray.

For efficient identification of genes that are really expressed differentially in vivo, it is critical to set the proper threshold of fold difference in relative expression levels between two samples being compared. To determine appropriate threshold settings, we examined the real expression pattern of about 200 genes with various fold difference by in situ hybridization (ISH). Based on the correlation between thresholds and the rate of “true-positive” genes, the number of segment-specific genes was estimated to be approximately 500. Among them, we have so far confirmed by ISH over 100 genes to be truly differential. Interestingly, the majority of the genes assessed as segment-specific were either up- or down-regulated specifically in the distal-most region, which has unique structures not found in other segments, such as claws. Potential link between the unique morphology and the distinct repertoire of gene expression will be discussed.

117

Dorsal-Ventral midline signaling and antagonistic transcription factors in the developing Drosophila eye. Atsushi Sato, Andrew Tomlinson. Dept Genetics & Development, Columbia Univ, New York, NY.

Boundaries between different cell types play key roles in varied developmental processes. They can be established by various mechanisms, and signaling between the different cell types can occur in a number of ways. One mechanism of cross-boundary signaling is controlled by the Notch (N)-modifying protein Fringe (Fng). In the Drosophila wing dorso-ventral border (D/V) the mechanism by which a Fng+/Fng- interface controls local N activation has been well characterized. A Fng+/Fng- interface that controls local N activation has also been described at the D/V border of the fly eye, but the mechanisms that establish it are different from the wing. Here we describe the ventral role of the Sloppy-paired (Slp) transcription factors, and their interactions with dorsally expressed Iroquois (Iro) transcription factors in the establishment and signaling of the Fng+/Fng- interface in the developing eye. The two classes of transcription factors are mutually repressive and initially abut at the D/V midline. However, signaling at the interface leads to a gap opening between the two expression domains resulting in three domains of expression; the dorsal Iro region, the ventral Slp region and the domain between them (the gap) which expresses neither. Serrate (Ser, a N ligand) is blocked by the expression of either transcription factor type, and thus the gap is the domain in which Ser levels can be increased. The gap phenomenon does not occur in the developing wing, and thus, although the wing and the eye share the use of a midline Fng+/Fng- mechanism, the modes by which the interfaces are established and maintained appear different.

118

Spatial and temporal regulation of ecdysone signaling revealed through analysis of TAI, the *Drosophila* homolog of AIB1, a breast cancer oncogene. Anna C-C Jang, Denise J. Montell. Dept Biological Chemistry, JHMI, WBSB, Baltimore, MD.

Ecdysone is the only known *Drosophila* steroid hormone directing many biological activities. In oogenesis, it is required for ovarian development beyond stage 8 and for border cell migration. However the spatial pattern of ecdysone-dependent gene expression has not been investigated in the ovary. We used the ecdysone reporter EcRE-lacZ (Kozlova and Thummel, 2003) to follow the pattern of ecdysone-induced gene expression during oogenesis. EcRE-lacZ activity was detected in a spatially restricted pattern. Beginning at stage 9 throughout border cell migration, β-galactosidase expression was observed at highest levels in border cells and in anterior nurse-cell-associated follicle cells.

TAI (*taiman*) is an ecdysone receptor coactivator that is required for border cell migration. TAI is homologous to p160 steroid hormone coactivators, including human AIB1 (amplified in breast cancer 1). The p160 family members have several conserved domains, such as the N-terminal bHLH/PAS domain and LXXLL motifs. The LXXLL motifs are responsible for interacting with the steroid hormone receptor in a ligand-binding dependent manner. Although the bHLH and PAS domains are the most highly conserved domains, their functions remain unclear. Here, we report that overexpression of a form of TAI lacking the bHLH domain [TAI(PLC)] caused not only failure of border cell migration but also precocious EcRE-lacZ expression in late stage 8 anterior follicle cells. Our data suggest that the bHLH domain of TAI is an essential negative modulator of ecdysone-dependent gene expression and that the truncated protein is hyperactive. Overexpression of TAI(PLC) and hopTum induced precocious border cell migration. This result suggests that bHLH domain is required to time the migration initiation through regulating ecdysone signaling.

119

Specific role of the SR protein splicing factor B52 in cell cycle control in *Drosophila*. Maxim Frolov, Vanya Rasheva, David Knight, Przemyslaw Bozko. Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL.

E2F and pRB are important regulators of cell proliferation; however, the function of these proteins and their regulation *in vivo* are not well understood. In *Drosophila*, there are two E2F genes, an activator *de2f1* and a repressor *de2f2*. The loss of *de2f1* gives rise to the G1/S block accompanied by the repression of E2F-dependent transcription. The *de2f1* mutant phenotype is rescued by the inactivation of dE2F2 suggesting that the cell cycle arrest in *de2f1* mutant cells is largely due to the repressor activity of the dE2F2/RBF complex. We carried out an F2 FLP/FRT screen to identify new regulators of cell proliferation that overcome the dE2F2/RBF-dependent G1/S block in *de2f1* mutant cells. One of isolates is the *B52* gene encoding a splicing factor SR protein. We show that B52 plays a highly specific role in regulation of the *de2f2* pre-mRNA splicing. The loss of *B52* restores S phases in clones of *de2f1* mutant cells and phenocopies the loss of the *de2f2* function *in vivo*. In B52 deficient cells, the level of dE2F2 protein is severely reduced and the dE2F2 specific targets are strongly derepressed. A re-expression of the intronless copy of dE2F2 in B52 deficient cells restores the dE2F2-mediated repression. In addition to B52, we have isolated mutant alleles of the *Doa* gene. Intriguingly, the *Doa* gene encodes a kinase that has been shown to phosphorylate B52 in vitro. These results uncover a previously unrecognized role of the splicing factors in maintaining the G1/S block *in vivo*. Our data exemplify how deregulation of splicing machinery observed in some tumor cells may lead to functional inactivation of the pRB pathway.

120

A gradient of EGFR signaling determines the sensitivity of rbf1 mutant cells to E2F-dependent apoptosis. Nam-Sung Moon, Luisa Di Stefano, Nicholas Dyson. Cancer Research Ctr, Massachusetts General Hosp, Charlestown, MA.

The inactivation of Rb family members sensitizes cells to apoptosis. This cell death affects the development of mutant animals and also provides a critical constraint to the malignant potential of Rb mutant tumor cells. The extent of apoptosis caused by the inactivation of Rb is highly cell type- and tissue-specific but the underlying reasons for this variation are poorly understood. Here, we characterize a specific time and place during Drosophila development where rbf1 mutant cells are exquisitely sensitive to apoptosis. During the third larval instar, many rbf1 mutant cells undergo E2F-dependent cell death in the Morphogenetic Furrow. This pattern of apoptosis is not caused by inappropriate cell cycle progression but instead involves the action of Argos, a secreted protein that negatively regulates Drosophila EGFR (DER) activity. We show that RBF1 and a DER/ras/raf signaling pathway co-operate in vivo to suppress E2F-dependent apoptosis, and that the loss of RBF1 alters a normal program of cell death that is controlled by Argos and DER. These results demonstrate that the importance of the contextual signals that determine when, and where, the inactivation of rbf1 results in dE2F1-dependent apoptosis. We are currently investigating other contextual signals that cooperate with the inactivation of rbf1.

121

In vivo and in vitro regulation of dMyc protein stability by Sgg/dGSK3 and Dco/CK1 kinases. Margherita Galletti[1], Florenci Serras[2], Jin Jiang[3], Pier Giuseppe Pelicci[4], Daniela Grifoni[5], Paola Bellosta[6]. 1) Uni of Modena, Modena, Italy; 2) Uni of Barcelona, Spain; 3) UT Southwestern Medical Center, Dallas, TX; 4) IEO European Institute of Oncology Milan, Italy; 5) Uni of Bologna, Italy; 6) City Collage-CUNY New York, NY.

Precise regulation of the Myc protein is essential to maintain normal cell functions, and its de-regulation can be detrimental. Myc has a half-life of 20-30 min. and studies have shown that Drosophila dMyc protein stability is regulated by the F box protein SCF-E3-ubiquitin ligase Archipelago-Ago (Fbw7/hCDC4) indicating that the pathway of ubiquitination and proteosomal degradation is conserved among humans and flies. We identified in the dMyc aminoacidic sequence putative consensus phosphorylation sites for the Sgg/Zw3/GSK3 and Dco/CK1 kinases. These dual pattern of recognition is often used to regulate protein downstream events: i.e. Armadillo in Wnt pathway, Ci in Hh signaling, or Period for the circadian rhythms, all these proteins are first phosphorylated and this event triggers their further function or fate. Our studies show that dMyc is phosphorylated in S2 cells by Sgg/Zw3/GSK3 and Dco/CK1 kinases and these events induce ubiquitination and degradation of dMyc. Furthermore our in vivo analysis shows that this mechanism of regulation of dMyc is conserved, data will be presented.

122

Mtrm: A Meiotic Inhibitor of Polo Kinase. Youbin Xiang[1], Jeffrey Cotitta[1,2], Stacie Hughes[1], Sue Jaspersen[1], R. Scott Hawley[1,2]. 1) Stowers Inst Medical Research, Kansas City, MO 64110; 2) Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

The mtrm gene exhibits dosage-sensitive effects on achiasmate segregation in Drosophila oocytes, as evidenced by high levels of achiasmate nondisjunction in mtrm heterozygotes. This phenomenon results from a defect in ensuring proper centromere co-orientation of achiasmate chromosomes at prometaphase I. While the deleterious effects of reducing the dose of Mtrm protein by 50% appear to be restricted to achiasmate segregation, complete loss of Mtrm protein results in defects in both the control of pre-meiotic nuclear division and precocious nuclear envelope breakdown in the oocyte. Consistent with multiple roles in the control of meiotic cell cycle, Mtrm exerts its functions in establishing co-orientation via an interaction with Polo kinase. as evidenced by the observation that reducing the amount of Polo in the oocyte suppresses the meiotic centromere co-orientation defects associated with reduced levels of Mtrm, and the over-expression of Polo strongly enhances the mtrm defect. Indeed, simple over-expression of Polo alone results in meiotic defects. Second, a mutation in one of the two putative Polo Binding Domains (PBDs) of Mtrm results in the complete loss of Mtrm function. Third, using antibody against Mtrm, we are able to pull down Polo with Mtrm by co-immunoprecipitation, in fashion that is dependent on this same PBD. Finally, live analysis of female meiosis shows that mtrm/+ oocytes proceed through nuclear envelope breakdown (NEB) and spindle formation far more rapidly than do wildtype oocytes, consistent with the observations by others that over-expression of Polo induces NEB and speeds up spindle assembly. We interpret these data according to a model in which Mtrm exerts its effect on meiosis by acting as a negative regulator of Polo.

123

The Role and Developmental Regulation of CORTEX, a Meiosis-Specific APC/C Activator. Jillian A. Pesin, Terry Orr-Weaver. Whitehead Institute and Department of Biology, M.I.T., Cambridge, MA.

During oogenesis in metazoans, the cell cycle arrests to ensure coordination between meiotic events and growth and development of the egg. The regulation of these arrests and progression through meiosis are poorly understood in Drosophila. *cortex* (*cort*) is required for the completion of meiosis and is expressed exclusively in oogenesis. Mutations in *cort* cause aberrant chromosome segregation in meiosis I and terminal arrest in metaphase II. CORT is a distant member of the Cdc20/FZY protein family and contains conserved key motifs required for activating the anaphase-promoting complex (APC/C). We found that CORT regulates the Drosophila APC/C *in vivo*. CORT physically associates with core subunits of the APC/C during oogenesis, and protein levels of mitotic APC/C substrates, mitotic Cyclins and PIM, the securin homolog, are increased in *cort* mutants. CORT activity appears to be highly regulated, suggesting a specific and crucial role for *cort* during female meiosis. We discovered that CORT protein levels are regulated through developmental control of its translation and degradation. Thus CORT is integral in release of meiotic cell cycle arrest in development, as appearance of the protein is likely to activate the APC/C and may target meiosis-specific substrates.

124

A genetic screen reveals novel regulators of autophagy. Andrew M. Arsham, Thomas P. Neufeld. Genetics, Cell Biology & Development, and Developmental Biology Center, University of Minnesota, Minneapolis, MN.

Autophagy, the starvation-induced process of cellular bulk waste removal and recycling, has recently been implicated in neurodegenerative disorders, cancer, and longevity, and is tightly linked to pathways of cell growth, division, metabolism, and energetics. Autophagy is also thought to suppress cell damage and senescence by eliminating damaged organelles and macromolecules. Most known metazoan autophagy genes have been deduced from homology to yeast genes. Using a mitotic mosaic approach, we have screened 383 lethal p-element insertions on chromosome 2L for dysregulation of autophagy. To the best of our knowledge, this is the first forward genetic screen for metazoan regulators of autophagy. The screen has revealed roles in regulating autophagy for genes involved in growth factor signaling, protein glycosylation, mitochondrial metabolism, and protein quality control, as well as a strong correlation between activation of autophagy and reduced cell size. Detailed characterization of the genetic pathways identified is underway, and will be presented.

125

Diverse signal transduction pathways link cell cycle progression and mitochondrial function. Edward Owusu-Ansah, Amir Yavari, Sudip Mandal, Utpal Banerjee. Dept MCDB, Univ California, Los Angeles, Los Angeles, CA.

Mitochondria are the predominant sites of ATP production and produce Reactive Oxygen Species (ROS) as by-products. The mitochondrial respiratory chain is made up of four electron transporting complexes (I-IV) and a proton translocating complex (V) which actually drives ATP synthesis. Given their central role, crucial cellular processes are usually integrated with the level of mitochondrial activity. To explore the various mechanisms by which mutations in mitochondrial proteins impinge on cell cycle progression, we analyzed clones of several mitochondrial mutants generated by ey-flp mediated recombination; and found several that failed to enter S-phase. Mutations in different mitochondrial complexes elicited remarkably different phenotypes. For instance, while mutations in complex IV proteins resulted in a significant drop in both ATP and ROS production, complex I mutants resulted in increased ROS production without significantly altering ATP production. In all mutants examined, neuronal differentiation proceeded normally, suggesting that the cell cycle phenotype is not a secondary effect of disruption of general cellular function. In addition, apoptosis was not significantly increased in any of the mutants. Our results show that mitochondria use multiple metabolic signals to enforce cell cycle checkpoints - at least, one involving a drop in ATP production and downregulation of cyclin E, and another involving increased ROS production, and upregulation of the cyclin E/CDK-2 inhibitor, Dacapo. The exact signal transduction pathways that are activated in response to different mitochondrial mutants to induce cell cycle arrest will be discussed.

126

RNAi: Quest for the golden locus. Michele Markstein[1], Chrysoula Pitsouli[1], Christians Villalta[1], Sue Celniker[2], Norbert Perrimon[1]. 1) Dept Genetics, Harvard Medical School, Boston, MA; 2) BDGP, Berkeley, CA.

A major obstacle to obtaining reliable RNAi in vivo is the extreme sensitivity of RNAi transgenes to position-effects. For example, individual RNAi hairpin constructs must often be randomly integrated into 10 or more loci to identify a single transgene that produces a robust RNAi phenotype. To overcome this problem, we decided to identify ideal attP "landing site" loci for the site-specific integration of UAS-RNAi transgenes. An ideal attP locus would be one that allows for sufficiently high Gal4 inducible expression but is otherwise quiescent. To identify such loci we developed a quantitative UAS-luciferase reporter assay to measure position effects at attP loci in the presence and absence of Gal4. We also integrated UAS-eGFP and a UAS-Notch hairpin into several of the same attP loci to correlate levels of luciferase activity with phenotypic outcomes. This analysis demonstrates that luciferase is a much more sensitive position-effect reporter than GFP, in that it allows detection of basal expression levels that are too low to be visualized by GFP, but that are high enough to yield phenotypic outcomes with the integrated UAS-Notch hairpin. Moreover, we found that we could exploit the position-effects measured by UAS-luciferase to create a predictable allelic series with the UAS-Notch hairpin. Altogether, we characterized basal and Gal4 inducible expression at 20 attP landing site loci. Of the loci analyzed, we identified one that exhibited very low levels of basal activity and yet was induced to significantly higher levels than any of the other loci in the presence of the ubiquitous Actin5C-Gal4 driver. This locus may therefore be a "golden locus," ideal for the expression of any UAS-RNAi hairpin, in any tissue. However, we found that position-effects resulting in low and high levels of Gal4 inducibility are tissue dependent, raising the possibility that there is no universal golden locus, but instead a number of golden loci each ideal for a different tissue. Using our UAS-luciferase assay, it is easy to identify such loci and to create predictable phenotypic outcomes.

127

Expanding the Gene Disruption Project collection with *Minos* insertion mutants. Robert W. Levis[1], Yuchun He[2,3], Joseph W. Carlson[4], Martha Evans-Holm[4], Soo Park[4], Kenneth H. Wan[4], Karen L. Schulze[2,3], Koen J.T. Venken[2,3], P. Robin Hiesinger[5], Roger A. Hoskins[4], Allan C. Spradling[1,3], Hugo J. Bellen[2,3]. 1) Dept Embryology, Carnegie Inst of Washington, Baltimore, MD; 2) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute; 4) Dept Genome Biology, Lawrence Berkeley National Laboratory, Berkeley, CA; 5) Dept Physiology, UT Southwestern Medical Center, Dallas, TX.

The Gene Disruption Project (GDP) is using a non-targeted transposon mutagenesis strategy to create a publicly available collection of insertion mutants. The goal is that each mutant will have a single marked transposon insertion disrupting a gene and the collection will contain a mutant affecting each gene (see Bellen et al (2004) Genetics 167: 761-781). All mutants in the collection are available from the Bloomington Stock Center. The collection can be searched online (http://flypush.imgen.bcm.tmc.edu/pscreen/) and new mutants may be requested while they are being balanced and rechecked.

The GDP has relied on the *P* element as the primary transposon mutagen. While *P* elements can insert throughout the genome, they have strong target preferences and a subset of genes have not been hit in our screens of >40,000 independent *P*-element insertions. To mutate the remaining genes, we have initiated a screen using a *Minos* enhancer-trap transposon (Metaxakis et al. Genetics (2005) 171: 571-581). *Minos* frequently inserts in genes that have been refractory to mutagenesis by *P* elements and also lacks the strong insertional bias for the promoters of genes characteristic of *P* elements. Of the first 1,717 mapped *Minos* insertions, 13% hit genes that had not been hit by our previous screens, which was five times the percentage of new gene hits in a concurrent *P*-element screen. As of November 1, 2006, we have generated 4,860 insertion mutants in the ongoing *Minos* screen and are producing about 400 new mutants per month.

128

Novel axon pruning mutants identified by a mosaic screen using *piggyBac*-based insertional mutagenesis. Oren Schuldiner, Jonathan Levy, Daniela Berdnik, Joy Wu, David Luginbuhl, Liqun Luo. Howard Hughes Medical Institute, Department of Biological Sciences, Stanford University, Stanford, CA.

Mosaic forward genetic screens are powerful tools for elucidating the functions of essential genes in a cell-dependent manner; however, these screens can be cumbersome due to the long mapping procedures and the inability to use *P*-elements as mutagens. We have adapted existing *piggyBac* tools (Hacker *et al*, 2003) to be compatible with MARCM, and improved their mutagenicity by adding splice acceptors and translation stops. Thus far we have generated ~2000 lines with transposon insertions inside ~1400 independent transcriptional units (including 1kb as a putative promoter). We find that ~25% of mutations are lethal, indicating that the mutagenicity of this screen is better than existing transposon based screening methods. Insertions in the 5'UTR are the most mutagenic (39% lethal), while insertions in the 3'UTR are the least (8%). Interestingly, intronic insertions are quite mutagenic (28% lethal), indicating that the engineered splice trap is increasing mutagenicity. All mutants we have generated are molecularly mapped and can be used in any mosaic or non-mosaic screen.

Using mushroom body (MB) development as an assay, we identified phenotypes in 19% of the lines screened, which include defects in neuroblast proliferation, cell survival, axon guidance and maintenance, and axon pruning. Specifically, we identified two subunits of the cohesin complex, which is essential for sister chromatid cohesion during cell division, to be essential for axon pruning. We are currently investigating whether the role of cohesin in axon pruning is through its function in sister chromatid cohesion, through transcriptional regulation, or through other novel functions.

129

Exploring the spatial and temporal diversity of embryonic gene expression patterns. Benjamin P. Berman[1], Pavel Tomançak[1], Amy Beaton[1], Erwin Frise[2], Richard Weiszmann[2], Elaine Kwan[1], Michael B. Eisen[1,3], Volker Hartenstein[4], Gerald M. Rubin[1,2], Susan E. Celniker[2]. 1) Dept MCB, HHMI, UC Berkeley; 2) BDGP and; 3) Genome Sciences, LBNL; 4) Dept of MCDB, UCLA.

Exhaustive spatial and temporal gene expression data are critical for understanding genomic regulatory networks. We collected and analyzed embryonic whole mount *in situ* hybridizations for an unbiased set of 6,003 genes representing almost half the fly genome. Two curators systematically annotated RNA expression patterns using a controlled vocabulary (CV). We show that the expression profiles captured by these CV annotations are extraordinarily diverse, and yet by using a computational clustering strategy and supplementing the spatial *in situ* data with a more quantitative microarry time-course, we were able to identify groups of genes with similar overall expression. Almost 60% of genes expressed in the embryo fall into one of ten “broad” or nearly ubiquitous patterns, while the remainder fall into one of 29 clusters exhibiting restricted expression. About 20% of these restricted genes appear to be patterned at the blastoderm stage, and common later stage patterns include pan-epithelial, nervous system specific, and muscle specific expression. We will present and discuss the functional and biochemical properties of the various expression clusters, which will ultimately serve as a rich data source for investigating regulatory mechanisms of the transcriptional network controlling animal development. A paper describing this work is in preparation (Tomancak, Berman & Rubin). We additionally used computational image processing techniques as a complementary method for understanding the diversity of expression patterns. We automatically segmented hybridized embryos from image files, then aligned each embryo to an elliptical mesh made up of small triangular regions, each defining a unique location within the embryo. By comparing signal intensity within corresponding triangles across multiple genes, we are able to define expression similarities and have developed an image based search and analysis tool for further studies (Frise & Celniker, *in preparation*).

130

cgChIP: A novel technique for analyzing the chromatin structure of developmentally regulated genes. Daniel J. McKay[1], Richard S. Mann[2]. 1) Integrated Program in Cellular, Molecular, & Biophysical Studies, Columbia University, New York, NY; 2) Department of Biochemistry & Molecular Biophysics, Columbia University, New York, NY.

Chromatin immunoprecipitation (ChIP) is a widely-used technique to identify transcription factor binding sites and to investigate the chromatin modifications associated with gene regulation. To study a gene in its *in vivo* context, chromatin is typically prepared from whole embryos or imaginal discs. However, many genes, especially those required for animal development, are usually expressed in a small subset of embryos or tissues, making interpretation of the signal’s source ambiguous. We have developed a novel technique to perform ChIP experiments in a cell and gene specific manner. The cgChIP approach utilizes the operator sequence from the *E. coli lac* operon to “tag” a DNA sequence of choice. Expression of an epitope-tagged Lac repressor protein under control of a Gal4 driver results in a tissue-specific DNA binding interaction. After formaldehyde fixation and chromatin preparation, antibodies recognizing the epitope-tagged Lac repressor protein are used to pulldown the *lac* operator and surrounding chromatin. This process results in the enrichment of the tagged chromatin specifically from the desired cells. We have used cgChIP to examine regulatory elements at the *Distalless* (*Dll*) gene and to demonstrate that distant enhancers physically interact with the *Dll* promoter in *Dll*-expressing cells.

131

CisDECODER reveals the regulatory logic underlying coordinate gene expression. Thomas Brody[1], Wayne Rasband[2], Kevin Baler[2], Alexander Kuzin[1], Mukta Kundu[1], Jermaine Ross[1], Ward Odenwald[1]. 1) Neural Cell-Fate Determinants, NINDS, NIH, Bethesda, MD; 2) Office of Scientific Director, IRP, NIMH, NIH, Bethesda, MD.

Identification of the regulatory elements that control coordinate gene expression is one of the major tasks currently being undertaken in the analysis of gene regulation. Use of the alignment tool EVOPRINTER (Odenwald, et al. PNAS 102: 14700-5, 2005) reveals that many of the regulatory sequences within identified enhancers are present as conserved sequence blocks (CSBs), averaging ~13 bases in length, suggesting that many of the larger CSBs are docking sites for multiple transcription factors; the average number of CSBs per enhancer ranges from ~13 to 20. We have developed an alignment tool, cisDECODER, that scans CSBs, generating libraries of cisDECODER tags (cDTs) that represents shared regulatory elements within enhancers. cisDECODER identifies elements that are shared by enhancers of coordinately regulated genes and identifies elements common to enhancers of divergently regulated genes. cisDECODER analysis of well-characterized enhancers identifies both known transcription factor binding sites and novel sequence elements. Many of these previously uncharacterized elements are proximal to or interlock with known transcription factor binding sites. cDT analysis of known enhancers reveals constellations of shared elements between enhancers of coordinately regulated genes. For example, cDTs can differentiate between *Drosophila* segmentation, neural, and mesodermal enhancers. We describe the adaptation of the biomolecular interaction network software Cytoscape (Shannon, et al. Genome Research 13: 2498-2505, 2003) to portray shared enhancer elements on a global basis, revealing the presence of shared elements in multiple enhancers of co-regulated genes. The results thus far suggest that cDT analysis can be used to parse the cis-regulatory code shared between enhancers of coordinately regulated genes.

132

Automated Image analysis of Multiple Gene activity patterns in developing animals. William Beaver[1], David Kosman[2], Gary Tedeschi[2], Adam Pare[2], Ethan Bier[2], William McGinnis[2], Yoav Freund[1]. 1) Department of Computer Science and Engineering, UCSD, La Jolla, CA 92093; 2) Cell and Developmental Biology Department, UCSD, La Jolla, CA 92093.

Combinatorial Transcriptional Fluorescent *In Situ* Hybridization (CT-FISH) is a confocal fluorescence imaging technique enabling the detection of multiple active transcription units in individual interphase diploid nuclei. Current CT-FISH methods in our laboratories are able to measure the activity levels of 5 genes in a single embryo or tissue section. Improved combinatorial labeling methods will allow simultaneous measurement of twenty gene activities.

Transforming confocal image stacks with multiple gene activity patterns into usable data is a labor intensive task that calls for computational analysis. Our analysis involves:

1) Segmentation of the cell nuclei using a variety of fluorescent nuclear markers,
2) Detection of transcription sites on chromosomes, removal of false positives, and classification of nascent transcription sites of specific genes by their fluorescent combinatorial codes,
3) Registration of image stacks from different embryos to a developmental model, in the process assessing the consistency of the temporal and spatial gene activity patterns.

Our image analysis involves a combination of image processing and machine learning algorithms. The machine learning algorithms allow experimentalists and computer scientists to reiteratively tune and improve the analysis system to reflect biological reality. Using such algorithms, we show that experimentalists can now overcome the initial analysis bottlenecks, automating steps 1 and 2, and in the near future we will implement step 3 and to develop detailed and accurate developmental gene expression atlases for Drosophila embryos and other complex tissues.

133

Small RNAS are involved in heterochromatin silencing in Drosophila. Christophe Antoniewski[1], Bassam Berry[1], Olivier Voinnet[2], Delphine Fagegaltier[1]. 1) Dev Biology,Jacques Monod Bldg, Institut Pasteur, Paris, CDX 15, FR; 2) IBMPC - CNRS, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, FR.

In plants and S. pombe, repeat-associated siRNA (rasiRNA) are mediators of heterochromatin nucleation and transcriptional gene silencing, through a complex process that involves the RNAi machinery components Dicers, Argonautes, and RNA-dependent RNA polymerases. RasiRNAs have been recently identified in drosophila. Their sequences match transposons, retrotransposons, as well as highly repeated short sequences, all characteristic of heterochromatic regions. Such small RNAs are sequestered in the germline by Piwi which suggests, together with genetic data, that this Argonaute protein may be a mediator of heterochromatin formation . We have investigated the role of small RNAs in heterochromatin formation in *Drosophila* by taking advantage of the ability of natural RNAi suppressor proteins from plant and insect viruses to sequester long or small RNAs. We found that expression of B2 or P19 viral proteins in flies i) suppresses the silencing of markers inserted into heterochromatic environments; ii) induces a dramatic change of the methylated histone patterns on polytene chromosomes (indicative of changes in heterochromatin landmarks) ; iii) sequesters in cultured cells rasiRNAs or their precursors. Together with studies of RNAi mutant effects on heterochromatin formation, the data presented provides direct evidence for involvement of rasiRNAs in establishing heterochromatin landmarks in *Drosophila*.

134

Identification of small RNAs associated with the *gypsy* chromatin insulator. Elissa P. Lei. Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD.

RNAi is a conserved silencing mechanism that can act through alteration of chromatin structure. Chromatin insulators promote higher order nuclear organization thereby establishing DNA domains subject to distinct transcriptional controls. Previously, a functional relationship was identified between RNAi and the *gypsy* insulator. Insulator activity is decreased when Argonaute genes are mutated, and insulator function is improved when the levels of the Rm62 helicase are reduced. Rm62 interacts physically with the DNA-binding insulator protein CP190 in an RNA-dependent manner. Finally, reduction of Rm62 levels results in dramatic nuclear reorganization of a compromised insulator. These results suggest a model in which RNAs processed by the RNAi machinery promote the ability of *gypsy* insulator complexes to form nuclear insulator bodies that serve as scaffolds for independent chromatin domains. Furthermore, recruitment of Rm62 to insulator complexes may remodel or release these RNA-protein interactions to negatively regulate insulator activity.

Several new observations have been documented, which provide a more thorough mechanistic understanding of how RNAi contributes to *gypsy* insulator function. First, Argonaute proteins Piwi and Argonaute2 interact physically with CP190 similarly to Rm62 but in an RNA-independent manner. In addition, Piwi can be found to colocalize with insulator bodies. Extensive analysis of known RNAi mutants has uncovered a role for a second helicase, Armitage, which is required for the maturation of RISC. Like Rm62, Armitage functions as a negative regulator of the *gypsy* insulator. Finally, purification of insulator complexes has identified associated small RNAs, which appear to be transcribed from uncharacterized intergenic regions. Current efforts are focused on identifying the sites of transcription of these RNAs and their functional relevance to insulator function.

135

DmSETDB1 is a euchromatin and chromosome 4-specific histone H3 lysine 9 methyltransferase in *Drosophila melanogaster*. Carole Seum, Emanuela Reo, Pierre Spierer, Séverine Bontron. Dept. Zoology & Animal Biology, University of Geneva, Geneva, CH.

Chromatin structure can be modulated by specific regulatory complexes that recognize and bind covalent modifications made on the protruding N-terminal histone tails of nucleosomes. Methylation on lysine 9 of histone H3 is one of these modifications and is associated with repression and heterochromatin formation. In *Drosophila*, SU(VAR)3-9 is responsible for H3K9 di- and trimethylation mainly at pericentric heterochromatin. However, histone methyltransferases responsible for H3K9 methylation at euchromatin, telomeres and at the peculiar chromosome 4 have not yet been identified. In this study, we characterize *Dm-setdb1*, a gene encoding an 842 amino acids protein bearing a tudor domain, an MBD domain and a bifurcated SET domain. Analysis of H3K9 methylation in a *Dm-setdb1* mutant generated by homologous recombination and in DmSETDB1 overexpressing lines, reveals that this enzyme is responsible for some of the H3K9 mono- and dimethyl marks in euchromatin and for most of H3K9 dimethylation on chromosome 4. We also show that DmSETDB1 represses variegating transgenes inserted on chromosome 4, but not on other chromosomes, supporting its role in chromosome 4-specific chromatin structure. This study characterizes a second H3K9 methyltransferase in *Drosophila* and shows that SU(VAR)3-9 and DmSETDB1 play distinct and complementary roles in H3K9 methylation.

136

Using Drosophila as a model to understand human laminopathies: a study of dMAN1. Belinda Pinto, Shameika Wilmington, Lori Wallrath, Pamela Geyer. Molecular Biology Program, Department of Biochemistry, University of Iowa, Iowa City, IA.

The integrity and organization of the nucleus depends upon the nuclear lamina, a protein network enriched at the inner nuclear membrane. Lamina proteins include the nucleus specific intermediate filament proteins called lamins, as well as LEM domain proteins, named for LAP2, emerin and MAN1. These proteins share a ~40 amino acid motif that binds Barrier-to-autointegration factor (BAF), a small, double stranded DNA binding protein. Through interactions with BAF, LEM domain proteins establish redundant protein interactions that bridge the nuclear envelope and interphase chromosomes. LEM domain proteins play a central role in tissue-specific functions of the nuclear lamina, as mutations in emerin cause X-linked Emery-Dreifuss muscular dystrophy (EDMD) and mutations in MAN1 cause autosomal dominant Buschke-Ollendorf syndrome characterized by increased bone density. The Drosophila genome encodes five LEM domain proteins. Of these, two are predicted inner nuclear envelope proteins: dMAN1 and Bocksbeutel, the putative emerin homologue. Our studies focus on dMAN1, as this protein is the most conserved LEM domain protein. dMAN1 contains a LEM, two transmembrane, an MSC and an RRM domains. Using yeast-two hybrid, we demonstrate that these domains mediate conserved interactions with components of the nuclear lamina (dBAF and Bocksbeutel) and transcription factors (Smads). To understand the function of dMAN1 in the nuclear lamina, we undertook a genetic characterization of *dMAN1*. We generated *dMAN1* mutants and found that loss of dMAN1 reduces viability and causes tissue-specific phenotypes, such as defects in wing patterning and positioning and male sterility. Interestingly, *dMAN1* mutants show reduced climbing ability, a phenotype that worsens with age, suggesting muscle dysfunction. Features of the *dMAN1* mutant phenotypes are reminiscent of human laminopathies, indicating that studies of Drosophila LEM domain proteins will provide insights into mechanisms underlying these diseases.

137

***Drosophila melanogaster* coilin.** Ji-Long Liu, Zheng'an Wu, Zehra Nizami, Joseph Gall. Department of Embryology, Carnegie Institution, Baltimore, MD 21218.

Cajal bodies (CBs) are nuclear organelles involved in assembly and maturation of the RNA processing machinery. They are often detected by the marker protein coilin, which has been identified in many organisms, including human, mouse, *Xenopus*, *Danio*, and *Arabidopsis*. By sequence comparison we recently identified the putative *Drosophila melanogaster* orthologue of coilin, gene CG8710. Flybase lists two possible isoforms; by RT-PCR we confirmed the existence of two different transcripts in whole adult flies. We obtained 20 independent P-element transgenic lines that express YFP-labeled long isoform under control of UAS/GAL4. Lines with YFP at the carboxy terminus (coilin-YFP) all show extremely high levels of expression with multiple clumps of protein, particularly in the cytoplasm. Lines with YFP at the amino terminus (YFP-coilin) show almost exclusively nuclear label. During early oogenesis, up to about stage 7-8, YFP-coilin is strongly expressed in the previously identified CBs of the nurse cells and oocyte. In later stages CBs fragment into multiple bodies that eventually disappear. YFP-coilin exists at a much lower concentration in the histone locus bodies (HLBs) of early nurse cells, but becomes a conspicuous component at later stages, particularly after nurse cell "dumping." In larval salivary gland nuclei YFP-coilin is expressed at high level in one major CB and 2-3 smaller foci near the chromocenter, and at a lower level in the single HLB, which is located at the histone gene locus. We are currently producing antibodies against *Drosophila* coilin to confirm the localization of the protein in wild type flies. These observations suggest that coilin may be a normal constituent of the HLB, and thus not a unique marker for CBs. Based on analysis of salivary gland and nurse cell nuclei we believe that the CB is associated with chromosome 2, either in or near the heterochromatin. Identification of a specific gene or DNA segment to which the CB is attached could prove useful for understanding the functions of the CB.

138

Genetic analysis reveals different cellular requirements of CAF-1 during development. Benjamin Klapholz, Bruce Dietrich, Catherine Schaffner, Jean-Pierre Quivy, Genevieve Almouzni, Nathalie Dostatni. Institut Curie, CNRS UMR218, Paris, FR.

The packaging of eukaryotic genomes into chromatin plays an important role in the maintenance of genome integrity, nuclear organization and gene expression. To better understand how chromatin organization is maintained through cell division during *Drosophila* development, we chose to investigate the function of the Chromatin Assembly Factor-1 (CAF-1), unique to assemble nucleosomes onto newly replicated or damaged DNA. CAF-1 is conserved from yeast to human but its requirement varies among species: it is not essential in the yeast *S. cerevisiae* and the plant *A. thaliana*, but the lack of its large subunit is lethal during early *xenopus* and mouse embryogenesis. We have generated a null allele of *p180* coding the largest subunit of CAF-1. Zygotic mutant larvae survive for 48hrs in the absence of maternal protein but die as small larvae, a phenotype that correlates with reduced DNA replication efficiency and growth defects of endo-replicating cells. Extending the mutant survival by nutrient-withdrawal at 24hrs of larval development demonstrates that the lethality is due to cell-cycle progression defects in endo-replicating cells. The mutant interacts genetically with an allele of the histone chaperon Asf1, defective for histone binding and, consistently, exhibits defects in nucleosomal compaction. The survival of the mutant larvae in the absence of maternal P180 provided us with a good model system to study CAF-1 requirement in mitotic cells. Our preliminary results indicate that the lack of P180 shows variable impacts on larval mitotic cells: although imaginal discs are too small to be analyzed in the mutants and fail to produce somatic clones, larval neuroblasts are still able to replicate DNA and eventually fail to undergo mitosis at 40hrs of larval development. Surprisingly, the mushroom-body neuroblast stem-cells are capable of replicating DNA in the absence of P180 after several days of nutrient-withdrawal, pointing out an intriguing prospect in which CAF-1 requirements would differ with cell identity. Our current work is aiming to shed light on these observations.

139

Drosophila MCM10 in heterochromatin dynamics and DNA replication. Tim Christensen, Helen Zhou, Jasmine Barrow, Gregory Kuzmik, Bike Tye. Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

MCM10 was initially characterized in the budding yeast as an abundant nuclear protein that plays an important role in the initiation of DNA replication. Recently it has also been demonstrated the MCM10 is required for the maintenance of silent chromatin in yeast. *Drosophila* MCM10, though it only shares 24% identity with yeast MCM10, is sufficient to complement a null mutant for growth but not does not rescue silencing defects in other yeast MCM10 mutants. Our *Drosophila* studies have shown that MCM10 interacts with components of the pre-replication complex and HP1. We have mapped the interaction regions of Mcm10 to the C terminal using two-hybrid analysis. In order to determine the functional role of Mcm10 in *Drosophila* RNAi studies have been performed. Depletion of MCM10 in culture leads to abnormal chromosome condensation and precocious sister chromatid separation. These defects may be the indirect result of problems in DNA replication that affect chromatin condensation. Additional work in tissue culture has shown that Mcm10 undergoes cell cycle specific re-localization. This has been followed up using transgenic flies with MCM10::YFP. To further understand the role of MCM10 in *Drosophila*, analysis of different mutant alleles of MCM10 is being performed. The first allele was identified in a sensitized chromosome inheritance modifier screen (Scim) (Dobie et al,2001). $MCM10^{Scim19}$ is a hypomorph of MCM10 and is homozygous viable. Brain squashes reveal that $MCM10^{Scim19}$ is defective for normal chromosome condensation. An additional mutant allele has also been identified that truncates the native protein by removing 85 amino acids from the C terminal. $MCM10^{d08029}$ is homozygous viable but demonstrates defects in DNA replication. Additional analysis including: localization of Mcm10 with replication proteins and HP1, PEV analysis, and chorion gene analysis will be presented. Drosophila MCM10 likely plays a multitude of roles. Understanding these will likely aid in understanding the relationship between DNA replication, chromosome condensation, and heterochromatic silencing.

140

ATM/ATR kinase activity at *Drosophila* telomeres. Sarah Oikemus[1], Daniel Savukoski[1], Joana Queiroz-Machado[2], Claudio Sunkel[2], Michael Brodsky[1]. 1) Dept PGF&E, University Massachusetts, Worcester, MA; 2) Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.

Analysis of terminal deletion chromosomes indicates that a sequence-independent mechanism regulates protection of *Drosophila* telomeres. Mutations in *Drosophila* DNA damage response genes such as *atm/tefu, mre11, nbs* or *rad50* disrupt telomere protection and localization of the telomere-associated proteins HP1 and HOAP, suggesting that recognition of chromosome ends contributes to telomere protection. We find that the *atm* and *atr-atrip* pathways act in parallel to promote telomere protection. Cells lacking both pathways exhibit a more severe defect in telomere protection compared to single mutants and fail to localize the protection protein HOAP to telomeres. Moreover chromosome fusion sites retain telomere specific sequences, demonstrating that loss of these sequences is not responsible for loss of protection. These results are consistent with an end-recognition model in which DNA damage response proteins interact with chromosome ends to help promote telomere function.

To probe ATM/ATR kinase activity at double strand breaks and at telomeres, we examined phosphorylation of H2Av, the *Drosophila* ortholog of H2AX. Phosphorylation of mammalian H2AX by ATM/ATR is one the earliest events in the DNA damage response. We demonstrate that the *Drosophila* ATM and ATR kinases mediate H2Av phosphorylation in response to DNA damage. Cells lacking the telomere protection protein HOAP exhibit increased phospho-H2Av at chromosome ends, indicating that the ATM/ATR kinases are active at unprotected telomeres. Furthermore, phospho-H2Av staining is also increased at telomeres in animals with a separation-of-function allele of HP1 that maintains telomere stability, but is defective for HP1 spreading. These results suggest that the ATM/ATR kinases are also active at protected telomeres. We propose that DNA damage response pathways and HP1 spreading cooperate to mediate epigenetic inheritance of telomere function.

141

COMBINATORIAL CODES SPECIFYING NEURONAL IDENTITIES. Magnus Baumgardt[1], Irene Miguel-Aliaga[2], Daniel Karlsson[1], Helen Ekman[1], Stefan Thor[1]. 1) Molecular Genetics, Linkoping University, Linkoping, SE; 2) Division of Mammalian Development, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom.

In the developing Drosophila ventral nerve cord, about 100 neurons express the LIM-HD gene apterous (ap). Approximately half of these ap neurons are peptidergic and these are divided into two cell types - the six Tv neurons, that express the neuropeptide FMRFamide, and the 28 Ap-let neurons, that express the dopamine receptor, DopR, and also a hitherto unknown neuropeptide gene. Previously, several genes were identified that regulate differentiation of the Tv neuron and its expression of FMRFamide, including ap, the zinc-finger gene squeeze, BMP retrograde signaling, the bHLH gene dimmed, and the dachshund and eyes absent transcriptional co-factors. Of these six regulators, only three (ap, dimm and eya) are expressed in Ap-let neurons, suggesting that other genes are acting to specify Ap-let identity and to regulate DopR and the unidentified neuropeptide gene. We report that the Ap-let neurons specifically express the novel neuropeptide gene Neuropeptide like precursor 1 (Nplp1) and the collier (col)transcription factor. col acts at early postmitotic stages to activate expression of some ap neuron determinants, such as ap and eya, but does not act to regulate dac and sqz. However, col also acts with ap and eya to activate dimm, and subsequently with ap, eya and dimm to activate Nplp1 and DopR expression. col thus plays a sequential role during ap neuron specification: first acting upstream to ensure activation of ap neuron determinants and subsequently acting combinatorially with these regulators to activate Nplp1 and DopR expression. Combinatorial misexpression reveals that both the Nplp1/DopR code and the FMRFamide code are highly potent and can activate the terminal differentiation genes in many postmitotic neurons, showing a surprising disregard for many global instructive cues. In spite of their overlapping composition, we furthermore find that the codes are remarkably specific, each code activating only the proper terminal differentiation gene(s).

142

Notch signaling controls the differentiation of sibling neurons at the Drosophila CNS midline. Scott R. Wheeler, Stephen T. Crews. Program in Molecular Biology and Biotechnology, UNC-Chapel Hill, Chapel Hill, NC.

It is well established that Notch signaling mediates asymmetric cell divisions in the CNS and PNS. However, little is known of the genes and molecular pathways that respond to Notch signaling and make sibling cells different. During the development of the CNS midline cells 5 midline precursor cells (MP1, MP3, and 3 VUM precursors) each divide once giving rise to 10 neurons of 7 types. These include glutamatergic, octopaminergic, and peptidergic motorneurons as well as dopaminergic and glutamatergic interneurons. Together with our identification of 290 genes expressed in midline cells and the advantages of Drosophila genetics and genomics, these cells are an excellent model system to study neurogenesis and neural diversity generation on a genome-wide scale. Temperature shift and misexpression experiments indicate that Notch signaling is required during stages 10-11 for MP asymmetric divisions. Loss of Notch or sanpodo (spdo) function during this critical period results in symmetric MP divisions. In these mutants, each VUM precursor gives rise to 2 VUM motorneurons (mVUM) instead of one mVUM and one VUM interneuron (iVUM) while the MP3 precursor gives rise to 2 H-cell neurons at the expense of its sibling, H-cell sib. In numb mutants the opposite phenotype is observed, iVUMs are duplicated at the expense of sibling mVUMs and H-cell sib is duplicated at the expense of its sibling H-cell. The normally symmetrically dividing MP1 neurons are unaffected. In these mutants, we have examined and categorized the expression of 30 midline genes expressed throughout embryonic development. These studies reveal that Notch signaling directly or indirectly activates the expression of transcription factors, signaling components, and neurotransmitter biosynthetic enzymes and receptors in the Notch-dependent cells while the expression of genes activated in Notch-independent cells is repressed. These studies indicate potential direct targets of Notch signaling and suggest genes and molecular pathways that are activated by Notch signaling leading to asymmetry in the differentiation of sibling neurons.

143

HOW mediates Ecdyson-dependent apoptosis of embryonic midline glia cells. Adriana Reuveny, Talila Volk. Dev. and Mol. Genetics, Weizmann Institute of Science, Rehovot , IL.

In the ventral nerve cord of the *Drosophila* embryo, the midline glial (MG) cells organize the commissural tracts into anterior and posterior commissural bundles. Here, we report that the RNA binding protein HOW (Held Out Wing) is expressed in the midline cells of the *Drosophila* embryo and it is involved in the regulation of MG cell number. The two protein isoforms of HOW, HOW(L) and HOW(S), have been shown to be required for the differentiation of different tissues in the *Drosophila* embryo. We have identified a novel requirement for HOW in the CNS. *how* mutant embryos exhibit excess of MG cells relative to wild type embryos. Our experiments indicate that only the repressor isoform of HOW, HOW(L), can rescue the excess MG cell phenotype, reverting it back to wild type. Further analysis indicates that in the MG cells of *how* mutants there is a reduction of active caspase-3 suggesting that the excess in MG cells stems from inhibition of apoptosis within these cells. Since it has been shown that in other embryonic tissues HOW(L) has a repressive effect on its target mRNAs , we hypothesized that in the MG cells HOW(L) may act by reducing the mRNA levels of a repressor of apoptosis. We have accumulated evidence suggesting that the *Drosophila* inhibitor of apoptosis (DIAP1) may be negatively regulated by HOW(L) activity: (a) DIAP1 levels are elevated in the MG cells of *how* mutant embryos (b) Reducing HOW levels in S-2 cells leads to an elevation of endogenous DIAP1 levels. Mutants in the Ecdysone (Ecd) dependent signaling pathway show a similar phenotype as *how* mutant embryos, where there is an access of MG cells. We found that in these mutants, HOW levels are significantly reduced, consistent with HOW being a target of the Ecd pathway. Taken together our results fit a model where the Ecd-signaling cascade induces MG apoptosis via HOW activity. This model is currently tested by the ability HOW(L) to rescue the Ecd-mutant MG phenotype. These results are consistent with a function for HOW(L) in repressing the levels of DIAP1 in MG cells, making them more susceptible to apoptotic signals.

144

Cytoplasmic and Mitochondrial Protein Translation in Development and Maintenance of Neuronal Terminal Arborization. Takahiro Chihara[1,2], David Luginbuhl[1], Liqun Luo[1]. 1) Howard Hughes Medical Institute, Department of Biological Sciences, Stanford University, Stanford, California 94305; 2) Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

In neurons, protein translation can occur near the cell body and in distal neuronal processes, and has been implicated in increasing aspects in neuronal development and function, such as axon guidance and synaptic plasticity etc. Furthermore, protein translation also occurs in the mitochondria for the mitochondrial genome. The function and regulation of mitochondria in synaptic transmission and plasticity have been reported, and mitochondrial dysfunction is a common feature in neurodegenerative diseases. However, because of the "house keeping" function of protein translation, the relative importance of cytoplasmic and mitochondrial protein translation for different neurobiological processes *in vivo* has not been investigated.

From a mosaic forward genetic screen to find mutants affecting neuronal morphogenesis, we identified a mutation in *glycyl-tRNA synthetase* (*gars*), the *Drosophila* homolog of the human Charcot-Marie-Tooth disease type 2D (CMT2D) associated gene. Loss of *gars* in neurons preferentially affects the elaboration and stability of terminal arborization of axons and dendrites without affecting their initial growth and guidance. Human and *Drosophila gars* genes encode both a cytoplasmic and a mitochondrial isoform. We show that cytoplasmic protein translation is required for terminal arborization of both dendrites and axons during development. By contrast, mitochondrial protein translation is essential for maintenance of dendritic, but not axonal, arborization in aging adults. We also provide evidence that human GARS plays equivalent functions in *Drosophila*, and CMT2D causal mutations exhibit loss-of-function properties. Our study highlights differential requirement of protein translation for the development and maintenance of axons and dendrites, and offers new insights into the pathogenesis of CMT2D.

145

***Dscam* is required for self-avoidance and proper dendritic field organization of dendritic arborization neurons.** Peter Soba[1], Sijun Zhu[1], Kazuo Emoto[2], Susan Younger[1], Shun-Jen Yang[3], Hung-Hsiang Yu[3], Tzumin Lee[3], Lily Jan[1], Yuh Nung Jan[1]. 1) Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA; 2) National Institute of Genetics, Mishima , Japan; 3) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA.

Dendritic field organization is a crucial developmental process to allow proper synaptic connectivity or sensory input. Several intrinsic mechanisms contribute to dendritic field organization: first, a neuron's dendrites typically do not cross one another (self-avoidance). Second, some classes of neurons respect each other's dendritic territory resulting in non-overlapping fields (tiling). Third, different classes of neurons often show overlapping dendritic fields (co-existence). Dendritic arborization (da) neurons of the larval peripheral nervous system are an ideal model system to study dendrite organization, since they feature all three mechanisms. The four classes of da neurons all exhibit dendritic self-avoidance, and class III and IV da neurons additionally show tiling, while the different classes extensively share their dendritic territories. In a candidate approach, we have identified *Down's syndrome Cell Adhesion Molecule* (*Dscam*) as a key player in dendritic self avoidance of all classes of da neurons. *Dscam* loss of function caused dendrite crossing and bundling resulting in disorganized dendritic fields. However, tiling of class IV da neurons was not affected by *Dscam*. Introducing one of the 38,016 Dscam isoforms to class I or class IV da neurons in *Dscam* mutants was sufficient to significantly restore self-avoidance. Remarkably, expression of the same Dscam isoform in da neurons of different classes prevented their dendrites from sharing the same territory. Our results therefore indicate that a single isoform of Dscam is sufficient for self-avoidance of dendrites, while co-existence of dendritic fields of different neuronal classes requires divergent expression of Dscam isoforms.

146

A candidate regulator of the transition from axon pathfinding to branching in *Drosophila* neuronal development: the BTB/POZ Zinc finger transcription factor broad-Z3. Janet Altman, James W. Truman. Department of Biololgy, University of Washington, Seattle, WA.

Neuronal arbor development consists of axon pathfinding to an initial target, interstitial and terminal sprouting to secondary targets, and finally terminal field elaboration and synaptogenesis. Axon pathfinding is primarily a noninteractive outgrowth mode involving reading a map of environmental cues, while later arbor branching modes are more interactive, involving bidirectional communication between neurons and their targets. Appropriate regulation of the developmental transitions between these stages is critical for building correct neuron shape and connectivity. However, we know very little about what factors shift neurons from one mode of growth to the next. We have identified the BTB/POZ Zinc finger transcription factor broad-Z3 (Br-Z3) as a candidate mediator of the transition from pathfinding to branching in the development of the adult central nervous system. We found Br-Z3 expression in all adult-specific interneuron lineages in the larval ventral nerve cord. Br-Z3 expression first appeared after initial stages of pathfinding, and was maintained in most immature neurons throughout the period of arrest with a single primary neurite extended to a primary target (Truman et al., Development, 2004). Br-Z3 was lost at metamorphosis, when these neurons underwent rapid sprouting and elaboration. We examined the function of Br-Z3 expression in the development of adult-specific interneurons using the MARCM technique (Lee and Luo, Trends Neurosci, 2001) to generate heat shock-inducible clones of either induced gene expression or homozygous mutation of Br-Z3 in otherwise unaffected animals. Br-Z3 overexpression clones showed defects including primary neurite errors and ectopic secondary branches from interstitial branching sites. Broad mutant clones (npr) showed inappropriate terminal field elaboration before metamorphosis. These results suggest that regulation Br-Z3 is important for transitioning from pathfinding to branching with appropriate timing.

147

Functional characterization of Syndecan, a Heparan Sulphate Proteoglycan (HSPG), in Slit/Robo Signaling. B Chanana, P Steigemann, H Jäckle, G Vorbrüggen. Dept Mol Dev Bio, Max Planck Inst, Göttingen, DE.

Syndecan is critical for the fidelity of Slit repellent signaling at the ventral midline, where it directs muscle and axonal patterning through receptor Robo. This study aims at elucidating the mechanism by which Syndecan functions in Slit/Robo signaling. Using tissue specific *sdc* rescue experiments we show that Syndecan is required on the target tissue, in a *cis-cis* configuration with Robo, and not in the Slit secreting cells or in the intermediate somatic tissue indicating that Syndecan acts as a co-receptor and has no function in Slit secretion or transport. Rescue experiments using *sdc* deletion transgenes reveals that the conserved cytoplasmic domain is not important for function proving that Syndecan does not participate directly in intracellular signaling or in modification of actin cytoskeleton of axonal growth cones. We further show that the conserved transmembrane domain serves only as an inert anchor for the extracellular domain and does not lead to the incorporation of the molecule into functional complexes. We thus conclude the extracellular domain to be the sole functional part of the molecule. Shedding of vertebrate Syndecan has been demonstrated to be essential for function *in vivo*. Contrarily, in our study, over-expression of a secretory form of Syndecan, equivalent to the shed form, had failed to rescue the *sdc* mutant phenotype. This suggests that D-Syndecan functions differently from its vertebrate homologs. We have therefore constructed a double-tagged *sdc* transgene lacking the putative cleavage site to determine if shedding of the extracellular domain is vital for function. We also explored the involvement of other HSPGs in Slit/Robo signaling and our double mutant analysis proved *dly* to be the second HSPG involved. Currently we are investigating the importance of the number of HS GAG chains. We have constructed *sdc* transgenes carrying increasingly fewer numbers of HS GAG chains and none at all. If Syndecan is the primary HSPG in Slit/Robo signaling due to the number of HS GAG chains then we expect to see a gradation of rescue with these transgenes.

148

***DE-cadherin*/*shg* in *Drosophila* larval brain development.** Siau-Min Fung[1], Fay Wang[2], Volker Hartenstein[1]. 1) Dept MCDB, Univ California, Los Angeles, Los Angeles, CA; 2) Gladstone Institue of Neurological Disease, Univ California San Francisco, San francisco, CA.

The *Drosophila* central brain consists of a great number of lineages that project their axons into the neuropile. Our lab has mapped these lineages in the late larval period. Each lineage is formed by the progeny of one neuroblast. Axons emitted by neurons of one lineage fasciculate in a tract, namely the secondary axon tract (SAT). The anatomy of each SAT consists of its neuroblast located near the brain surface, the progeny of these neuroblast form the secondary tract which innvervate the neuropile. In the neuropile, tufts of filopodia appear at specific positions along the SAT (proximal branchpoint), typically where the SAT meets the neuropile glia. In the pupal period, SAT grows out to form arbors at proximal and terminal branchpoints in the neuropile.

The comprehensive map of the secondary lineages allows us to study the function of *DE-cadherin*/*shg* in the larval brain. We have used the MARCM technique to generate *shg* clones in specific secondary neurons. Shg antibody has been used to further validate the clones. In the cortex, *shg* mutant clones have inappropriate layering of the cortex, detouring of SAT before entering the neuropile and axon bundles do not fasciculate together. In addition, at the boundary of cortex and neuropile, *shg* mutant clones display ectopic branching and *shg* SAT form premature terminal arbors in the neuropile. Furthermore, we have also obtained data to suggest *shg* control axon bundling through the catenin complex (arm) instead of the *wg* pathway.

149

HNF4 coordinates nutrition with fat metabolism in *Drosophila*. Laura Palanker, Carl S. Thummel. Dept of Human Genetics, University of Utah, Salt Lake City, UT.

The *Drosophila* orphan nuclear receptor HNF4 represents the single ancestral ortholog of vertebrate transcription factors that are highly enriched in liver, known as hepatocyte nuclear factors 4 α and γ. Mutations in the genes that encode these proteins lead to insulin-resistant diabetes in humans and to impaired glucose tolerance, liver overgrowth, and reduced serum lipid concentrations in mice. We found that *Drosophila HNF4* null mutants die as pharate or newly eclosed adults under normal feeding conditions and are highly sensitive to starvation during larval stages. Immunohistochemistry reveals that HNF4 is expressed broadly in *Drosophila* larvae and prepupae, including the midgut and fat body, which play important roles in nutrient uptake, storage, and metabolism. We use a Gal4-HNF4 fusion protein to demonstrate that the transcriptional activity of HNF4 can be regulated by diet in these tissues, acting as a readout of the metabolic status of the animal. Upon starvation, *HNF4* mutant larvae contain reduced levels of glucose and fatty acids, which are essential energy sources under nutrient-deficient conditions. Mutant animals abnormally retain triglycerides in the midgut and fat body and thus appear unable to efficiently hydrolyze stored fat to make the fatty acyl-CoA substrates for β-oxidation. Metabolic profiling by GC/MS suggests that enzymes involved in long chain fatty acid metabolism may be misregulated in starved *HNF4* mutants. We are currently undertaking microarray studies under fed and starved conditions to identify HNF4 transcriptional targets that may be involved in energy homeostasis. We are also testing candidate fatty acid ligands for their ability to regulate HNF4 activity. Our data supports a model for HNF4 as a sensor that regulates fat metabolism in response to nutritional status.

150

Reduction of p53 activity in the brain of adult D. melanogaster extends life span as part of the Sir2 life span-extending pathway. Johannes Bauer, Chenyi Chang, Suzanne Hozier, Siti Nur Sarah Morris, Sandra Andersen, Joshua Waitzman, Stephen Helfand. Department of Molecular Biology, Cell Biology and Biochemistry Division of Biology and Medicine Brown University 70 Ship Street, Room 408 Providence, RI 02903.

We have recently shown that expression of dominant negative (DN) versions of D. melanogaster p53 (Dmp53) in the adult brain significantly extends the life span of the fly. Here we show that this life span extension is abolished when DN-Dmp53 is expressed in a Dmp53 null background. This suggests that reduced Dmp53 function, rather than unspecific effects of the different DN-Dmp53 proteins causes life span extension. Preliminary data showed that the mechanism of DN-Dmp53 dependent life span extension might be related to the Calorie Restriction (CR) pathway. Life span extension by DN-Dmp53 expression is not additive to CR and life span extension by CR is partly blocked in Dmp53 null flies. In Drosophila, the CR pathway is mediated by the histone deacetylase Sir2. Interestingly, one target of Sir2 is p53 itself. Deacetylation of p53 by Sir2 leads to p53 inactivation. Overexpression of Sir2 or treatment of flies with the Sir2 activator resveratrol leads to extended life spans. However, concomitant expression of DN-Dmp53 in flies either treated with resveratrol or overexpressing Sir2 does not further extend the life span of these long lived flies. Taken together, these results suggest that p53 is indeed part of the CR/Sir2 life span extending pathway. In addition, we identified the Drosophila insulin producing cells (IPC) as the smallest subset of neurons in which DN-Dmp53 expression still leads to life span extension. This suggests that life span regulation by Dmp53, in addition to being a part of the CR pathway, might also be related to insulin signaling, thus providing a possible mechanistic link between these two life span extending pathways.

151

Germline Regulation of Aging in Drosophila melanogaster. Thomas Flatt[1], Michael Rocha[2], Kyung-Jin Min[1], Michael Grunwald[3], Ruth Lehmann[3], Leanne Jones[2], Marc Tatar[1]. 1) Dept Ecology & Evolution, Brown Univ, Providence, RI; 2) The Salk Institute for Biological Studies, Laboratory of Genetics, La Jolla, CA, USA; 3) Howard Hughes Medical Institute, Developmental Genetics Program, Skirball Institute of Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, NY, USA.

While elimination of the entire gonad has no effect on C. elegans survival, ablation of germline precursor cells dramatically extends adult life span. This suggests that signals from the germline interact with signals from the somatic gonad to affect whole-organism aging; however, the identity of these signals is poorly understood. Here we report that eliminating the germ line also increases the life span of both sexes in Drosophila melanogaster. Genetic ablation of the germ line at the larval to adult transition extends life span, whereas loss of the germline at earlier stages does not. Conversely, over-proliferation of germline tissue in the adult shortens adult life span. Importantly, increased longevity resulting from loss of the germ line is not due to reduced investment in reproduction since female-sterile mutants with an intact germline are not long-lived. In addition, we find that germline loss in the adult modulates the production of Drosophila insulin-like peptides by median neurosecretory cells, suggesting there is likely an endocrine feedback loop between germline and brain. From these data we propose that the germline modulates aging by repressing longevity assurance signals produced by the mature somatic gonad.

152

4EBP mediates alterations in translation and lifespan due to dietary restriction in Drosophila. Brian Zid[1], Aric Rogers[2], Seymour Benzer[1], Pankaj Kapahi[2]. 1) Caltech, Pasadena, CA; 2) Buck Institute, Novato, CA.

Dietary restriction (DR) extends lifespan in a multitude of organisms, yet its molecular underpinnings are still poorly defined. We find that the eukaryotic translation initiation factor binding protein, 4EBP, is upregulated under DR, and is necessary and sufficient for the lifespan extension of DR. Upon investigating the genome-wide translational changes under DR, it was found that genes translationally downregulated have extensive amounts of 5'UTR secondary structure, while upregulated genes have weakly structured 5'UTRs. Mitochondrial oxidative phosphorylation transcripts were a subset of mRNAs which showed upregulation upon DR and contain little 5'UTR secondary structure. Translational upregulation of mitochondrial processes under DR was confirmed at the post-translational level and was d4EBP dependent. We show that d4EBP has an important role in DR, and that differential control of translation by 5'UTR secondary structure appears to be a key output.

153

Subcellular regulation allows the *Drosophila* PI3-kinase/Akt signalling to perform different metabolic and developmental functions. Clive Wilson[1], Natalia Vereshchagina[1], Noelia Pinal[2], Lucy Collinson[2], Yasuyuki Fujita[2], Iain Cox[1], Franck Pichaud[2], Deborah Goberdhan[2]. 1) Dept Physiology, Anatomy & Genetics, Le Gros Clark Building, Univ Oxford, Oxford, GB; 2) MRC LMCB, Dept of Anatomy and Developmental Biology, UCL, Gower Street, WC1E 6BT, London.

Insulin/insulin-like growth factor signalling (IIS) globally regulates cell growth and metabolism in *Drosophila* . These effects are thought to be mediated by cell surface activation of the Class I PI3-kinase/Akt signalling cassette. Detailed analysis of mutants affecting this pathway suggests that it also has cell type-specific roles. For example, the establishment of proper rhabdomeric morphology at the apical surface of photoreceptors requires normal IIS, a function that has recently been shown to be conserved in mammalian epithelia. In flies, we have found that a specific isoform of PTEN, a direct antagonist of PI3-kinase function, binds to focal adhesion structures flanking the photoreceptor apical region via the PDZ domain-containing protein Bazooka and is essential for controlling levels of activated, phosphorylated Akt (P-Akt) specifically within this cell surface domain. By contrast, nurse cells of the developing egg chamber express P-Akt within their cytoplasm. Levels of cytoplasmic P-Akt are massively upregulated in *PTEN* mutant cells. This upregulation correlates with a redistribution of stored lipid into large (up to 15 μm) lipid droplets, an effect that is not observed when Akt is selectively activated at the cell surface. We show that LSD2, the homologue of the lipid droplet coating protein perilipin, is upregulated in *PTEN* mutant nurse cells. These phenotypes mirror the effects on lipid droplet formation and perilipin expression seen in adipocytes in response to insulin and suggest that the nurse cell may be an excellent model to study the genetics of lipid storage. In summary, our data reveal an important role for subcellular regulation of PI3-kinase/Akt signalling, which acts in combination with other cell type-specific factors to determine the metabolic and developmental response of cells to insulin-like molecules.

154

Genetic analysis of AMPK and LKB1 function in Drosophila melanogaster. Michelle Bland, Julie Magallenes, Morris Birnbaum. Dept. of Medicine, University of Pennsylvania, 415 Curie Blvd., Philadelphia, PA.

The AMP-activated protein kinase (AMPK) senses decreased cellular energy levels and activates a switch leading to ATP conservation and production during metabolic stress. Despite its role as a critical regulator of cellular energy metabolism, relatively little is known about the in vivo functions of AMPK, owing in part to the redundancy of AMPK subunit genes in mammals. AMPK and one of its upstream kinases, LKB1, are well conserved in the model organism *Drosophila melanogaster*, and each *Drosophila* AMPK subunit is encoded by only one gene. Therefore, we have taken a genetic approach in *Drosophila* to study the in vivo consequences of loss of AMPK and LKB1 function. Imprecise excision of a P element inserted 2.4 kb downstream of the *Drosophila* AMPK α subunit gene (*dAMPKα*) resulted in deletion of the 39 C-terminal amino acids and the 3'UTR of *dAMPKα*. Hemizygous *dAMPKα*$^{\Delta 39}$ mutant larvae lack detectable AMPK phosphorylation and die near the beginning of the third larval instar. The lethality of the *dAMPKα*$^{\Delta 39}$ mutation can be completely rescued by ubiquitous expression of a wild type *dAMPKα* subunit transgene but not by limited transgene expression in fat body, muscle or neurons, alone or in combination. As adults, *dAMPKα*$^{\Delta 39}$ heterozygotes display altered responses to stressors compared with wild type flies. In contrast to *dAMPKα*$^{\Delta 39}$ hemizygotes, flies with homozygous null mutations in *lkb1* die as wandering third instar larvae. Furthermore, AMPK α is still phosphorylated, albeit at lower levels, in *lkb1* homozygous larvae compared with *lkb1* heterozygous larvae. These results demonstrate that *dAMPKα* is an essential gene in *Drosophila* and suggest that other kinases can activate AMPK in cells lacking LKB1. Mosaic analysis is being undertaken to allow study of the cell-autonomous requirements for LKB1 and AMPK signaling while circumventing the lethality of these mutations. Using these genetic approaches, we hope to identify additional members and roles of the AMPK signaling pathway.

155

The Role of Autophagy in the Adult Nervous System During Oxidative Stress and Aging. Kim Finley[1], Anne Simonsen[2], Robert Cumming[1], David Schubert[1]. 1) Cellular Neurobiology Lab, Salk Inst Biological Studies, La Jolla, CA; 2) Institute for Cancer Research Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway.

Previous work has shown that mutations in the blue cheese (bchs) gene results in extensive neural degeneration and the accumulation of insoluble ubiquitinated protein aggregates (IUP) in the adult CNS. Using a Bchs-based modifier screen several mutations in genes associated with of lysosomal trafficking pathways are shown to modify a dominant Bchs-eye phenotype. The identified genes included several members that participate in the macroautophagic pathway (autophagy). Autophagy is a vesicle-based proteolytic pathway that is constitutively active and upregulated during conditions of stress. Recent studies showed that mice with targeted disruption of key autophagic genes exhibit neurodegeneration and motor dysfunction accompanied by accumulation of ubiquitinated proteins. Here we demonstrate that mutations in the Drosophila autophagic pathway can also result in progressive accumulation of IUP aggregates throughout the CNS and shortened adult lifespans. These defects as well as mutations in bchs also increase sensitivity to oxidative stress. Enhance expression of individual autophagy genes in the mature CNS (using the GAL4/UAS system), resulted in an extension of lifespan by as much as 60% when compared to wildtype control animals. Enhancing the autophagic pathway is also protective under oxidative stress conditions and decreases both IUP and carbonylated protein profiles. In addition, increased autophagy gene expression suppresses polyglutamine induced eye toxicity. Our results indicate that both mutant and age-related decreases in autophagic function are associated with the accumulation of cellular damage in neurons that can be blocked by increasing rate-limiting components of the pathway. This work demonstrates that autophagy plays a key role in maintaining neuronal health and response to oxidative stress during aging.*blue cheese bchs*.

156

Lipid Storage Droplet (Lsd2) Mutants are Lean, Long-lived, and Benefit from Dietary Restriction. Danielle Skorupa[1], Beverly Patuwo[2], Sergiy Libert[1], Jessica Zwiener[1], Scott Pletcher[1]. 1) Huffington Center on Aging, Baylor College of Medicine, Houston, TX; 2) Center for Educational Outreach, Baylor College of Medicine, Houston, TX.

The *Drosophila* fat body, an endocrine organ analogous in function to mammalian adipose tissue and liver, is involved in the coordination of cellular growth and survival of the entire animal. It has been established that adult-only reduction of insulin/insulin-like signaling (IIS) in the peripheral or pericerebral fat body by overexpression of the forkhead transcription factor FOXO is sufficient to extend lifespan (Ginnakou et *al.*, 2004; Hwangbo et *al.*, 2004). Many mutants in various components of the IIS pathway (i.e., melted) likewise have starvation or triglyceride content phenotypes (Teleman et *al.*, 2005). Furthermore, dietary restriction (DR) may be beneficial to the lifespan of organisms partially because of the reduction in adipose tissue mass observed with DR regimens. Lsd2 (lipid storage droplet) mutants are lean, similar to the orthologous Perilipin null mice (Tansin et *al.*, 2001) and have defects in lipid droplet trafficking (Welte et *al.*, 2005). We chose to study lean $Lsd2^{51}$ mutants to observe changes in longevity associated with leanness and its relationship to dietary restriction. We determined that homozygous, hemizygous, and heterozygous null flies are all long-lived compared to their revertant controls. Correspondingly, Gal4-drivern Lsd2 overexpression shortens lifespan in a tissue-specific manner. The lifespan of $Lsd2^{51}$ mutants can further be extended by the application of a DR regimen suggesting that Lsd2 is acting in a parallel or distinct pathway from that of DR and that leanness can be beneficial in addition to DR application. Further studies will be aimed at determining the tissue-specific requirements for rescue of this longevity and DR phenotype.

157A

An expression-based approach to identifying factors that mediate cell competition. Claire de la Cova, Laura A. Johnston. Genetics and Development, Columbia University, New York, NY.

Cell competition is a process that results in elimination of "losing" cells and survival of "winning" cells within growing organs of both vertebrates and *Drosophila melanogaster*. In a mosaic *Drosophila* wing imaginal disc, cells expressing high levels of dMyc compete against and eliminate their neighbors with lower dMyc levels. "Losing" cells die frequently and their competitive elimination requires the pro-apoptotic gene *hid*. Because little is known about the signal that kills "losing" cells, and no biological markers specific to cell competition exist, we sought to better characterize "winning" and "losing" cells using a gene expression approach. We have generated and isolated competing cell populations from the wing imaginal disc and used gene expression microarrays identify mRNA expression changes in "winning" dMyc cells and wildtype "losing" cells. A large number of significant expression changes occur in dMyc-expressing cells (750 genes at ≥1.5-fold). On the other hand, when growing near dMyc-expressing cells, wildtype "losing" cells display a comparably small number of significant expression changes (58 genes at ≥1.5-fold). We have selected several candidate genes whose expression levels are altered in "losing" cells and used a simple clonal assay of cell competition in the wing imaginal disc to explore their roles in the competitive process. We find that some of our candidate genes contribute to the cell death or to the growth disadvantage of "losing" cells, while others are required for the survival of dMyc-expressing "winning" cells. This data raises the possibility that both "winning" and "losing" cells produce signals necessary for cell competition.

158B

Characterization of a Mutation that Produces Cell Competition. Yassi Hafezi, Iswar Hariharan. Molecular and Cell Biology, Univ. of California, Berkeley, Berkeley, CA.

The goal of this study is to address the mechanistic basis of a phenomenon known as cell competition. In Drosophila melanogaster, viable yet slow-growing clones of cells can be eliminated from a tissue when they are adjacent to certain faster-growing cells. The basis of competitive ability and the mechanism by which cells eliminate other cells are unclear. Our laboratory has previously identified mutants in over twenty loci in an extensive screen for genes that negatively regulate growth in the developing Drosophila eye. In this study we characterize one viable mutation from this screen and show that it is involved in cell competition. We are currently trying to map the mutation to identify the gene responsible for this phenotype. We are also testing the effects of this mutation on molecules previously implicated in cell competition, dMyc and Decapentaplegic (Dpp), to determine whether it competes in the same way or through a novel mechanism. Ultimately, we hope to better understand this interesting cell behavior.

159C

A functional analysis of cell competition using *Drosophila* cell culture. Nanami Senoo-Matsuda, Laura A. Johnston. Department of Genetics & Development, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Our studies have revealed that developing wing cells in *Drosophila melanogaster* that differ in expression levels of the growth regulator dMyc can compete, leading to the apoptotic death of the cells with less dMyc ("losers") and over-representation of cells with more dMyc ("winners") in the wing (de la Cova *et al.*, 2004). This phenomenon, called cell competition, seems to play a crucial role in the control of organ size. To identify the genetic logic underlying cell competition, we have developed an S2 cell-culture based assay for cell competition to use in a genome-wide, functional RNAi screen. We have made stable cell lines that inducibly express either dMyc or the PI3K, Dp110, or constitutively express GFP, and established an *in vitro* model of cell competition. Using a variety of co-culture assays we find that cell competition is induced in a dMyc-concentration and time-dependent manner. Our results indicate that cell competition does not require direct cell-to-cell contact and is the result of factors secreted from both the "winner" and "loser" cell population. We will discuss these results and our efforts to identify genes required for competition to occur.

160A

Studies on the regulation of dMyc expression by Insulin and Nutrients signaling. Rajendra Chilukuri[1], Federica Parisi[2], Daniela Grifoni[2], Paola Bellosta[1]. 1) City College-CUNY,New York, NY; 2) University of Bologna, Italy.

myc is a gene whose deregulation is prominent in cancer, and is a critical regulator of growth in flies and mammals. Genetic studies in vertebrates and invertebrates suggest that signals from the conserved patterns organizing morphogens such as Insulin, BMP/Dpp/TGF-b Wnt/Wingless, and Hedgehog contribute to this program, but it is unclear how they monitor and regulate growth. Our and others microarray analysis revealed that the majority of Myc target genes play a role in ribosome biogenesis, protein synthesis and metabolism, consistent with dMyc's role in cellular growth. We recently demonstrated in vitro using S2 cells, that stimulation of cells with Insulin increases Myc protein levels and this event is dependent on Tor signaling. Our data are consistent with a role of dMyc on Insulin and Nutrients signaling. Most recent data will be presented.

161B

Drosophila TCTP is a new component of the TSC pathway. Ya-Chieh Hsu[1], Kwang-Wook Choi[1,2]. 1) Program in Developmental Biology, Baylor College Med, Houston, TX; 2) Molec & Cell Biol, Baylor College Med, Houston, TX.

Cellular growth and proliferation are properly coordinated during organogenesis. Misregulation of these processes leads to pathological conditions such as cancer. Tuberous Sclerosis (TSC) is a benign tumor syndrome caused by mutations in either TSC1 or TSC2. Recent studies in Drosophila and other organisms have identified TSC signalling as a conserved pathway for growth control. Activation of the TSC pathway is mediated by Rheb (Ras homologue enriched in brain), a Ras superfamily GTPase. TSC2 has been shown to be the GTPase activating protein (GAP) for Rheb, but a guanine nucleotide exchange factor (GEF) that facilitates the GDP/GTP exchange on Rheb has not been identified. We have found genetic and biochemical evidence which suggests that the highly conserved protein Translationally Controlled Tumor Protein (TCTP) is a new component of the TSC pathway. Reducing dTCTP levels affects cell size, cell number and organ size, which mimics the Drosophila Rheb (dRheb) mutant phenotypes. dTCTP is genetically epistatic to TSC1 and dRheb, but acts upstream of ds6k, a downstream target of dRheb. In addition, dTCTP directly associates with dRheb and displays GEF activity to it both in vivo and in vitro. Expression of the human TCTP (hTCTP) can rescue dTCTP mutant phenotypes. Since hTCTP is also able to interact with human Rheb (hRheb) and stimulates its GTP/GDP exchange, the function of TCTP in the TSC pathway is likely to be conserved throughout evolution. Currently we are identifying critical amino acids mediating the function of dTCTP and dRheb.

162C

The role of CUL4-DDB1 in the control of growth and CDT1/DUP levels during *Drosophila* development. Hyun O Lee, Sima Zacharek. GMB, University of North Carolina, Chapel Hill, NC.

CDT1/DUP is an essential replication licensing factor that is degraded at the onset of S phase via ubiquitin-mediated proteolysis to ensure that the genome is replicated only once per cell cycle. The $CUL4^{DDB1}$ E3 ubiquitin ligase is necessary for the regulated proteolysis of CDT1/DUP after DNA damage, but whether it plays an essential role in the destruction of CDT1/DUP at the beginning of S phase is unclear. In order to examine this issue and to determine the in vivo function of $CUL4^{DDB1}$ we isolated and characterized mutations in the essential *Drosophila Cul4* and *Ddb1* genes. *Cul4* and *Ddb1* null mutants develop until the 1st or 2nd larval instar stage, and then display phenotypes consistent with a growth defect: The mutant animals can survive for up to 10 days without developing further and fail to incorporate BrdU in most cells. Clones of *Ddb1* null mutant cells generated by mitotic recombination in larval imaginal discs are reduced in size relative to control clones. Similarly, *Cul4* mutant cells grow slowly and are eventually eliminated from the imaginal epithelia most likely via competition with phenotypically normal neighboring cells. Depletion of either CUL4 or DDB1 in homozygous mutant larvae or by RNAi in cultured S2 or HeLa cells results in mild hyper-accumulation of CDT1/DUP. DDB1 and CDT1/DUP were detected in CUL4 immunocomplexes. Thus, we were surprised to find that clones of either *Ddb1* or *Cul4* mutant imaginal cells demonstrated normal CDT1/DUP degradation at the G1-S transition, suggesting that $CUL4^{DDB1}$ is not necessary for cell cycle regulated CDT1/DUP degradation and that the observed hyper-accumulation may be due to growth or cell cycle arrest. Recent results in vertebrate systems suggest redundancy between CUL4 and CUL1 E3 ligases in the control of CDT1/DUP degradation during the cell cycle. However, cells in *Cul1* or *Cul1 Cul4* double mutant clones also fail to hyper-accumulate CDT1/DUP.

163A

Identification and characterisation of novel regulators of insulin signalling. Shivanthy M Visvalingam, Deborah C.I Goberdhan, Clive Wilson. Department of Physiology, Anatomy & Genetics, Le Gros Clark Building, Oxford University, Oxford, GB.

The Insulin/Insulin-like growth factor signalling (IIS) cascade is a highly conserved pathway which regulates growth and metabolism in response to the availability of nutrients. Investigating this pathway has immense importance in understanding tumorigenesis, a process which is frequently upregulated, and type 2 diabetes where IIS is reduced. Our laboratory is interested in identifying and characterising novel growth regulators, which might mediate their effects through IIS. Random and candidate gene overexpression screens using the GAL4/UAS system have highlighted a number of genes involved in protein trafficking and cytoskeletal remodelling, which can promote growth and appear to interact genetically with the IIS pathway. Some of these genes interact with other signalling cascades such as the Notch signalling pathway which can also modulate growth. We describe the characterisation of these genes and their effects on different signalling pathways. These experiments have indicated a role for protein shuttling in regulating multiple functions of IIS in Drosophila.

164B

The Drosophila Hus1 is required for double strand DNA repair during meiosis. Lihi Gur-Arie[1,2], Uri Abdu[1,2]. 1) Life science, Ben-Gurion University, Beer-Sheva, IL; 2) The National Institute for Biotechnology in the Negev, Beer-Sheva, IL.

In Drosophila un-repaired double-strand DNA breaks (DSBs) activate a meiotic checkpoint which affects egg chamber polarity and organization of the oocyte DNA. The Rad9-Rad1-Hus1 checkpoint complex is known as the sensor of DNA damage. In previous work we studied the role of *hus1* in activation of the meiotic checkpoint and found that the *hus1* mutation suppressed the dorsal-ventral pattering defects caused by mutations in DNA repair enzymes. Interestingly, we found that the *hus1* mutant exhibits similar oocyte nuclear defects as those produced by mutations in DNA repair enzymes. These results demonstrate that *hus1* is essential for the activation of the meiotic checkpoint and that *hus1* is also required for the organization of the oocyte DNA, a function that may be independent of the meiotic checkpoint. In this study we analyzed the function of *hus1* during meiosis and discovered that the synaptonemal complex (SC) disassembles abnormally in *hus1* mutants. We showed that the oocyte nuclear and the SC defects in *hus1* mutants can be suppressed by blocking the formation of DSBs, suggesting that the *hus1* oocyte nucleus defects are DSB dependent. Eliminating the checkpoint activity by using mutations in *chk2* or *mei-41* suppressed the oocyte nucleus and SC defects of *hus1*, suggesting that these processes are dependent on *chk2* and *mei-41* checkpoint activity. Moreover, we showed that in *hus1* DSBs during meiosis are not processed efficiently, this defect was not suppressed by mutations in either *chk2* or *mei-41*. Finally, we found a significant increase in the level of X non-disjunctions in *hus1* mutants. We proposed that the un-repaired DSBs in the *hus1* mutant led to activation of *chk2* and *mei-41*-dependent checkpoint that is required for organization of the oocyte DNA and for the disassembly of the synaptonemal complex.

165C

BubR1, but not Mad2, is required for recruiting and localizing of Fzy to the kinetochores in Drosophila melanogaster. Deyu Li[1], Roger Karess[2], Michael Whitaker[1], Jun-Yong Huang*[1]. 1) Cell and Molecular Biosciences, Faculty of Medical Sicences, Newcastle, GB; 2) CNRS, Centre de Génétique Moléculaire, Ave de la Terrasse, 91198 Gif sur Yvette, France.

Mad2 and BubR1, two important spindle checkpoint proteins, have been reported to inhibit anaphase promoting complex/cyclosome (APC/C) activity by binding to its activator Cdc20 (or Fzy in Drosophila), which results in suspension of the metaphase/anaphase transition until all the kinetochores are properly attached to microtubules and the sister chromatids aligned properly at metaphase plate. However, it is still unclear how these three proteins interact on kinetochores in time and space to generate this inhibitory signal. Here we report that the dynamic distribution and localization of GFP-Mad2 in Drosophila syncytial embryos is consistent with that reported in other systems though in addition it predominantly localizes with the nuclear regions throughout the cell cycle. Drosophila Mad2 associates with kinetochores at prophase and starts to disappear from early metaphase kinetochores. Interestingly, we found that the dynamic distribution of average fluorescent intensities of CFP-Mad2 and YFP-Fzy in the nucleus regions oscillates out of phase with each other in transgenic Drosophila syncytial embryos that co-express these two proteins. Surprisingly, Mad2 does not appear to be an essential protein for mitotic progression; flies without Mad2 (Mutant: EY21687) develop normally. Furthermore, we have found the recruitment and kinetochore localization of Fzy is dependent on BubR1, but not Mad2, when we examined the localizations of GFP-Fzy in Mad2 or BubR1 null mutant embryos and neuroblast cells.

166A

Activation of Mitotic checkpoints by impaired mitochondrial function. Sudip Mandal, Kevin Yackle, Utpal Banerjee. Dept MCDB, Univ California, Los Angeles, Los Angeles, CA.

The regulation of cell cycle progression from one phase to the next is intricately controlled and has many checkpoints that take into account cellular status and environmental cues. In a genetic screen we isolated mutations in several genes encoding mitochondrial proteins that cause a cell cycle arrest during late larval stages in Drosophila. Mutations in Cytochrome oxidase Va (CoVa), a member of complex IV of the electron transport chain, lead to reduction in the level of ATP and activates a specific pathway that eventually cause a block in G1-S transition. Interestingly, mutations in genes coding for Glutaminyl-Amido Ttransferase A and Arginyl-tRNA Ligase that are involved in mitochondrial protein synthesis affect both G1-S and G2-M progression. Importantly, this block in cell division is specific because other cellular functions like growth and differentiation remain unaffected and it also does not invoke apoptosis. The genetic pathway leading to the activation of these checkpoints will be presented.

167B

Drosophila MEK and ERK function in an intrinsic cell-cycle checkpoint pathway. Vladic Mogila, Fan Xia, Willis X. Li. Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642.

Cell cycle checkpoints are surveillance mechanisms that safeguard genome integrity. The presence of extrinsic pathways that halt the cell cycle when DNA damages are detected has been well documented to involve the ATM and ATR checkpoint kinases. In contrast, the intrinsic pathways that monitor the orderly and timely progression of cell cycle events are not well understood. Here, we demonstrate that Drosophila MEK and ERK constitute an essential intrinsic checkpoint pathway that restrains cell cycle progression in the absence of DNA damage, and also responds to ionizing radiation to arrest the cell cycle. Embryos lacking MEK exhibit faster and extra division cycles and fail to undergo timely midblastula transition (MBT) or arrest following ionizing radiation. Conversely, constitutively activated MEK causes cell cycle arrest. Further, MEK activation in the early embryo is cell cycle-dependent and Raf-independent, and increases in response to ionizing radiation or in the absence of Chk1. Thus, MEK/ERK activation is required for multiple checkpoints and is essential for orderly cell cycle progression.

168C

The fruit fly Drosophila melanogaster as a model system for studying the role of 9-1-1 complex in DNA damage checkpoints. Ronit Tokarsky[1,2], Uri Abdu[1,2]. 1) Life science, Ben - Gurion University, Beer -Sheva, IL; 2) The National Institute for Biotechnology in the Negev, Beer -Sheva, IL.

The Rad9, Rad1 and Hus1 complex (9-1-1 complex) has been characterized as a sensor of DNA damage required for initiation of the DNA damage checkpoint. Evidence provided by molecular modeling and electron microscopy analysis suggested that human Hus1, Rad1, and Rad9, may form a PCNA-like doughnut-shaped heterotrimeric complex arranged in a head to tail manner. In this work we would like to study the mechanism by which the Drosophila Rad9, Hus1 and Rad1 protein complex acts in response to DNA damage. For this reason, the three proteins Hus1, Rad1, and Rad9 were expressed in the Drosophila embryo-derived Schneider S2 cell line, and their localization was studied by immuno-fluorescence. We found that Hus1 is a cytoplasmic protein; Rad1 is localized both to the cytoplasm and to the nucleus and Rad9 localizes to the nuclear envelope. When the three proteins were co-expressed, they were detected on the nuclear envelope, suggesting that Rad9 determines the complex localization. To determine the localization of Hus1, Rad1 and Rad9 *in vivo*, we generated transgenic flies carrying HA-Hus1 and Flag-Rad9 under the control of the Gal4/UAS system and examined the localization of the proteins by immuno-fluorescence in the fly ovary. We found that Hus1 localized to the cytoplasm of the nurse cells and Rad9 localized to the nuclear envelope of the nurse cells. These results further support the work done in Drosophila S2 cells. In order to examine the ability of the Drosophila Rad9, Hus1 Rad1 proteins to form a complex, we examined the interaction between these proteins by co-IP assays. We were able to co-IP Rad9 with Hus1 and Rad1, suggesting that the Drosophila 9-1-1 proteins form a biochemical complex. The work on the function of this complex in response to DNA damage events is still in process.

169A

Fragile X mental retardation protein controls *trailer hitch* expression and cleavage furrow formation in *Drosophila* embryos. K. Monzo[1], O. Papoulas[1], G.T. Cantin[2], Y. Wang[1], J.R. Yates III[2], J.C. Sisson[1]. 1) The Section of MCD Biology and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 2) The Department of Cell Biology, The Scripps Research Institute, La Jolla, CA.

During the cleavage stage of animal embryogenesis cell numbers increase dramatically in the absence of cell growth and a shift from maternal to zygotic genetic controls occurs called the mid-blastula transition (MBT). We demonstrate that *Drosophila* fragile X mental retardation protein (dFMRP) is required in early embryos for cleavage furrow formation and functions within dynamic cytoplasmic ribonucleoprotein (RNP) bodies during the MBT. We observe that dFMRP colocalizes with the RNP body components Trailer Hitch (TRAL) and Maternal Expression at 31B (ME31B) in the cytoplasm of cleavage stage embryos. Complementary biochemistry demonstrates that dFMRP does not associate with polyribosomes in cleavage stage embryos, consistent with the reported exclusion of polyribosomes from many cytoplasmic RNP bodies. When the normal cytoplasmic accumulation of zygotic transcripts is disrupted during the MBT by using a conditional mutation in *small bristles* (*sbr*), which encodes an mRNA export factor, large dFMRP/TRAL-associated structures are observed in the cytoplasm, suggesting that dFMRP and TRAL dynamically regulate RNA metabolism during this time in development. Furthermore, we demonstrate that dFMRP associates with endogenous *tral* mRNA and is required for normal TRAL expression levels and localization, revealing *tral* mRNA as a new target of dFMRP control. Finally, we show genetically that *tral* itself is required for cleavage furrow formation. Together these data suggest that in cleavage stage *Drosophila* embryos dFMRP functions by regulating the availability and/or competency of specific transcripts for translation. Additional proteomics-based approaches have been initiated to identify more targets of dFMRP regulation during the MBT, which should enhance our understanding of the mechanisms of dFMRP activity and cleavage furrow formation.

170B

Mutation of the microRNA *let-7* in *Drosophila melanogaster*. Elizabeth E. Caygill, Laura A. Johnston. Department of Genetics & Development, Columbia University College of Physicians and Surgeons, New York, NY.

The microRNA *let-7* was initially characterized in the nematode *C. elegans*, where it is expressed in hypodermal blast cells and regulates the timing of their cell cycle exit and adoption of adult cell fate. In *D. melanogaster* the functional 21 nucleotide *let-7* sequence and the general developmental timing of it's expression are conserved. Expression begins at the end of the larval growth period, and peaks midway through pupal development. This conservation of sequence and temporal expression pattern, and its role in regulating proliferation in *C. elegans* led us to hypothesize that *let-7* contributes to the cessation of growth at the end of *Drosophila* larval development. Experiments of *let-7* over-expression are consistent with this model, but to determine its true role in fly development we used homologous recombination to generate a *Dm let-7* mutant. Homozygous *Dm let-7*mutants exhibit pleiotropic phenotypes including sterility, shortened life span, growth defects, and neurological defects. We will discuss our efforts to determine the basis for these phenotypes, and to identify genes regulated by *Dm let-7* with a directed genetic screen.

171C

Cullin3 suppresses Hedgehog signaling to pattern the Drosophila retina. Wang Chien-Hsiang[1,2], Ou Chan-Yen[1], Chien Cheng-Ting[1,2]. 1) Academia sinica, Institution of Molecular Biology, Taipei, TW; 2) National Yang Ming University, Institution of Neuronscience, Taipei, TW.

Drosophila eye development is under delicate regulation by several signaling pathways from larval to adult stages. Hedgehog (Hh) signaling promotes morphogenetic furrow (MF) proceeding and initiates eye differentiation through regulating the downstream effector Cubitus interruptus (Ci). At the mid-pupal stage, mature ommatidia are enclosed by 3 sensory organ precursors, 6 secondary pigment cells and 3 tertiriary pigment cells, collectively named interommatidial cells (IOCs). Cullin3 (Cul3) interacts with Roc1 and BTB-domain proteins to form a complex, functioning as ubiquitin E3 ligases. Our previous study showed that Cul3 down regulates Ci protein levels behind the MF in larval eye discs. Here we report that Cul3 mutants display ectopic IOCs in mid-pupal eyes. Elevation of Ci or Hh also induces extra IOCs. Reversely, in Ci dsRNA knocked down mutants or in eye-specific hh mutants the IOC numbers are reduced. More over, expression of Ci dsRNA suppresses the phenotype of ectopic IOCs in Cul3 mutants, consistent with that Cul3 downregulates Ci levels in IOC formation. IOCs are derived from a pool of precursors in the second mitotic wave (SMW) behind the MF. In Cul3 mutants, ectopic cell division in the SMW had been observed, as assessed by ectopic BrdU incorporation and Cyclin B accumulation. We also found that Hh signaling also regulates the cell cycle progression in the SMW. In conclusion, we suggest that Cul3 suppresses Hh signaling to ensure only one finale cell cycle for precursor cells in the SMW. It has been suggested that the BTB-protein Rdx/Hib function as the substrate receptor for Cul3 ligases in ubiquitinating Ci. However, while Ci level is elevated behind the MF in rdx and hib mutants, the effect of ectopic IOCs is less prominent, suggesting the existence of other BTB-domain proteins in downregulating Ci levels during retinal development.

172A

Characterization of *whale* a spontaneous cell growth mutant. Deborah K Hoshizaki[1], Alia Jabali[1], Cheryl Gustafson[2], Paul Lawson[1]. 1) School of Life Sciences, University of Nevada, Las Vegas, NV; 2) Unversity of Nevada School of Medicine, Reno, NV.

We have identified a spontaneous growth control mutant designated *whale*. The identification of the *whale* mutant was based on the presence of gigantic larvae in a *w*; *Gal4* stock designated F9. By a series of genetic crosses the *whale* mutation was extracted from the F9 stock, identified as a second chromosome recessive larval lethal mutation, and established in a second chromosome balanced stock. We have demonstrated by measuring fecundity that persistence of *whale* in the F9 stocks is likely due to the selective advantage of *whale* heterozygotes over F9 individuals.

whale mutants exhibit normal growth rates during the initial larval growth phase but the last larval instar is prolonged. *whale* mutants persist as third-instar larvae and continue to grow resulting in an accumulation of enlarged larvae. By day 10 where >95.5% of *whale* heterozygotes have eclosed as adults, ~90% of the *whale* mutants are present as enlarged third-instar larvae. The *whale* larvae do not pupariate and die as larvae with a shrunken fat body and miniature imaginal disc.

We have mapped *whale* using the Bloomington deficiency kit to a 75kb region within 25C8-D1. Contained within this region is the TGF-B type I receptor thick vein. Further characterization of *whale* is under investigation and includes testing whether *whale* is a neomorph of *thick vein* and measuring the growth parameters of the *whale* larvae.

173B

Control of proliferation during the transition from undifferentiated into progenitor cell state during *Drosophila* eye development. Carla S. Lopes[1], Fernando Casares[1,2]. 1) CABD-Centro Andaluz de Biologia del Desarrollo, Universidad Pablo de Olavide, Seville, Spain; 2) IBMC-Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.

In *Drosophila*, eye differentiation starts in early L3 and progresses, throughout L3 and early pupa stages, as a moving signalling center (the morphogenetic furrow (MF)) sweeps the eye primordium (eye disc) in a posterior-to-anterior direction, leaving on its wake differentiated photoreceptors and accessory cells. This progressive differentiation is tightly coupled to the control of proliferation. The most anterior cells in the eye primordium are undifferentiated and proliferate asynchronously. Immediately anterior to the MF, cells withdraw synchronously from the cycle, at the “first mitotic wave (FMW)”and arrest in a G1 state. The domain of G1 arrest corresponds to the domain of eye progenitor cells, in which cell cycle withdrawal coincides with the expression of the proneural gene atonal, and of retinal determination markers. Despite the increasing knowledge on the factors required for eye fate determination and photoreceptor differentiation, little is known about the factors and mechanisms involved in the maintenance of the undifferentiated state, that characterizes the anterior eye disc population. Previous studies have shown that Homothorax (Hth), a homeodomain transcription factor, acts as a repressor of retinal genes and is required for proliferation of cells in the anterior domain of the eye disc (Pai *et al.,*2000; Pichaud and Casares, 2000; Bessa *et al.*,2002), suggesting that Homothorax is implicated in the maintenance of the undifferentiated/proliferative cell state. In agreement, ectopic expression of Hth results in overgrowths, maintenance of asynchronous proliferation, and delayed retinal differentiation. Our study, by investigating the cell cycle steps controlled by Hth and identifying specific cell-cycle regulators as putative Hth targets, addresses the mechanisms through which Hth maintains the undifferentiated state of eye disc cells and how cell cycle withdrawal is coordinated with the acquisition of the progenitor cell fate.

174C

Rbf1-independent termination of E2f1 target gene expression during early *Drosophila* embryogenesis. Shusaku Shibutani, Lisa M. Swanhart, Robert J. Duronio. Dept Biology, Univ North Carolina, Chapel Hill, NC.

The initiation and maintenance of G1 cell cycle arrest is a key feature of animal development. In the *Drosophila* ectoderm, G1 arrest first appears during the 17th embryonic cell cycle. The initiation of $G1_{17}$ arrest requires the developmentally-induced expression of Dacapo, a p27-like Cyclin E/Cdk2 inhibitor. The maintenance of $G1_{17}$ arrest requires Rbf1-dependent repression of E2f1-regulated replication factor genes, which are expressed continuously during cycles 1-16 when S phase immediately follows mitosis. The mechanisms that trigger Rbf1 repressor function and mediate the maintenance of $G1_{17}$ arrest are unknown. We show that the initial down regulation of expression of the E2f1 target gene *RnrS*, which occurs during cycles 15 and 16 prior to entry into $G1_{17}$, does not require Rbf1 or $p27^{Dap}$. This suggests a mechanism for Rbf1-independent control of E2f1 during early development. E2f1 protein is destroyed in a cell cycle dependent manner during S phase of cycles 15 and 16. E2f1 is destroyed during early S phase, and requires ongoing DNA replication. E2f1 protein re-accumulates in $G1_{17}$ arrested epidermal cells, and in these cells the induction of $p27^{Dap}$ activates Rbf1 to repress E2f1 target genes to maintain a stable G1 arrest.

175A

The proneural bHLH proteins Atonal/Daughterless and the canonical EGFR signaling coordinately regulate photoreceptor specification and cdk inhibitor expression. Madina Z. Sukhanova, Dilip K. Deb, Gabriel M. Gordon, Miho Matakatsu, Wei Du. Ben May Institute for Cancer Research, University of Chicago, Chicago, IL.

Proper formation of organs requires coordinated regulation of cell proliferation and differentiation, but the developmental mechanisms that link these processes have not been well characterized. In the developing *Drosophila* eye, cell cycle exit during photoreceptor differentiation is redundantly regulated by the fly retinoblastoma protein RBF and the fly cyclin dependent kinase inhibitor Dacapo (Dap), which expression is transiently induced during photoreceptor specification. Surprisingly, we found that Dap expression in various photoreceptor cell types is differentially regulated. We focused on identifying the mechanisms that regulate Dap expression during R2 and R5 photoreceptor specification. Interestingly, Dap expression in these cells is regulated by the proneural bHLH proteins Atonal (Ato) and Daughterless (Da) and the canonical EGFR signaling, which have been previously shown to be required for proper R2/3/4/5 specification. Our results suggest that the direct control of key cell cycle regulators by differentiation factors is a simple and general mechanism that ensures coordinated regulation of cell cycle and differentiation.

176B

Growth regulation in the *Minute* mutants of *Drosophila melanogaster*. Meng-Ping Tu, Debra A. Smith, Laura A. Johnston. Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The *Minutes (M)* are a large class of dominant mutations, primarily in ribosomal protein-encoding genes. *M* mutants grow slowly and are delayed in development, presumably due to impaired protein synthesis, but eventually reach a relatively normal adult size. To understand the mechanisms behind the slow growth of *M* mutants, we have initiated a detailed study of larval and imaginal disc growth in several different *M* mutants, including the *rpl14 (M66D), rps3 (M95A), rps5*^{2} *(M15D), and rpl19 (M60E)*. We find that although larval development in most of these mutants occurs with normal kinetics for the first two instars, imaginal disc growth and patterning is aberrantly uncoupled from larval growth and severely delayed. However, a prolonged growth period during the third instar allows the discs to reach a final size slightly larger than wildtype controls. *M* imaginal discs, but not larval tissues, have significantly increased cell death, which may contribute to their slow growth. Consistent with this idea, when cell death is reduced with the *H99* deletion (removing one copy of *hid, reaper* and *grim*) the larval developmental delay is rescued by approximately 20%. In addition, measurements of cell doubling times under these conditions indicates that wing disc cells from *M* mutants proliferate at the same rate as wildtype, although they spend a disproportionate amount of time in S phase. To study the de-regulated coordination of larval and imaginal growth in *M* mutants, we are carrying out experiments with rescuing transgenes in various *M* tissues using tissue-specific GAL4 drivers. Preliminary results suggest that coordination of larval and imaginal development requires hormonal regulation.

177C

Characterization of a novel conserved cyclin in *Drosophila*. Dongmei Liu, Russell Finley. Dept CMMG, Wayne State Univ, Detroit, MI.

Cyclins are a conserved family of proteins that interact with and activate Cyclin dependent protein kinases (Cdks). We are studying the function of a *Drosophila* protein, CG14939, which has a conserved cyclin-like domain. We have shown by yeast two-hybrid (Y2H) screens that CG14939 interacts with a poorly characterized Cdk called Eip63E. Here we confirmed the interaction by co-affinity purification (co-AP) and further showed that they preferentially interact with each other relative to other Cdks and cyclins. A mutation of Eip63E, Gly243, which is adjacent to the putative cyclin-interacting PFTAIRE domain, almost eliminated interaction with CG14939, supporting the notion that Eip63E/CG14939 constitute a bona fide Cdk-cyclin pair. To further characterize the function of CG14939, we generated gene knock-down mutant animals by the Gal4/UAS-dsRNA system. We found that gene knock-down with two independent non-overlapping dsRNA resulted lethality. Furthermore an imprecise excision also produced larval lethality, suggesting that this gene plays an important function during development. Further characterization is ongoing.

178A

MAPK activity during Drosophila egg activation. Katharine Sackton, Norene Buehner, Mariana Wolfner. Dept Molecular Biol & Genetics, Cornell Univ, Ithaca, NY.

The transition from mature oocyte to dividing embryo requires the coordination of multiple events, in a process called "egg activation". This process, which is triggered by calcium in all animals examined, includes resumption of meiosis, initiation of translation of some maternal mRNAs and degradation of others, and changes in the egg's outer coverings. The molecular pathways that trigger these changes are only poorly understood, but studies in marine invertebrates and in vertebrates suggest that a drop in MAPK activity may prompt the resumption of meiosis. We have shown that all three of Drosophila's MAPKs are active in mature oocytes, and that activity of all three decreases upon egg activation. Using mutants that arrest during activation, we have identified which of these activation-related genes act upstream of the changes in MAPK activity and which genes are independent or downstream of this aspect of egg activation. Two Drosophila proteins that regulate the onset or characteristics of embryo mitosis are known MAPK substrates that become dephosphorylated during activation. However, our data show that the drop in MAPK activity that occurs during activation is not sufficient for the dephosphorylation of these proteins.

179B

***Shattered*, Anaphase Promoting Complex-1, is indispensable for proper photoreceptor cell differentiation through cell cycle synchronization.** Miho Tanaka-Matakatsu[1], Barbara J. Thomas[2], Wei Du[1]. 1) BMICR, University of Chicago, Chicago, IL; 2) Genes, Genomes and Genetics IRG, CSR, OER, NIH.

The Shattered (Shtd) is required for the proteolysis of mitotic Cyclins and Cdc25/String in/ahead of the morphogenetic furrow (MF) in developing Drosophila eye disc. The failure of proteolysis of mitotic CycA in the MF leads the G1 cells undergo premature S phase entry, while the failure of Stg and CycA degradation causes the cells delay/arrest upon the exit of mitosis. Interestingly, *shtd* mutation disrupts cell cycle exit of the developing photoreceptor cells, leading to an additional cell division of the already specified photoreceptor neurons. We showed that *shtd* encodes Apc1, the largest subunit of the Anaphase-promoting complex/Cyclosome (APC/C). This is consistent with the observation that mutations of APC/C activator *fizzy-related (fzr)* also lead to accumulation of Stg and CycA. Our study suggests that APC/C activation during M-G1 phase is indispensable to G1 cell cycle synchronization to ensure proper photoreceptor differentiation.

180C

Cell biological analysis of Cyclin E/Cdk2-mediated replication-dependent histone mRNA biosynthesis. Anne E. White[1], Michelle E. Leslie[2], Brian R. Calvi[5], William F. Marzluff[1,2,3,4], Robert J. Duronio[1,2,4]. 1) Department of Biology; 2) Curriculum in Genetics and Molecular Biology; 3) Department of Biochemistry and Biophysics; 4) Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, NC; 5) Department of Biology, Syracuse University, Syracuse, NY.

Metazoan histone mRNA biosynthesis is tightly regulated during the cell cycle, such that the majority of histones are made only during S phase. We are using *Drosophila* to explore the molecular details of cell cycle regulated histone mRNA biosynthesis during animal development. Cyclin E/Cdk2 is necessary for histone gene expression during embryogenesis, but precisely how it regulates histone mRNA biosynthesis is not known. The monoclonal antibody MPM-2 recognizes a phospho-epitope found on several proteins during interphase and mitosis. In *Drosophila*, nuclear MPM-2 staining requires Cyclin E/Cdk2 activity. We show that in early embryos MPM-2 detects one or two Cyclin E/Cdk2-dependent nuclear foci that co-localize with nascent histone transcripts. These foci are coincident with the histone locus body (HLB), a Cajal body-like nuclear structure in *Drosophila* associated with the histone locus and enriched in histone pre-mRNA processing factors such as Lsm11, a core component of the U7 snRNP. Using MPM-2 and anti-Lsm11 antibodies, we demonstrate that the HLB appears precisely when zygotic histone transcription begins during nuclear cycle 11 in syncytial embryos. Whereas the HLB is found in all cells after its formation, MPM-2 labels the HLB only in replicating cells that contain active Cyclin E/Cdk2. These data indicate that MPM-2 recognizes a cell cycle-regulated component of the HLB. Interestingly, MPM-2 and Lsm11 foci are present in embryos lacking the histone locus, indicating that the HLB organizes into a sub-nuclear structure independently of histone mRNA biosynthesis. However, in these embryos the MPM-2 foci are smaller, and some Lsm11 foci are not associated with MPM-2 foci, suggesting that histone expression is important for HLB integrity.

181A

An RNAi screen to identify regulators of MEI-S332 localization. Hannah R. Cohen, Thouis Jones, Robert Lindquist, David Sabatini, Terry Orr-Weaver. Whitehead Institute, Cambridge, MA.

Drosophila MEI-S332 is the founding member of the shugoshin family of centromeric cohesion proteins. It is essential for accurate chromosome segregation in meiosis and contributes to this process in mitosis. In mitotic cells, MEI-S332 is loaded onto centromeres at the onset of prophase and is unloaded at the metaphase/anaphase transition. We are using a living cell microarray to identify genes necessary for the correct localization of MEI-S332. Spots of dsRNA targeting 12,000 *Drosophila* genes are printed on a set of glass slides. *Drosophila* tissue culture cells are then allowed to adhere to the slides, and one gene is knocked down on each spot. MEI-S332 localization is assayed by immunofluorescence and analyzed computationally. We expect this approach to identify novel factors that regulate the loading and unloading of MEI-S332 from centromeres. We predict that factors identified in this screen will be essential for chromosome segregation in mitosis and/or meiosis.

182B

An analysis of chromosome pairing dynamics in meiosis and in the early embryo. Justin Blumenstiel[1], William Theurkauf[2], R. Scott Hawley[1]. 1) Stowers Inst, Kansas City, MO; 2) UMass Medical School, Worcester, MA.

To ensure the proper segregation of chromosomes during meiosis, it is essential that chromosomes become paired with one another. In many organisms, this pairing is restricted to meiosis and mediated by crossing over but in *Drosophila* and other dipterans, somatic pairing is ubiquitous. Recent work using the LacI-GFP system has indicated that meiotic chromosome pairing is established in the mitotically dividing germline. This suggests that chromosome pairing in *Drosophila* may be established in the early germline and that the mechanism that establishes somatic chromosome pairing may also establish meiotic pairing. One model for the initiation of homolog pairing - capture in *trans* of nascent RNA transcripts derived from one homolog by the RNAi machinery assembled on the other homolog - is suggested by the fact that 1) the RNAi machinery plays a role in heterochromatin formation and heterochromatic associations stabilize pairing between non-crossover chromosomes in meiosis and 2) the RNAi machinery mediates long range chromosomal interactions between homologous Polycomb group response elements. To test this model, we have analyzed chromosome pairing dynamics using FISH in early embryos and during meiotic prophase of flies that are mutant for various components of the RNAi machinery. Specifically, we have analyzed chromosome pairing dynamics in flies that are mutant for components of both the siRNA and rasiRNA pathway. To date, we find no evidence that either the siRNA machinery, or the rasiRNA machinery, are necessary for the initiation of somatic pairing in the early embryo, meiotic pairing within the germline or the maintenance of heterochromatic associations through meiotic prophase. While we have yet to rule out functional redundancy between these different aspects of the RNAi machinery, these results seem to suggest that homology sensing between homologous chromosomes is established by a mechanism that is independent of the mechanism necessary for the clustering of homologous Polycomb group response elements and RNAi mediated heterochromatin formation.

183C

Why Don't Null Alleles of Axs have a Phenotype? Susan M. Flynn, Stacie E. Hughes, R. Scott Hawley. Stowers Institute for Medical Research, Kansas City, MO.

Axs (*abnormal X segregation*), the founding member of a novel *Drosophila* family of transmembrane proteins, localizes to the ER in early embryos and to a membranous sheath-like structure associated with the oocyte meiotic spindle. A dominant mutation in this gene, *Axs* ^{D}, increases achiasmate-specific chromosome nondisjunction and exhibits defects in cell cycle progression and spindle formation. We have generated knock-outs of *Axs* on both the *X* chromosome and the achiasmate *FM7* chromosome. Curiously, neither chiasmate nor achiasmate homozygous *X* chromosomes nondisjoin at high frequency in such females; and thus being homozygous for a null allele of *Axs* does not mimic the phenotype of the *Axs*^{D} mutant. We suggest two possible hypotheses as to why the *Axs*^{D} mutation causes meiotic defects whereas the *Axs* knock-outs do not. The *Axs*^{D} mutation may be neomorphic, resulting in a protein product that has acquired a new function which disrupts meiosis. Alternatively, since Axs is a member of a family of 5 transmembrane domain proteins, it may have a functionally redundant partner that can act in place of Axs when it is knocked out. For the three most distant homologs, antibody localization experiments and analysis of strong loss-of-function alleles failed to show roles for these genes in meiosis. Until now, we have not been able to test the closest homolog, *CG15270*. However, three putative loss-of-function alleles of *CG15270* have been generated by the FLY-TILL Project at FHCRC. We will present data on our analysis of these alleles for segregational defects in a homozygous or deficiency background as well as in the presence of the *Axs* knock-out mutant. Mutations in the human Axs-like gene *GDD1* cause bone anomalies suggestive of a defect in calcium regulation. Likewise, the structure and localization of Axs suggest that it could function as a calcium channel and the *Axs*^{D} mutation may allow improper calcium signaling during meiosis leading to defects in meiotic cell cycle progression, spindle formation and achiasmate chromosome segregation.

184A

The *ald/mps1* and *polo* Kinases Form Filaments in *Drosophila* Female Meiosis. William Gilliland[1], Jeffrey Cotitta[1,2], Stacie Hughes[1], Youbin Xiang[1], R. Scott Hawley[1,2]. 1) Stowers Inst, Kansas City, MO; 2) U. Kansas Medical Center, Kansas City, KS.

The *Drosophila* gene *ald* encodes the fly homolog of *mps1*, a conserved kinetochore-associated kinase required for the meiotic and mitotic spindle checkpoints. We demonstrate using live imaging that in the absence of the Ald/Mps1 protein, oocytes enter anaphase I immediately upon completing spindle formation, without allowing nonexchange bivalents to congress to the metaphase plate, an observation that explains the heightened sensitivity of non-exchange chromosomes to the meiotic effects of hypomorphic *ald* alleles. After germinal vesicle breakdown (GVBD) in wildtype oocytes the Ald/Mps1 protein localizes to the outer edge of highly stretched meiotic kinetochores and to numerous filaments throughout the oocyte; this localization is greatly reduced in *ald* mutant females. While these novel filamentous structures are not observed in mitotic cells, similar filaments were observed in live oocytes bearing a *polo-GFP* transgene. Fixed oocytes demonstrate colocalization of Polo-GFP and Ald/Mps1 protein to the same filaments, confirming that these proteins are found in the same structures. However, the kinetochore-associated proteins Aurora-B and Incenp do not colocalize to these filaments, nor did the structural filaments Actin, Tubulin or the septins Peanut, Sep1, Sep2, or Sep4. While the function of these filaments is unknown, one possibility is that these filaments protect stored kinetochore components from degradation during the syncitial nuclear divisions.

185B

A work in progress: Live imaging of chiasmate and achiasmate co-orientation during *Drosophila* female meiosis. Stacie E. Hughes, Jeffrey Cotitta*, William Gilliland, R. Scott Hawley. Stowers Institute for Medical Research, Kansas City, MO.

Using live imaging we investigated the behavior during prometaphase in *Drosophila* oocytes of two classes of X chromosomal bivalents, those held together by chiasmata and achiasmate chromosomes, which are conjoined only by heterochromatic pairing. During meiosis I, chiasmate bivalents were found to co-orient immediately on the anastral spindle and appeared to define the metaphase plate of the developing spindle. We interpret these observations to mean that chiasmata predispose bivalents to establish proper co-orientation prior to nuclear envelope breakdown. Although achiasmate homologs are usually well separated by metaphase (i.e. positioned between the plate and opposite poles), during early prometaphase their movement is considerably more dynamic, with movements that often cross the metaphase plate, before becoming stably balanced on the two halves of the spindle. In situ hybridization studies suggest that during prometaphase these homologs are in fact connected by long fibers of heterochromatin, which may control their movement in a fashion analogous to two spheres connected by a rubber band.

186C

Identification of proteins that interact with Nod, the chromokinesin-like protein essential for achiasmate chromosome segregation. Li-Jun Huo, Youbin Xiang, Kimberly Collins, R. Scott Hawley. Stowers Institute for Medical Research, Kansas City, MO.

The mechanisms which maintain homologs on the metaphase plate until anaphase are crucial to ensure high-fidelity chromosome segregation in meiosis. In metaphase of meiosis I, the physical linkages between homologs that result from recombination, called chiasmata, function to prevent aberrant and precocious segregation. In the absence of chiasmata, the distributive system functions to properly segregate achiasmate chromosomes. In *Drosophila* female meiosis, the chromokinesin-like protein called Nod is essential to the distributive system. Nod is an important component of the polar ejection force; a force that emanates from the spindle poles and is directed towards the metaphase plate in mitosis and meiosis. Polar ejection forces contribute to maintaining achiasmate chromosomes on the metaphase I spindle by balancing the opposing poleward forces exerted by the kinetochore microtubules. While the polar ejection force has long been known to exist, the mechanisms which regulate it are largely unknown. To investigate Nod regulation, we sought to identify proteins that interact with Nod, either physically or genetically. To discover physical interactors, we have identified proteins that interact with Nod-FLAG in oocyte extracts by analyzing co-precipitating proteins with Mass Spectroscopy. In parallel, we are performing two screens to identify genetic modifiers of Nod function. The screens are based on the observation that overexpression of the antimorphic allele Nod^{DTW} in the eye under the control of the GAL4 driver leads to a narrow and reduced eye phenotype. We are currently screening the Bloomington and Exelixsis deletion stocks for haploinsufficiencies that dominantly enhance or supress the Nod^{DTW} eye phenotype. We have identified several modifiers and are currently in the process of mapping the responsible loci.

187A

The fly that came in from the cold: *trade embargo* (*trem*), a new cold-sensitive meiotic mutant. Cathleen M. Lake[1], Rachel J. Nielsen[1], Kathy Teeter[1], Scott Page[2], R. Scott Hawley[1]. 1) Stowers Inst Medical Research, Kansas City, MO; 2) Comparative Genomics Centre, James Cook Univ., Townsville, Australia.

An allele of *CG4413* was uncovered in the course of a genetic screen engineered to identify meiosis-defective mutations in both essential and non-essential genes. This cold-sensitive meiotic mutant, which we have named *trade embargo* (*trem*^{F9}), shows high levels of *X* chromosome nondisjunction, presumably as a consequence of the large decrease in recombination. InDel and deficiency mapping placed the mutation within the interval 92A2-93F4. A *PBac* insertion which affects the 5'end of *CG4413* failed to complement the nondisjunction phenotype. Sequence analysis of the *CG4413* gene revealed a single C-T transition when compared to the target chromosome which corresponds to a proline to leucine change. *CG4413* is predicted to encode a zinc finger gene and is located in a cluster of four zinc finger genes. This mutation is in a conserved residue in the linker region between the first and second zinc finger domains. This mutation shows normal localization of the synaptonemal complex protein C(3)G, however, it is defective in both double stand break formation and recombination. Five additional alleles were obtained through TILLING. Two alleles which mutate critical residues in the first zinc finger domain also show high levels of *X* nondisjunction. We will summarize our efforts in analyzing these additional alleles in terms of synaptonemal complex formation, double strand break formation, recombination frequency, cold sensitivity and chiasmate and achiasmate chromosome segregation. These analyses will enable us to determine the role of *trem* in the meiotic recombination pathway.

188B

The chromatin insulator protein Dtopors is required for nuclear lamina assembly, centrosome regulation and meiotic chromosome segregation in males. Maiko Matsui[1], Krishn Sharma[1], Barbara Wakimoto[2], John Tomkiel[1]. 1) Biology, UNC Greensboro, Greensboro, NC; 2) Zoology, University of Washington, Seattle, WA.

Mutations in the .*nuclear blebber*(*nbl*) gene cause fourth chromosome loss in male meiosis and an unusual nuclear dysmorphology in primary spermatocytes. We demonstrate that all chromosomes segregate essentially at random in meiosis in *nbl* males, but female meiosis is unaffected. We mapped the *nbl* mutations, and show that they are missense mutations in dtopors, the Drosophila homolog of the human suppressor Topors (Topo-isomerase I interacting arginine-serine rich protein). Topors is a RING finger protein that in humans can function as an E3 ubiquitin ligase or a sumo ligase. We show that the abnormal nuclear morphology in *dtopors* primary spermatocytes is accompanied by alteration in the distributions of both Lamin Dm0 and Lamin C. Additionally, centrosome replication or division is abnormal, resulting in tetrapolar meiosis I spindles and consequently errors in chromosome segregation. Dtopors has recently been shown to interact directly with Lamin Dm0, as well as the chromatin insulator proteins Su(Hw) and Mod(mdg4)2.2, and is required for insulator function (Capelson and Corces 2005). We show that Dtopors protein is localized to intranuclear foci in late prophase, a subset of which are associated with the lamina and the meiotic chromosomes. The numbers and distribution of these structures are similar to those of insulator bodies observed in somatic cells. We also find that Dtopors localization is disrupted in *teflon* mutants, in which autosomal homologs prematurely separate in late prophase. We discuss parallels between chromatin insulators and male meiotic chromosome segregation, and suggest a model in which chromatin insulators form the basis for chromosome/lamin interactions that facilitate meiotic pairing and/or homolog conjunction.

189C

The Cdc20/Cdh1-related protein, Cort, cooperates with Cdc20/Fzy in cyclin destruction and anaphase progression in meiosis I and II. Andrew Swan[1], Trudi Schüpbach[2]. 1) Dept of Biological Sciences, University of Windsor, Windsor, Canada; 2) Dept of Molecular Biology, Princeton Universtiy, Princeton, NJ.

Meiosis is a highly specialized cell cycle that requires a significant reorganization of the canonical cell cycle machinery and the use of meiosis-specific cell cycle regulators. The Anaphase Promoting Complex (APC) and a conserved APC adaptor, Cdc20/Fzy are required for anaphase progression in mitotic cells. The APC has also been implicated in meiosis, though it is not yet understood how it mediates these non-canonical divisions. Cort is a diverged Cdc20 homologue expressed in the female germ-line of Drosophila where it functions with the Cdk1-interacting protein, Cks30A, to drive anaphase in meiosis II. Here we show that Cort functions together with the canonical mitotic APC adaptor, Cdc20/Fzy to target the three mitotic cyclins for destruction in the egg and drive anaphase progression in both meiotic divisions. In addition to controlling cyclin destruction globally in the egg, Cort and Fzy appear to both be required for local destruction of cyclin B on spindles. We find that cyclin B associates with spindle microtubules throughout meiosis I and meiosis II, and dissociates from the meiotic spindle in anaphase II. Fzy and Cort are required for this loss of cyclin B from the meiotic spindle. Our results lead to a model in which the germline specific APCCort cooperates with the more general APCFzy both locally on the meiotic spindle, and globally in the egg cytoplasm to target cyclins for destruction and drive progression through the two meiotic divisions.

190A

A genetic screen for modifiers of *teflon*, a gene required for proper segregation of autosomes at meiosis I in males. Amanda L. Thomas, John E. Tomkiel. Dept Biol, The University of North Carolina at Greensboro, Greensboro, NC.

For most organisms, recombination between homologs during prophase I establishes the connections necessary for proper segregation at anaphase. Recombination-deficient organisms, such as male *Drosophila melanogaster*, must employ a different method to ensure that paired homologs remain associated until anaphase. The *teflon* (*tef*) gene is required specifically for ensuring adhesion between autosomes in male meiosis. In *tef* mutants, autosomal homologs pair, partition normally to separate domains at the nuclear periphery, but separate prior to metaphase, resulting in random segregation of homologs at meiosis I . To identify genes that interact with *tef*, we have performed a screen for dominant second site modifiers of a hypomorphic allele, tef^{P1150}. We have tested the third chromosome deficiency kit, which spans ~90% of the third chromosome, as well as a collection of previously identified male meiotic mutants (Wakimoto et al. 2004). We identified 11 regions containing Enhancers, and 6 regions containing Suppressors of *tef*. One of the enhancing deletions removes *mod(mdg4)*, and null *mod(mdg4)in meiosis* alleles produce the same effect. This supports a model proposed by Thomas et al. (2005) that suggests that *mnm* and *tef* act in the same pathway to ensure autosomal conjunction. We are currently refining the mapping of the remaining Modifiers to identify new genes involved in the male segregation pathway.

191B

Cdk1 phosphorylation sites on Cdc27 are required for correct chromosomal localisation and APC/C function in syncytial Drosophila embryos. Jun-Yong Huang, Gary Morley, Michael Whitaker. Cell and Developmental Physiology, Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, University of Newcastle upon Tyne, Catherine Cookson Building, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK.

We have previously reported that Cdc27 and Cdc16, two core components of APC/C, are differentially localised during mitosis in transgenic Drosophila syncytial embryos by demonstrating that GFP::Cdc27 associates with chromosomes, but that its closely related partner GFP::Cdc16 does not (Huang and Raff, 2002). Here, we report that two potential Cdk1 kinase phosphorylation sites are required for the chromosomal localisation of GFP::Cdc27 during mitosis. Either or both of the highly conserved prolines in the Cdk1 phosphorylation consensus sequence motifs were mutated to alanine (Cdc27 P304A or P456A). The singly mutated fusion proteins, GFP::Cdc27P304A and GFP::Cdc27P456A , can still localise to mitotic chromosomes in a manner identical to wild type GFP::Cdc27. These mutant fusion proteins are functional in that they can rescue the phenotype of the cdc27L7123 mutant in vivo. However, when both of the Cdk1 phosphorylation sequence motifs were mutated, the resulting GFP::Cdc27P304A,P456A construct was not localised to the chromosomes during mitosis. More importantly, it was no longer functional, as it failed to rescue mutant phenotypes of the cdc27L7123 gene. Western blot results show high levels of cyclin B and cyclin A accumulated in mutant third instar larvae brain samples compared to its wild type control. These results show for the first time that the two potential Cdk1 phosphorylation sites on Drosophila Cdc27 are required for its chromosomal localisation during mitosis and imply that these localisations specific to Cdc27 are crucial for APC/C functions. $^{P304AP456AP304AP456AL7123}cdc27^{P304A,\ P456A}cdc27^{L7123}DrosophilaDrosophila$.

192C

A genetic analysis of the E2F1 mediated transcriptional activation. Jun-yuan Ji, Fajun Yang, Anabel Herr, Anders Näär, Nick Dyson. Massachusetts General Hospital Cancer Center, Charlestown, MA 02129.

The Rb-E2F pathway plays important roles in regulating the G1-S phase transition. This is largely achieved by regulating the phosphorylation status of Rb family proteins and by activating the transcription of E2F target genes. The mechanisms of how the Rb-E2F pathway responds to growth factor stimulation and the functions of E2F target genes in regulating the G1-S phase transition have been extensively studied. However, it is largely unknown exactly how E2F communicates with RNA polymerase II and activates transcription. It is also not clear how this process is turned off in vivo. To address these questions, we took advantage of the relative simplicity of the Rb-E2F pathway in Drosophila. Specifically, we have established an inducible RNAi system that specifically knocks down endogenous E2F1 activity in a tissue-specific manner. When combined with different Gal4 lines, dE2F1- RNAi generates reliable phenotypes that can be modified by known factors of the Rb-E2F pathway in expected ways. Using these phenotypes, we genetically tested mutations in transcription factors and cofactors in order to find ones that can dominantly modify the E2F1-RNAi phenotypes. One of the potent suppressors is Cdk8, which is a subunit of the repressive submodule of the Mediator complex. Other subunits of the repressive submodule also show varied degrees of suppression of the dE2F1-RNAi phenotypes. The Mediator complex is believed to function as a bridge between certain transcription activators and the general transcriptional machinery. Currently, we are using biochemical approaches to investigate whether the Mediator complex is directly involved in E2F1-mediated transcriptional activation and what role Cdk8 may play in this process.

193A

Loss of Drosophila Myb interrupts the progression of chromosome condensation. Joseph Lipsick, John Manak, Hong Wen, Tran Van, Laura Andrejka, Wai Choi. Dept Pathology & Genetics, Stanford Univ, Stanford, CA.

Completion of chromosome condensation is required prior to segregation during the mitotic cell cycle in order to insure the transmission of genetic material with high fidelity in a timely fashion. In higher eukaryotes this condensation is regulated by phosphorylation of histone H3 on serine 10 (H3-S10). This phosphorylation normally begins in the late replicating pericentric heterochromatin and then spreads to early replicating euchromatin. We now show that these phases of condensation are genetically separable in that the absence of Drosophila Myb causes cells to arrest with H3-S10 phosphorylation of heterochromatin but not euchromatin. In addition, we used mosaic analysis to demonstrate that although the Myb protein can be removed in a single cell cycle, the failure of chromosome condensation occurs only after many cell divisions in the absence of Myb protein. The Myb protein is normally located in euchromatic but not heterochromatic regions of the nucleus, implying that Myb performs an essential modification of euchromatin that is required for the spread of chromosome condensation.

194B

Isolation and characterization of new maternal mutants defective for blastoderm cellularization. Ana Rita Marques[1], Rui Tostões[1], Thomas Marty[2], Rui Gonçalo Martinho[1]. 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Skirball Institute, NYU, NYC, USA.

Our research goal is to further elucidate the molecular and cellular mechanisms underlying Drosophila blastoderm cellularization. In order to achieve this goal, we took advantage of a maternal screen previously done in the laboratory of Ruth Lehmann (NYU, USA). In a primary screen, mutants were isolated when the soma looked extremely abnormal but where the primordial germ cells were formed normally. The secondary screen involved the isolation of mutants defective for the production of cuticle. Analysis of these mutants allowed the identification of at least six complementation groups. We expect that the isolated mutations will affect the function of genes required for nuclei division and/or migration, transcriptional regulation, and membrane invagination and/or establishment of cell polarity.

Through a candidate gene approach it was concluded that one of the complementation groups is allelic to scraps (1). scraps encodes Anillin and is likely to play an important role in the coordination between the actin cytoskeleton and membrane invagination. Through the use of the 2R deficiency kit and a candidate gene approach we also concluded that another of the isolated complementation groups is allelic to DRhoGEF2. The DRhoGEF2 gene was shown to be required for the correct assembly of actin filaments during blastoderm cellularization (2). The fact that two genes with clear functions during Drosophila blastoderm were isolated in this screen suggests that it was successful. We will present data concerning the phenotypic characterization of these and other mutants defective for blastoderm cellularization.

(1) Development, 132: 2849-2860 (2005); (2) Development, 132: 1009-20 (2005).

195C

Isolation and characterization of *Drosophila melanogaster* kinetochore proteins. Lucia Mentelova[1,3], Gonçalo Costa[1], Fatima Pereira[1], Ana Roque[1], Alvaro Tavares[1,2]. 1) Cell Division Group, Inst.Gulbenkian Ciencia, Lisboa, Portugal; 2) Chemical Eng. Inst. Superior Tecnico, Lisboa, Portugal; 3) Dept. of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

Kinetochores are large complex protein structures that assemble at the centromeric regions of each sister chromatid and perform three key functions : kinetochores attach chromosomes to the spindle, co-ordinate microtubule dynamics to chromosome movement along the spindle, and generate the 'wait' signal that prevents anaphase onset until all the chromosomes are correctly aligned on the spindle. Recent proteomic studies suggest that kinetochores of Saccharomyces cerevisiae are comprised of at least 60 proteins. Although some of these proteins are conserved from yeast to humans, the majority of proteins in high eukaryote kinetochores are still to be identified. In order to identify and characterize kinetochore proteins in Drosophila we have developed a method to obtain enriched mitotic kinetochore protein fractions. The protein profile pattern of such fractions was characterized by separation by two-dimensional electrophoresis followed by peptide mass fingerprinting. Partial sequencing information was then used as a protein identification strategy. We have identified 92 proteins in the protein fraction. Some (like CID and Cenp-E) had already been described as kinetochore components. Others, like Sgt1, had been described in other organisms but not in Drosophila. Finally, 16% of the proteins show no obvious homologues in other organisms. We have characterized in detail the function of DmSgt1 and four of these new genes. GFP-tagging followed by expression in S2 cells, show that three also co-localize with the mitotic chromatin and one (Gc70) is an inner kinetochore protein. Mutant analysis and RNAi studies show that mutations in the Gc70 or DmSgt1 genes cause early embryonic or 1st instar larval lethality. Taken together the results show that the approach to isolate kinetochore proteins was succefull.

196A

Nopo is a candidate E3 ubiquitin ligase required for genomic stability during early embryogenesis in Drosophila. Julie Merkle, Jamie Rickmyre, Audrey Frist, Erin Loggins, Laura Lee. Department of Cell and Developmental Biology, Vanderbilt University Medical School, Nashville, TN.

A collection of Drosophila maternal effect-lethal mutants was screened for cell cycle defects in early embryogenesis, and several new mutants were identified. We have given the name "*no poles*" (*nopo*) to one of these mutants. Embryos from *nopo* females undergo mitotic arrest with a high frequency of acentrosomal spindles, misaligned chromosomes, and tripolar spindles. Genetic studies show that the checkpoint kinase, Chk2 (product of the *mnk* gene) is activated in *nopo* mutants, suggesting that *nopo* plays a role in maintaining genomic integrity. We have identified the gene responsible for the *nopo* phenotype by genetic mapping and sequencing of candidates. We found a point mutation resulting in a non-conservative amino acid change in *CG5140*, a homolog of the human *TRIP* gene (TRIP=TRAF-interacting protein, TRAF=TNF-α receptor-associated factor). We have identified additional *nopo* alleles, including generation of a null allele by imprecise excision of a P-element insertion in the 5'-UTR of *CG5140*. To definitively demonstrate that *TRIP* is the *nopo* gene, transgenic rescue experiments are currently underway.

Human TRIP, which was originally implicated in TNF signaling by virtue of a two-hybrid interaction with TRAF, has been shown to block TNF signaling *in vitro*. A conserved RING finger domain in the amino terminus of Nopo, which is mutated in our EMS allele, is not required for this activity, suggesting additional biological roles for this protein. The RING domain of Nopo resembles that of known E3 ubiquitin ligases. To test whether Nopo has E3 ligase activity, auto-ubiquitination assays are currently in progress. Phenotypic analysis and yeast two-hybrid results suggest that Ben, an E2 conjugating enzyme, forms an ubiquitination complex with Nopo. Future goals include identifying Nopo targets and interactors so as to elucidate the mechanism by which Nopo promotes genomic stability.

197B

Microcephalin (MCPH1) is required for cell-cycle progression in the early Drosophila embryo. Jamie L. Rickmyre, Audrey Y. Frist, Laura A. Lee. Cell & Developmental Biology, Vanderbilt University Med Ctr, Nashville, TN.

Mutations in human *MCPH1* result in a form of autosomal recessive primary microcephaly, a disorder of fetal brain growth characterized by severely reduced cerebral cortex with mental retardation. Both human and Drosophila MCPH1 contain BRCA1 C-terminal domains (BRCT domains), which are found in many proteins that function in DNA repair and cell-cycle control. To date, no animal models for primary microcephaly due to mutation of *MCPH1* have been reported.

We have identified maternal-effect lethal mutations in Drosophila *MCPH1*. Early Drosophila embryogenesis consists of rapid S-M cell cycles driven by stockpiles of maternal RNA and protein that occur without gaps or cytokinesis. These streamlined cycles as well as the genetic tractability of Drosophila offer ideal conditions in which to study cell-cycle regulation during development. Embryos from mutant *MCPH1* females undergo mitotic arrest in the early syncytial divisions and contain short, rounded spindles that lack centrosomes. This phenotype is reminiscent of spindle changes that occur in response to activation of Checkpoint kinase 2 (Chk2) in the early embryo. Known triggers of this kinase include mitotic entry with DNA damage or incomplete DNA replication. To test this model, we created flies doubly mutant for *MCPH1* and *mnk*, which encodes Chk2. Embryos from *mnk MCPH1* females exhibit dramatic suppression of the *MCPH1* phenotype (both developmental arrest and cell-cycle defects), suggesting that loss of MCPH1 results in genomic instability with Chk2 activation. Current efforts are directed towards elucidation of the mechanism by which mutation of *MCPH1* leads to Chk2 activation and placement of MCPH1 within a molecular framework. Our data suggest MCPH1 functions in a common pathway with Mei-41 and Grapes (ATR and Checkpoint kinase 1, respectively) to regulate unperturbed cell cycles in the early Drosophila embryo but not in the response to DNA damage. We hypothesize that MCPH1 is necessary throughout early Drosophila embryogenesis to delay mitotic entry until DNA replication is complete.

198C

Characterization of Drosophila cyclin J. Govindaraja Atikukke[1], Russell L Finley, Jr.[1,2]. 1) Biochemistry and Molecular Biology, Wayne State University, Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State University,Detroit, MI.

Cyclin J is a poorly characterized cyclin originally identified in *Drosophila*, and conserved in metazoans including human, mouse, and mosquito. In *Drosophila*, cyclin J is found exclusively in the female germ line and early embryo, suggesting a role for the protein during oogenesis or the unique division cycles of early embryogenesis, which lack gap phases and certain checkpoints. Consistent with this possibility, we previously showed that injection of embryos with cyclin J inhibitory aptamers or antibodies results in cell cycle defects. To further explore the role of cyclin J, we are characterizing a piggyBac-element insertion allele, cycJA138. We have shown that cycJA138 is a strong cyclin J loss-of-function allele, which is partially female sterile and which can be rescued with a cyclin J transgene. Homozygous mutant female ovaries show defects in oogenesis. Embryos laid by mutant females are delicate, significantly smaller than normal, and show a range of dorsal appendage abnormalities. These embryos have dramatically reduced hatching rates and DNA segregation defects as revealed by DAPI staining. To obtain an independent cyclin J loss-of-function allele, we have created a deficiency strain that lacks cyclin J and the two neighboring genes (*armi* and CG14971). Oogenesis is completely disrupted in females that are homozygous for the 3-gene deletion and this can be partially rescued by introduction of cycJ and CG14971 transgenes. Finally, we are using a protein interaction map as a guide to identifying the cyclin J genetic regulatory networks. We have shown that cycJ genetically interacts with several of the genes encoding proteins in the physical interaction network, including genes already known to play roles in oogenesis or early embryonic development.

199A

The Role of Centriole and Centrosome in Cilium Inheritance and Formation. Tomer Avidor-Reiss, Stephanie Blachon. Cell Biology, Harvard Medical School, Boston, MA.

Centrioles are believed to perform two distinct roles: they act as precursors to basal bodies, the cellular organelles that give rise to cilia, and together with the pericentriolar material constitute the core component of centrosomes, the major microtubule organization centers in animal cells that participate in mitosis. In Drosophila melanogaster, all cells have centrioles, but centrioles are only transformed to a basal body in sensory neurons and in the male germ line. These basal bodies participate in the formation of motile and mechanosensory cilia as well as in male meiosis. To study early events that govern cilia biogenesis, we screened for mutations that generated adult flies showing a characteristic proprioception phenotype of ciliary mutants, exhibiting immotile sperm flagellum and are unable to carry out meiosis. We found three mutants in different genes, in all of which, basal bodies and cilia are absent. Surprisingly, we found that the centrioles in these mutants are either abnormal or absent. These findings demonstrate that centrioles are not required for fly development bur are essential for ciliogenesis. The proteins encoded by the candidate genes are localized specifically to the centriole and basal body, showing a distinct spatial and temporal localization, suggesting they involve in the events governing centriole initiation and scaffolding of the centriole wall.

200B

Search for components of a nutrient sensor of the Drosophila larval fat body. Marc Bourouis, Marianne Bjordal, Sophie Layalle, Pierre Léopold. Institute for Signalling, Developmental Biology and Cancer -CNRS UMR 6543- Université de Nice, France.

By a “genetic starvation” method involving the silencing of the amino acid transporter gene *slif*, we previously assigned a nutrient-sensing function to the Drosophila larval fat body which was dependent on TOR signaling. The fat body sensor, in turn, triggered peripheral growth via a modulation of insulin/IGF signaling (Colombani et al. Cell, 2003).

We are searching for new components of the fat body sensor including its humoral relay part by a combination of microarray analysis and genetic screenings. Analysis of transcriptional outputs from fat bodies starved by *slif* inhibition and from fat bodies starved by forced expression of the TOR inhibitor TSC1/2 revealed extensive similarities. Common regulated genes as well as other selected sets of genes were then tested for their ability to modify the starvation phenotype caused by fat body-directed *slif* inhibition using misexpression or RNAi-silencing strains. Recovered modifiers include targets of TOR or its upstream regulators and other genes tied to endocrine and metabolic functions specific of the fat body.

201C

An interaction map guided screen for novel genetic interactions. Stephen Guest[1], Jingkai Yu[1], Russell Finley Jr.[1,2]. 1) Center for Molecular Medicine and Genetics; 2) Department of Biochemistry and Molecular Biology Wayne State University School of Medicine, 540 East Canfield, Detroit Michigan, 48201.

Genome-wide RNA interference based screens in cultured *Drosophila* cells have been successfully used to identify novel regulators of diverse cellular processes. To date, these screens have targeted primarily individual genes in a one-gene-at-a-time approach. Double RNAi, i.e. using RNAi to simultaneously target two genes in the same cell, is possible and has been shown to be an effective method for identifying pairs of genes that interact genetically. Using double RNAi to test the complete set of potential *Drosophila* gene pairs is however not feasible using current methods. In this study, we have identified a set of putative novel genetic interaction pairs *in silico*, which we can target by double RNAi. We accomplished this by first integrating the available protein-protein and genetic interaction maps of yeast, worm, fly and human, and then searching the resulting combined map for network motifs likely to be biologically meaningful. This analysis has yielded >1600 predicted genetic interaction pairs. We are currently testing these predicted pairs using double RNAi in cultured *Drosophila* cells and have successfully identified novel genetic interactions using this approach.

202A

The Drosophila MRL adapter protein Pico promotes insulin- and Egfr-dependent cell growth and proliferation. Daimark Bennett, Ekaterina Lyulcheva. Department of Zoology, Oxford University, Oxford, GB.

The construction of properly sized and functional tissues and organs during animal development requires tight control of cell growth, proliferation, differentiation and death. These processes are co-ordinated by networks of intracellular signal transduction pathways that respond to various secreted ligands and cell surface proteins. Multivalent adapter proteins that lack intrinsic enzymatic activity play key roles in intracellular signal transduction by directly linking upstream events, for example at growth factor receptors, with downstream effectors via protein-protein or protein-lipid binding domains. Mig10/RIAM/Lamellipodin (MRL) proteins represent one such family of molecular adapters, which are thought to be capable of organizing intracellular responses to local signals. We have found that reduction in levels of pico, the only MRL homologue in Drosophila, results in reduced cell division rates, growth retardation and lethality. Conversely, overexpression of pico promotes coordinated cell growth and proliferation without affecting cell cycle phasing, leading to net increase in tissue size. pico is dependent on insulin-like peptide and Egf receptor signaling for its function, but acts in parallel to canonical InR-Pi3K and Ras-MAPK pathways. Our data reveal a novel role for an MRL protein in co-operating with multiple signals to promote tissue and organismal growth. The functional properties of pico, which are distinct from those of other growth regulators, may help to explain the previously unaccounted for effects of insulin and Egfr signaling on cell proliferation. Furthermore, identification of pico as a novel component of a signaling network downstream of insulin peptides and Egfr, which stimulates hyperplastic growth, indicates that the MRL proteins may play a role in the pathogenesis of certain cancers and may represent potential molecular targets for therapeutic intervention.

203B

Genetic and molecular analysis of *bene*, a glutamyl-tRNA (Gln) amidotransferase homolog required for growth and maturation in Drosophila. Leah Bergman, Anna Kruyer, Mikhail Gertsberg, Adriana Guigova, Jason Z. Morris. Dep't of Natural Sciences, Fordham University, New York, NY.

The gene *benedict* (*bene*) was initially isolated in a clonal screen for ovary germ cell growth and cell cycle defects (Morris, et al., 2003). Subsequently, we showed that *bene* is required for larval growth and survival. Wild type Drosophila larvae grow 200-fold before pupariation. Most of that growth relies on the endocycle, a specialized cell cycle in which cells repeatedly undergo DNA replication without cell division. Both endocycling and mitotic tissues in *bene* homozygous mutant larvae grow very slowly, and endocycling cells never attain high DNA contents. In addition, *bene* mutants exhibit molting delays and die before pupariation. We molecularly and genetically mapped four *bene* alleles and determined that all the alleles disrupt the *gatA* gene. This gene encodes a protein predicted to be required for translation of the 13 genes encoded in the Drosophila mitochondrial genome (see poster by Kruyer et al.). The *bene* growth and maturation defects could be due to a non-specific requirement for mitochondrial energy production. Alternatively, dysfunctional mitochondria in *bene* mutants may specifically affect cell cycle regulation. In order to distinguish these models, we are currently assaying for genetic interactions with *dMyc*, *cycD* and *PTEN* to determine if up-regulation of the major growth pathways can suppress the *bene* mutant growth defects. Morris, JZ, Navarro C, Lehmann R. 2003. Identification and characterization of novel genes required for oocyte specification in Drosophila. *Genetics* 164: 1435-1446.

204C

Cell-autonomous growth suppression by the *Drosophila tsg101* ortholog *erupted* via cell polarity and cell cycle pathways. M. Melissa Gilbert, Caroline Krisel, Kenneth H. Moberg. Department of Cell Biology, Emory University, Atlanta, GA.

The *Drosophila* endocytic gene *erupted* (*ept*) is an ortholog of human Tumor Susceptibility Gene-101 and suppresses tissue growth in two distinct ways: patches of *ept* mutant cells drive hyperplasia of wild type cells and eye imaginal discs composed entirely of *ept* mutant cells overgrow into amorphous tissue masses. The latter phenotype indicates that *ept* autonomously limits cell proliferation and affects the organization of cells in developing tissues, although the mechanisms underlying this effect are not known. Here we show that *ept* is required cell autonomously to maintain the eye disc as a monolayer epithelium, and that *ept* masses exhibit neoplastic phenotypes including persistent proliferation and a failure to express differentiation markers. Genetic and molecular analysis demonstrates that the *stat92E* and *crb* pathways play critical roles in the etiology of these *ept* growth and architectural phenotypes, and that Stat92E may be activated in *ept* cells by a Notch-independent mechanism involving endosomal sequestration of the Domeless receptor. *stat92E* and *crb* are also required for the appearance of low molecular weight forms of Cyclin E in *ept* tissue that are similar to cleaved forms of human Cyclin E found in epithelial cancers. Genetic data indicates that these truncations activate Cyclin E in *ept* mutant cells. These results identify the *stat92E* and *crb* pathways as critical downstream targets of *ept* that collaborate to regulate apical membrane dynamics and tissue architecture, and also implicate Stat92E and Crb in the regulation of Cyclin E processing in developing epithelia.

205A

JNK signaling is necessary for the activation of a developmental checkpoint in response to tissue damage. Adrian Halme, Iswar Hariharan. Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Drosophila melanogaster imaginal discs have a remarkable capacity to withstand significant damage during their growth within the larvae. Even when a substantial portion of the cells within an imaginal disc are induced to undergo programmed cell death, the animal is still capable of producing a qualitatively normal adult organ derived from the remaining tissue. One component of this tissue homeostasis is the induction of a developmental delay, which lengthens the larval stage to accomodate repair of the damaged tissues. To better understand the pathways that regulate this developmental checkpoint, we have initiated a series of small-scale screens to identify either dominant or recessive viable mutations that produce an attenuated delay after tissue damage induced by ionizing radiation. Several of the mutants isolated in this screen contain mutations of the gene *basket*, which encodes the *Drosophila* Jun N-terminal kinase. We are currently characterizing the role of the JNK pathway in regulating both repair of damaged tissues and the timing of larval development.

206B

Fat acts through Hippo signalling to regulate tissue size. Fisun Hamaratoglu[1,2], Maria Willecke[1,3], Madhuri Kango-Singh[1], Ryan Udan[1,2], Chiao-lin Chen[1,4], Chunyao Tao[1], Xinwei Zhang[1], Georg Halder[1,2,4]. 1) Dept Biochem & Molec Biol, MD Anderson Cancer Ctr, Houston, TX; 2) Prog in Dev Biol, BCM, Houston, TX; 3) Interfakultäres Institut für Zellbiologie, Abt. Genetik der Tiere, Universität Tübingen, Tübingen, Germany; 4) Prog in Genes and Dev, UT MD Anderson Cancer Ctr, Houston, TX.

Fat (Ft) is a large protocadherin required for several processes during the development of Drosophila imaginal discs; it acts as a tumor suppressor to restrict imaginal disc growth, it is required for the establishment of normal planar cell polarity and for correct proximal-distal patterning of appendages. However, the pathway through which Ft acts to carry out these functions has been unknown. Here, we report that Ft acts through the Hippo (Hpo) tumor suppressor pathway to regulate tissue size. Hippo signaling is a key pathway that controls tissue size via coordinate regulation of both cell proliferation and apoptosis. The FERM-domain proteins Merlin (Mer) and Expanded (Ex) were the most upstream components of the pathway known prior to the following findings. We found that the overgrowth phenotypes of ft mutants are similar to those of mutants in Hpo pathway components: ft mutant cells display continued proliferation and deregulate Hpo target genes such as cyclin E and diap1. These similarities suggested that Ft may act as a receptor for the Hippo pathway. Consistent with this hypothesis, Ft acts genetically upstream of Expanded (Ex) and Hpo in vivo and regulates the phosphorylation of Warts and the activity of the transcriptional coactivator Yorkie in cell culture. We also found that Ft is required for proper localization of Ex, but not Mer, to the plasma membrane. The ft mutant phenotypes very closely resemble the ex mutant phenotypes and we found that Ft, like Ex, acts partially redundant with Mer. In our current model, Ft acts mainly through Ex, and in parallel to Mer, to regulate Hpo signaling. Taken together, our data identify a cell surface molecule that may act as a receptor of the Hippo signaling pathway. *Drosophilaftcyclin Ediap1in vivoex*.

207C

Mutations in the novel gene *gang of four* deregulate growth in the Drosophila eye. Carolyn A. Krisel, Kenneth H. Moberg. Department of Cell Biology, Emory University, Atlanta, GA.

The *Drosophila* eye is an excellent model system in which to isolate and study mutations in genes that normally restrict cell proliferation and tissue growth during development. We have used the *eyFLP* system to recover four alleles of a recessive lethal complementation group that we have named *gang of four* (*gfr*). Cells homozygous mutant for *gfr* have a subtle, but reproducible, growth advantage over adjacent wild type cells in the eye imaginal disc. Examination of the cellular architecture of *gfr* clones reveals that *gfr* mutations affect cell number and organization in the pupal retina, with particular effects on cells of the interommatidial bristle complex. *gfr* clones contain extra BarH1-positive and Senseless-positive cells, suggesting that *gfr* may be involved in Notch signaling in the sensory organ precursor lineage. Consistent with this, we have found that *gfr* alleles dominantly modify Notch-dependent wing phenotypes. We also find that *gfr* controls the levels of a putative MAP kinase substrate in eye cells. Collectively, these phenotypes suggest that the protein encoded by the *gfr* gene may function to modulate signaling through both the Notch and MAPK pathways, thus controling cell specification and cell proliferation in the developing eye. Mapping of *gfr* is ongoing. The gene has been localized by meiotic and deficiency mapping to a small genomic interval, and we are in the process of testing candidate ORFs to determine which one corresponds to *gfr*.

208A

Molecular, cellular and biochemical analysis of the function of *bene/gatA*, a gene required for growth and maturation in Drosophila. Anna Kruyer, Leah Bergman, Mikhail Gertsberg, Grace Vernon, Jason Morris. Department of Natural Sciences, Fordham University, New York, NY.

Loss-of-function mutations in *benedict (bene)* cause growth defects and larval lethality (see poster by Bergman, et al.). *bene* encodes the Drosophila glutamyl-tRNA (Gln) amidotransferase subunit A. This enzyme is required in mitochondria and in many species of bacteria for proper translation. In the absence of gatA activity, glutamine tRNAs are charged with glutamate. We are characterizing the translation defects in *bene* mutants by analyzing abundance and pI of the mitochondrially translated respiratory chain protein, COX1, on isoelectric focusing gels. We are determining the *bene* expression pattern via RTPCR and mRNA in situ hybridization. Finally, we are using electron microscopy to compare the morphology of mitochondria in *bene* and wild type salivary glands and brains.

209B

Expression profiling of Bowl-induced hyperplastic wing imaginal discs reveals multiple deregulated processes. Elzbieta Kula-Eversole[1], Victor Hatini[1,2]. 1) Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA; 2) Program in Cell, Molecular and Developmental Biology.

Tumors of epithelial origin show many alterations in gene expression, cell behavior, and tissue organization, but the specific changes essential for the initiation of tumor growth are not known. The epithelia of the Drosophila imaginal discs provide a tractable model system in which to explore the underlying mechanisms involved in nascent tumor formation. In our lab we discovered that the zinc finger transcription factor Bowl promotes overgrowth of the wing and eye imaginal discs. Since Bowl is a transcription factor, the identification of its targets could explain the effect of Bowl on cell proliferation and epithelial organization observed in those tissues. Using a candidate gene approach we found that the Wingless (Wg) ligand was produced ectopically in Bowl expressing clones. Removing Wg from these clones reversed the hyperplastic phenotype (D.N & V.H). We hypothesized that additional genes and pathways cooperate with Wg to promote hyperplastic growth in Bowl-expressing clones. In order to identify these targets in an unbiased way, we analyzed the gene expression profile of Bowl-induced hyperplastic wing discs using Affymetrix microarrays. We uncovered a number of deregulated pathways in these discs. Notably, we discovered upregulation of several components of the JAK/STAT pathway including the ligands unpaired 1, unpaired 2 and unpaired 3 and their inducible feedback antagonists ken-and-barbie and Socs36E, which provide a signature of pathway activation. We therefore propose that Unpaired ligands cooperate with the Wg ligand to promote hyperplastic growth using autonomous and non-autonomous mechanisms. We will present functional studies to test whether the activation of the JAK/STAT pathway is necessary, and whether coexpression of wg and unpaired is sufficient to promote hyperplastic growth. Finally, we will also examine the potential relationships between Bowl and other pathways that control growth in the wing imaginal disc.

210C

The role of the JAK/STAT pathway in growth control in *Drosophila*. Aloma Rodrigues, Erika Bach. Pharmacology, New York Univ. School of Med., New York, NY.

Previous work in *Drosophila* indicates that imaginal discs have an intrinsic mechanism to maintain their overall size by balancing proliferation, mass accumulation and apoptosis (Johnston and Gallant, 2002, *Bioessays*, 24:54). Analyses of loss- and gain-of-function mutations in the JAK/STAT pathway ligand *unpaired* suggest that this pathway is involved in growth control in *Drosophila* (Bach et al., 2003, *Genetics* 165:1149). To determine whether the JAK/STAT pathway regulates growth autonomously, we specifically removed *stat92E* function at specific times during early larval development and analyzed clone and twin spot areas in third instar eye and wing discs. We find that Stat92E has an early autonomous requirement in growth of these tissues. *stat92E* clones induced 30 hours after egg deposition (AED) were only ~15% the size of twin spots, and at later time points they were still significantly smaller than the twin spots. Importantly, we find that *stat92E* clones do not undergo apoptosis, suggesting that either proliferation and/or mass accumulation are decreased when JAK/STAT pathway activity is inhibited. In support of this hypothesis, we find autonomous, increased expression of CycE (a G1 cyclin) and of Myc (which promotes mass accumulation) in flip-out clones expressing the JAK Hopscotch (Hop), which autonomously activate Stat92E. *hop* flip-out clones also induce autonomous tissue over-growth but only in the dorsal region of the eye and in the notal region of the wing. Moreover, in imaginal discs *hop*-expressing clones frequently form autonomous, independent ectopic structures with smooth boundaries, suggesting that hyper-activation of the JAK/STAT pathway alters cell affinities and/or adhesion. Consistent with this, we show that the cell adhesion molecule DE-Cadherin is specifically increased within *hop* flip-out clones. These latter data raise the intriguing possibility that the JAK/STAT pathway controls growth through regulating levels of adhesion molecules.

211A

The Fat cadherin acts through the Hippo tumor-suppressor pathway to regulate tissue size. Maria Willecke[1,3,5], Fisun Hamaratoglu[1,4,5], Madhuri Kango-Singh[1], Ryan Udan[1,4], Chiao-lin Chen[1,2], Chunyao Tao[1], Xinwei Zhang[1], Georg Halder[1,2,4]. 1) Dept of Biochemistry & Molecular Biology; 2) Program in Genes and Development MD Anderson Cancer Center Houston, TX; 3) Interfakultäres Institut für Zellbiologie Abteilung Genetik der Tiere Universität Tübingen, Germany; 4) Program in Developmental Biology Baylor College of Medicine, Houston, TX; 5) These two authors contributed equally to this work.

We are interested in understanding the molecular mechanisms that control the size of imaginal disc derived tissues. The recently discovered Hippo pathway controls tissue size by coordinately regulating cell proliferation and apoptosis. Expanded and Merlin are the most upstream components of the pathway known so far. Genes that act upstream of Expanded and Merlin such as transmembrane receptors have not yet been identified. Here we report that the atypical cadherin Fat acts as an upstream component in the Hippo pathway. Fat is a known tumor suppressor protein in *Drosophila*, and *fat* mutants have severely overgrown imaginal discs. We found that the overgrowth phenotypes of *fat* mutants are similar to those of mutants in Hippo pathway components and that *fat* mutant cells show deregulation of the same set of target genes as in *hippo* mutants. Furthermore, genetic epitasis experiments and biochemical assays place Fat upstream of the transcriptional coactivator Yorkie, the Hippo and Warts kinases, and the FERM domain-containing protein Expanded. In conclusion, our study links the growth control function of Fat to the Hippo tumor suppressor pathway. Since Fat is a transmembrane protein it may act as the receptor of the Hippo pathway.

212B

***Drosophila* Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation.** Ryan O. Andersen[1], Cheng-Yu Lee[1,3], Clemens Cabernard[1], Laurina Manning[1], Khoa D. Tran[1], Marcus J. Lanskey[1], Arash Bashirullah[2], Chris Q. Doe[1]. 1) Institutes of Molecular Biology and Neuroscience, Howard Hughes Medical Institute, University of Oregon, Eugene, OR; 2) Department of Human Genetics, Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT; 3) Center for Stem Cell Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI.

The precise regulation of stem cell self-renewal versus differentiation is critical for embryonic development and adult tissue homeostasis. *Drosophila* larval neuroblasts divide asymmetrically to self-renew, and can be used to identify genes regulating self-renewal. Here we show that mutations in *aurora-A*, encoding a conserved kinase implicated in human cancer, produce neuroblast brain tumors. In vivo clonal analysis and timelapse imaging show that single neuroblasts generate multiple neuroblasts to expand the neuroblast population. This phenotype is due to (1) defects in Numb and aPKC cortical polarity; and (2) failure to align the mitotic spindle with the cortical polarity axis. *aurA* neuroblast overgrowth is strongly suppressed by Numb overexpression, although spindle orientation defects persist, showing that altered cortical polarity is the primary cause of the phenotype. We conclude that Aurora-A and the Notch-antagonist Numb are novel inhibitors of neuroblast self-renewal, and that lack of either Aurora-A or Numb results in neuroblast tumors.

213C

Fat tumor suppressor pathway regulates Warts stability and activity. Yongqiang Feng[1], Eunjoo Cho[1], Cordelia Rauskolb[2], Kenneth Irvine[1]. 1) HHMI, Waksman Inst/Rutgers Univ, Piscataway, NJ; 2) Waksman Institute, Rutgers, The State University of New Jersey, Piscataway New Jersey 08854, USA.

Fat is a transmembrane protein classified as a protocadherin. Mutation of *fat* influences tissue polarity and imaginal disc growth. A potential ligand of Fat, Dachsous, and the Golgi protein Four-jointed are also involved in both tissue polarity and growth regulation. However, aside from a requirement for the unconventional myosin Dachs, downstream components regulated by Fat signaling were unknown. The Hippo tumor suppressor pathway regulates growth and cell death through the kinase Warts and transcription factor Yorkie. The tumor suppressor proteins Expanded and Merlin have also recently been linked to the Hippo pathway. We found that *fat* shares similar phenotypes with *hippo*, *warts*, *salvador*, *mats*, *ex* and *Mer* in terms of overgrowth, regulation of downstream targets genes and inhibition of apoptosis. We have found that the Fat tumor suppressor pathway regulates growth by modulating Warts protein stability and activity. Loss of *fat* induces the degradation of Warts *in vivo*, which can be suppressed by *dachs* mutation. Specific mutations of the casein kinase I δ/ε, *dco*^{3} also destabilizes Warts protein *in vivo*, and genetically *dco* is upstream of *dachs*. Loss of *ex* promotes cell growth but does not affect the stability of Warts protein. Mutation of *fat* influences the localization or stability of Ex protein on the apical membrane but only at later stages of wing imaginal disc development. Thus, the influence of Fat on Ex levels or localization does not appear to make a significant contribution to growth regulation. Overexpression of *warts* completely rescues *fat* or *dco*^{3} induced overgrowth and partially rescues *ex* growth phenotypes. These data suggest that Fat, Dco and Ex regulate growth all through Warts but by two different mechanisms. Fat may regulate Warts activity in some contexts by affecting Ex protein localization or stability, but regulation of the stability of Warts appears to be the major mechanism by which the Fat tumor suppressor pathway influence growth.

214A

The effector caspase *DCP-1* has functions in *Tor* mediated autophagic cell death in *Drosophila* . Young-Il Kim, OokJoon Yoo. Dept Life Sci, KAIST, DeaJeon, DeaJeon, KR.

Caspases are known as essential component to occur apoptosis in vertebrates and invertebrates. *DCP-1* is studied long times as effecter caspase to make apoptosis by cleaved by initiator caspase *Dronc* with *drICE* in *Drosophila melanogaster*. We tried to find out the suppressor of apoptosis *in vivo* by *DCP-1* with *GMR-GAL4* mediated modifier screening. The majority of positives are apoptosis inducing genes and signaling genes. But, Remarkably, *DCP-1* mediated phenotype on Drosophila retina is suppressed by essential autophagy genes *Tor*(target of rapamycin) over expression. Moreover, in the absence of Tor by deletion of *Tor*, the over-expression of *DCP-1* in eye shows lethal effect. Thus, these data demonstrate the effector caspase *DCP-1* has role also in autophagic cell death and suppressed by Tor.

215B

Functional antagonism of E2F and armadillo/β-catenin signaling in apoptosis regulation. Erick J. Morris, Nicholas J. Dyson. Lab Molecular Oncology, Massachusetts Gen Hosp Cancer Ctr, Charlestown, MA.

Retinoblastoma (pRB) and E2-promoter binding factor (E2F) family members are important regulators of G1-S phase progression. Deregulated E2F also sensitizes cells to apoptosis but this aspect of E2F function is poorly understood. Among the eight mammalian E2F genes, E2F1 alone possesses strong pro-apoptotic activity. Beyond its direct transcriptional output however, little is known regarding signaling pathways that might control E2F1-dependent apoptosis. Due to its effects on both cellular proliferation and apoptosis, E2F1 can function as both an oncogene and tumor suppressor. Hence, cellular context is an important determinant of E2F1-dependent phenotypes. An important context-dependent regulator of tumorigenesis and metastasis is cellular adhesion survival cues. We therefore investigated potential links between adhesion-related survival signaling and apoptosis induced by acute E2F1 activation. Our results demonstrate that armadillo/β-catenin is down-regulated by E2F1 expression in both fly and human cells. Co-expression of armadillo or TCF/pangolin or re-expression of stable tumor-derived β-catenin mutants is sufficient to suppress E2F1-dependent apoptosis. These interactions are likely to affect the canonical WNT-β-catenin-TCF signaling pathway regulating cell survival. Taken together, these results demonstrate functional antagonism between E2F1 and armdillo/β-catenin signaling during apoptosis and may have important implication during tumorigenesis. Currently, we are investigating the mechanism of this interaction and its relevance in human cancer.

216C

The effector caspase *dcp-1* has functions in *Beclin* mediated autophagic cell death in *Drosophila*. JuHyun Shin, OokJoon Yoo. Life Sci, KAIST, DeaJeon, DeaJeon, KR.

Caspases are known as essential component to occur apoptosis in vertebrates and invertebrates. *DCP-1* is studied long times as effecter caspase to make apoptosis by cleaved by initiator caspase *Dronc* with *drICE* in *Drosophila melanogaster*. We tried to find out the suppressor of apoptosis *in vivo* by *DCP-1* with *GMR-GAL4* mediated modifier screening. The majority of positives are apoptosis inducing genes and signaling genes. But, Remarkably, *DCP-1* mediated phenotype on Drosophila retina is suppressed by essential autophagy gene *beclin* over expression. *DCP-1* mediated cell death was reduced by the *beclin* co-expression. Thus, these data demonstrate the effector caspase *DCP-1* has role also in autophagic cell death and suppressed by *beclin*.

217A

Apoptosis activator *hid* is a target for Rb-E2F mediate transcriptional repression. Miho Tanaka-Matakatsu, Jinhua Xu, Wei Du. BMICR, University of Chicago, Chicago, IL.

The cues to coordinate cell cycle progression and cell death to ensure proper tissue differentiation remain elusive. Our series of *rbf* modifier screening identified apoptosis activator *hid* and *dronc* as suppressors. Loss of *rbf* in developing eye dramatically increased apoptotic cells in the morphogenetic furrow, whereas the double mutant of *rbf* and *hid/dronc* suppressed *rbf* dependent apoptosis. The *rpr* is not responsible for this *rbf* dependent cell death. Transgenic animals contain wild-type *hid cis*-regulatory element displayed quite low levels of reporter gene expression in eye disc, while the mutation of putative E2F motif dramatically increased reporter gene expression. Chromatin IP and gel shift assay further support that Rbf-E2F complex regulates *hid* transcription through the direct binding to its E2F motif in 5' *cis*-regulatory element. Interestingly, *rbf* mutant animals accumulate high level of *hid* mRNA in the whole eye disc while high level of apoptosis was restricted near the morphogenetic furrow. We will show the mechanisms that regulate this domain specific apoptosis.

218B

Mob as Tumor Suppressor is Directly Activated by Hippo Kinase for Growth Inhibition. Xiaomu Wei[1], Takeshi Shimizu[2], Zhi-Chun Lai[1,2,3]. 1) Genetics Program, Pennsylvania State University, University Park, PA; 2) Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA; 3) Dept of Biology, Pennsylvania State University, University Park, PA.

Tissue growth and organ size are determined by coordinated cell proliferation and apoptosis in development. Recent studies have demonstrated that Hippo (Hpo) signaling plays a crucial role in coordinating these processes by restricting cell proliferation and promoting apoptosis. In our previous studies, we have shown that the Mob as tumor suppressor protein, Mats, functions as a coactivator of Wts/Lats protein kinase to mediate growth inhibition (Lai et al., Cell 120: 675-685, 2005). To further test an idea that Mats is a key component of the hpo pathway, we have carried out genetic analysis and found that mats is epistatic to hpo. Thus, mats functions downstream of or in parallel to hpo. Co-expression analysis indicated that Mats can indeed potentiate Hpo-mediated growth inhibition in vivo. Furthermore, we found that Mats associates with Hpo in a protein complex and is a direct target of the Hpo serine/threonine protein kinase. Mats phosphorylation by Hpo increases its affinity with Wts/Lats protein kinase and ability to up-regulate Wts/Lats catalytic activity to target downstream proteins such as Yorkie (Yki). Our results support a model in which Mats is directly activated by Hpo through phosphorylation for growth inhibition and this regulatory mechanism is conserved from flies to mammals.

219C

Bcr-Abl interacts with Rho GTPases to alter cell migration during *Drosophila* development. Nicholas B. Artabazon, Sara Tittermary, Katherine D. Miller, Traci L. Stevens. Biology Department, Randolph-Macon College, Ashland, VA.

Bcr-Abl is an activated fusion protein linked to leukemia in humans and results from a reciprocal translocation between chromosomes 9 and 22 that fuses most of the *abl* gene to the *bcr* gene. Normal, cellular Abl (c-Abl) is a protein tyrosine kinase that regulates cell migration by direct interactions with the actin cytoskeleton and indirectly by phosphorylation of proteins that regulate actin dynamics. Compared to c-Abl, Bcr-Abl has increased tyrosine kinase activity. Studies in culture suggest that altered actin dynamics and abnormal cell migration in cells expressing Bcr-Abl may be key events in promoting leukemia, though the exact processes are still unknown. Our studies in *Drosophila* support a role for Bcr-Abl in regulating cell migration and adhesion through its effects on the actin cytoskeleton. *Drosophila* embryos expressing Bcr-Abl in the epithelium die with defects in dorsal closure, a process that requires cell migration and regulated cellular adhesion. Furthermore, expression of Bcr-Abl dramatically altered actin-based projections in migrating epithelial cells during dorsal closure. The overall goal of our research is to gain insight into the molecular pathways that lead to altered actin structure and cell migration in cells that express Bcr-Abl. Rho GTP-binding proteins are a family of actin regulators required for dorsal closure, and studies in cell culture suggest that Rho proteins and Bcr-Abl may act in together to remodel the actin cytoskeleton. In order to understand the relationship between these two proteins, we examined the effects of mutant versions of Rho on Bcr-Abl signaling. In epithelial cells lacking Rho activity, we found that Abl kinase activity was mis-localized. In addition, levels of Ena, a target of Bcr-Abl phosphorylation, were altered in *rho1* mutants. Furthermore, the phenotypes of double mutants expressing Bcr-Abl and mutant versions of Rho indicate that these proteins function in the same pathway. Taken together, these studies suggest that at least some of the effects of Bcr-Abl on cell migration may be through the Rho family of actin regulators.

220A

Projectin assembly, domain interactions and elastic domain in IFM myofibril. Agnes Ayme-Southgate, Danielle Adler, Richard Southgate. Dept Biol, Col Charleston, Charleston, SC.

Projectin is a giant (~1,000 kDa) sarcomeric protein with multiple possible functions. One projectin molecule is long enough to be embedded within the Z-band, span the I band to the edge of the thick filaments in the myofibrils of insect flight muscles (IFM). Its amino-terminal region contains a PEVK domain, as well as two clusters of Immunoglobulin (Ig) domains. The PEVK domain and/or the Ig domains might confer elastic properties to the protein. We will present data characterizing the stretchable domain(s) within the IFM-specific isoform. We will also present data supporting the *in vivo* interactions of GFP-projectin fusions with different regions of the sarcomeres in both wild type and sarcomeric mutant flies. We will discuss these data in relation to projectin assembly within the IFM sarcomere during the myofibrillogenesis process, as well as the functional significance of the elastic domain(s) in the stretch-activation mechanism.

221B

Muscle LIM Protein cooperates with Titin to maintain the structural integrity of muscle. Kathleen Clark[1,2], Jennifer Bland[1], Mary Beckerle[1,2,3]. 1) Huntsman Cancer Inst, University of Utah, Salt Lake City, UT; 2) Department of Biology, University of Utah, Salt Lake CIty, UT; 3) Department of Oncology, University of Utah, Salt Lake CIty, UT.

Muscle LIM Protein is a cytoskeletal "LIM-only" protein found at the intercalated disc and Z-line in cardiac muscle. Genetic ablation of murine MLP produces dilated cardiomyopathy (DCM) and heart failure. Mutations in human MLP are also associated with cardiac hypertrophy and DCM; however, the molecular mechanism by which MLP functions in normal and diseased muscle is still not established. We developed the fly system to probe the role of MLP in a genetically tractable model. We generated null mutations in *mlp84B* and found that the protein is essential for post-embryonic muscle function. The *mlp84B* mutants cannot fully contract their body wall muscles during pupariation, and make a long, thin pupal case. Most animals arrest development at this point, and exhibit only limited muscle contractions. A few mutants eclose as adults, but have impaired flight and cardiac dysfunction. In order to identify the essential molecular functions for MLP, we looked for mutations in other genes that would modify the *mlp84B* phenotype. Flies null for *mlp84B* and heterozygous for a mutation in *D-titin* show marked enhancement of the *mlp84B* phenotypes. Surprisingly, the *mlp84B* mutant muscles on their own do not have any observable structural defects, but co-reduction in D-titin activity produces a severe disruption of muscle structure, with most fibers displaying some loss of sarcomeric organization and/or tearing. We have also discovered a novel function of Mlp84B in directing actin filament dynamics. Forcing Mlp84B into the nucleus can result in the production of intricate actin filaments that also contain Mlp84B. A report from Montana and Littleton demonstrates that Mlp84B, the related protein Mlp60A, and proteins that regulate actin dynamics are all upregulated in damaged muscle. Together these observations suggest a model by which the fly MLPs promote normal muscle function by regulating actin dynamics during muscle repair.

222C

Unexpected complexity in the mechanisms that target assembly of the spectrin cytoskeleton. AMLAN DAS, CHRISTINE BASE, SRILAKSHMI DHULIPALA, RON DUBREUIL. BIOLOGICAL SCIENCES, UNIV. OF ILLINOIS AT CHICAGO, CHICAGO, IL.

Mutations in the Drosophila α and β spectrin genes are lethal and produce striking phenotypes in epithelia and neurons. Prevalent models suggest that spectrin contributes to the formation of plasma membrane domains with specialized composition and function. Consequently targeting mechanisms are important to spectrin function, but it has been difficult to identify cues that are responsible. Here we used transgene rescue to test the contribution of individual spectrin domains to its assembly. This approach has uncovered a surprising complexity in mechanisms accounting for polarized spectrin assembly in midgut epithelium, salivary gland, and the cells surrounding the larval optic lobe. We produced a battery of modified β spectrin transgenes and tested their ability to rescue lethal β spectrin mutations and their competence for polarized assembly. First, we replaced the putative ankyrin-binding domain of β spectrin and found no effect on spectrin targeting in any cells (including epithelia and neurons). It was surprising because ankyrin is widely believed to be a primary attachment site between spectrin and plasma membrane proteins. In fact, mutants rescued by this transgene often survived as adults. Second, we truncated the C-terminal pleckstrin homology (PH) domain of β spectrin and this mutation was usually lethal. It resulted in mislocalization of spectrin and ankyrin in midgut copper cells, but not in salivary gland or in the optic lobe epithelium. Third, we produced a double mutant transgene and found that its targeting was normal in the salivary gland, but abnormal in the optic lobe. These results indicate that targeting in the salivary gland is dependent on yet another site in the β spectrin molecule, but that the PH domain and the ankyrin-binding site made overlapping contributions to targeting in the optic lobe. Thus in three cell types that we have studied we have uncovered three distinct mechanisms of spectrin targeting. These different modes of assembly may allow spectrin to make unique contribution to membrane function in diverse cells.

223A

How Do Cells Shape Actin-Based Protrusions? Stacie A Dilks, Stephen DiNardo. University of Pennsylvania, Philadelphia, PA.

The ability of cells to organize actin filaments into stable structures is vital to many cellular functions such as nutrient absorption, hearing, and sensory input. Although the actin-based protrusions that underlie these processes have distinct, elaborate shapes, it is unknown how cells form shaped protrusions. To better understand this process, we are studying the *Drosophila* ventral embryonic epidermis. In this tissue, certain cells produce an actin-based protrusion (called a denticle) that exhibits a reproducible, row-specific hooking shape. Although the transcription factor shavenbaby is sufficient for denticle production, *shavenbaby* expression alone is NOT sufficient for denticle shaping. We hypothesize that the regulation of certain cytoskeletal components is required for the proper shaping of actin-based protrusions.

twinstar encodes the protein Cofilin, which regulates actin dynamics by severing long actin filaments and enhancing the actin depolymerization rate. Twinstar is negatively regulated by Lim Kinase and activated by the phosphatase encoded by *slingshot (ssh)*. We now show that *ssh* mutations result in both misshapen denticles and an expansion of the denticle field, indicating that the dynamic turnover of actin filaments is necessary for denticle shaping.

zipper (zip), encoding the Myosin II heavy chain, also plays a role in denticle shaping. *zip* mutants show dramatically misshapen denticles, but it is unclear how *zip* participates in denticle formation. We show that *zip* mutants mislocalize the actin bundling protein, Forked, which is required at growing tips to initiate bundle formation. Other proteins associated with elongating filaments are properly localized in *zip* mutants, however, indicating that *zip* may be required specifically for actin bundle formation.

Our studies are currently focused on how modulation of Myosin II and Cofilin activity control denticle shape.

224B

Trc/Fry and Wts/Mats have opposite effects on wing cell shape and timing of hair initiation in Drosophila melanogaster. Xiaolan Fang, Paul Adler. Department of Biology, University of Virginia, Charlottesville, VA.

tricornered (trc), which encodes the Drosophila Ndr (Nuclear Dbf2-related) serine/threonine protein kinase, is required for the normal morphogenesis of epidermal hairs, bristles, arista laterals and sensory neuron dendrites. Previous studies indicated that Trc functions in concert with the Furry protein to regulate the organization of the cytoskeleton during the outgrowth of these structures. Two other genes, *wts* and *mats*, are closely related to trc function. We have found that in the pupal wing, *trc*, *fry*, *wts* and *mats* mutations produce complementary phenotypes. Mutations in *trc* or *fry* result in cell width increased cross section and delayed hair initiation. Mutations in *wts* or *mats* result in cell width decreased cross section and earlier hair initiation.

225C

Ena promotes actin filament elongation during epithelial morphogenesis. Julie Gates[1], Connie Barko[1], Stuart Hollenshead[1], Justine Lu[1], Frank B. Gertler[2], Mark Peifer[3]. 1) Biology, Bucknell University, Lewisburg, PA; 2) Biology, MIT, Cambridge, MA; 3) Biology, UNC-CH, Chapel Hill, NC.

During development the actin cytoskeleton must be regulated to accommodate the remarkable changes in cell shape, cell rearrangements and cell migrations that occur as the embryo is shaped. If actin dynamics are not properly regulated, morphogenesis is disrupted and normal development fails. Numerous proteins have been identified that influence actin dynamics including members of the Ena/VASP protein family. Mammalian Ena/VASP proteins have been proposed to promote the continued elongation of actin filaments by binding to their barbed end and preventing the binding of Capping Protein. Drosophila has a single Ena/VASP family member, Ena. Using genetic loss and gain of function techniques, as well as a mislocalization strategy, we have identified several processes that are influenced by the level and/or localization of Ena. When we generate animals in which the maternal and zygotic contributions of Ena are depleted the resulting embryos display defects in head involution, germ band retraction and dorsal closure. While adhesion and the actin cytoskeleton appear roughly normal, the cell shape changes that drive morphogenesis are disrupted in a subset of embryos. Altering Ena's level and/or localization later in development results in defects during dorsal closure. During dorsal closure the leading edge cells produce actin-rich membrane protrusions in the form of broad lamellipodia that give rise to long, thin filopodia. Using GFP-actin to visualize these protrusions in living embryos we have found that altering Ena localization alters the type of actin-rich protrusion formed. Pulling Ena away from its normal location results in the formation of mainly lamellipodia and very few filopodia, while concentrating Ena at the membrane or overexpressing it results in the formation of mainly filopodia and very few lamellipodia. We are currently examining how Ena may function with other actin regulators during dorsal closure.

226A

The role of a short peptide gene, *polished rice*, during imaginal development. Yoshiko Hashimoto[1], Takefumi Kondo[1], Yuji Kageyama[1,2]. 1) Nara Institute of Science and Technology, Ikoma, Nara, Japan; 2) PREST, Japan Science and Technology Agency.

It has been shown that short peptide genes are involved in various biological events. For example, soybean *ENOD40* (12 aa) is engaged in root nodule formation and *Arabidopsis ROT4* (53 aa) regulates cell proliferation and is required for proper leaf development. *mille pattes (mlpt)* in *Tribolium* is another example in animal development and functions as a segmentation gene. However, how these peptides exert their effects is poorly understood.

polished rice (pri) is a novel small peptide gene in *Drosophila*, which encodes at least four small peptides (11 and 32 aa) and shows extensive similarities with *mlpt*. Contrary to biological function of the *Tribolium* counterpart, *pri* is required for proper development of epithelial structures rather than segmentation: *pri* mutant embryos completely lack denticle belts and dorsal hairs, as well as taenidial folds in the tracheal system. In *pri* mutants, localized signals of F-actin is not observed, suggesting that *pri* is involved in cell shape control through reorganization of actin filaments. Since transcripts of *pri* are detected also in larvae and adult flies by northern analysis, *pri* may function in a variety of developmental processes.

To investigate the function of *pri* during imaginal development, we first examined expression patterns by *in situ* hybridization and revealed that *pri* is strongly expressed in imaginal discs in specific patterns. As *pri* mutants are embryonic lethal, we next performed mosaic analyses to assess the physiological roles of the gene during imaginal development. Although small *pri* mutant clones showed normal morphology, collapse of ommatidia was occasionally observed at the center of large clones. Mutant cells that abut wild type cells are morphologically normal, suggesting that *pri* functions in a non-cell autonomous fashion. Our results indicate that short peptide gene pri functions also in postembryonic stages, probably by regulating cytoskeletal organization *via* cell-cell communication.

227B

Diaphanous, a link between the actin and myosin cytoskeleton. Catarina Homem, Mark Peifer. Dept Biol, UNC, Chapel Hill, NC.

Studies of cultured mammalian cells are revealing how adhesion, cytoskeletal dynamics and cell contractility are interrelated. It is increasingly relevant to study these processes in whole animals during morphogenesis. It is unclear how changes in the actin cytoskeleton are coordinated with contractility and altered cell adhesion. Here we address a mechanism for this coordinate regulation during embryogenesis. Formins are important regulators of the actin cytoskeleton. Diaphanous-related formins, like Drosophila Diaphanous (Dia), are regulated by Rho, which is also an important myosin (myo) and Adherens Junctions (AJs) regulator. Using a constitutively active form of Dia we examined Dia's role during morphogenesis. As expected, active Dia alters the actin cytoskeleton, yet we also observed unexpected changes in myo levels and localization and changes in AJs. Interestingly, the changes induced by active Dia were different in different cell types. Amnioserosal cells apically constrict during dorsal closure while epidermal cells elongate. Expression of active Dia in amnioserosal cells induces apical myo accumulation, affecting both myo localization and levels and stabilizes AJs. These changes induce precocious apical cell constriction. In epidermal cells, which unlike amnioserosal cells are planar polarized, myo does not accumulate uniformly around the membrane but accumulates in a planar polarized fashion. Strikingly while myo is normally anterior/posterior planar polarized, in cells expressing active Dia myo planar polarity is altered, accumulating preferentially at dorsal/ventral cell boundaries, where AJs also accumulate. We next investigated the mechanism by which Dia works, examining whether Dia regulates myo levels, or stabilizes myo at AJs. To test these hypotheses we overexpressed myo or induced myo activation. Neither is sufficient to change myo planar polarization or stabilize AJs, but myo activation does mimic the cell contraction phenotype of cells expressing active Dia. Thus Dia regulates cell shape, contractility, and adhesion not only by regulating actin but also myo levels, planar polarization and perhaps myosin activity, thus helping establish cell contractility and planar cell polarity.

228C

polycistronically-encoded small peptides regulate actin-based morphogenesis in *Drosophila*. Takefumi Kondo[1], Kagayaki Kato[3], Yoshiko Hashimoto[1], Shigeo Hayashi[3], Yuji Kageyama[1,2]. 1) Grad. Sch. Biol. Sci., NAIST, Nara, JP; 2) PREST, Japan Science and Technology Agency; 3) Riken Center for Developmental Biology, Kobe, JP.

During the development of multicellular organisms, each cell acquires specific identity and changes its cell shape. It is well known that cell shape depends on cytoskeletal architecture, cell adhesion and extracellular matrix. However, regulation mechanisms of cell shape are still elusive.

In the course of studies for uncharacterized transcripts in *Drosophila*, we identified *polished rice* (*pri*), which is required for proper embryogenesis. *pri* is expressed in epidermis, trachea and gut during embryogenesis and *pri* null mutants show complete loss of denticle formation and collapse of tracheal network. In *pri* mutants, denticle cells did not show specific accumulation of F-actin required for the formation of cell protrusion, although expression of *shaven baby*, the master regulator of denticle formation, was not affected. These results indicate that *pri* acts independently of *shaven baby* to play an essential role in actin dynamics in denticle formation.

All ten ORFs found in *pri* transcripts is very short (< 49 amino acid), and *pri* thus has been originally identified as non-coding RNA. However, we found that ORF1~3 (11 a.a. each), ORF4 (32 a.a.) and the N-end half of ORF5 (49 a.a.) are highly conserved among *Drosophila* species. In addition, ORF1~4 peptides contain a conserved septa peptide motif, LDPTGQ/TY. We present evidence that ORF1-4 (, but nut ORF5,) are functional cistrons in S2 cells. Furthermore, overexpression of minigenes containing either ORF1 or ORF4 alone was sufficient for complete rescue of the denticle phenotype. These result suggest that *pri* is transcribed as polycistronic mRNA and translated to multiple small peptides. Taken together, our data uncover the importance of small peptide-coding genes, hidden in the genome annotation, in regulation of cell shapes through cytoskeleton.

229A

Identification and characterization of the role of TRAF1 in Drosophila ventral furrow formation. Sam J. Mathew, Thomas C. Seher, Maria Leptin. Institute for Genetics, University of Cologne, Germany.

Gastrulation in Drosophila begins with ventral furrow formation. Although several genes required for ventral furrow formation are already known, it was evident that as yet unidentified genes are involved in this process. Thus, a screen to identify new loci involved in ventral furrow formation was performed previously, implicating the cytogenetic region 24-25 among others. Genetic and molecular analysis of this region resulted in the identification of TRAF1 (TNF Receptor Associated Factor 1) as the gene responsible for the observed defects. Previous reports suggest that the vertebrate homolog of TRAF1, TRAF4 is required during early development in mice. We found that TRAF1 is a transcriptional target of *twist*, a gene required during ventral furrow formation. Although TRAF1 is known to function in the JNK signaling cascade, the ventral furrow defects associated with TRAF1 were found to be independent of this pathway. We are investigating the signaling downstream of TRAF1 which leads to the observed ventral furrow defects. Furthermore, we are also generating a null mutant for TRAF1 in order to better understand its function during Drosophila development.

230B

Plasma membrane diffusion barriers in the precellularizing Drosophila embryo. Manos Mavrakis[1], Richa Rikhy[1], Bob Phair[2], Jennifer Lippincott-Schwartz[1]. 1) Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892; 2) Integrative Bioinformatics Inc, Los Altos, CA 94024.

In this study we focus on the organization of the plasma membrane (PM) in the precellularizing Drosophila embryo. We have previously shown that each nuclei in the early embryo possess functionally compartmentalized secretory units in the absence of physical barriers. It is unknown whether proteins delivered to the PM are able to freely diffuse to all regions of the embryo, or whether the PM is compartmentalized over individual nuclei. To follow plasma membrane dynamics, we generated flies expressing fluorescently-tagged integral membrane proteins or fluorescently-tagged proteins associated with the inner leaflet of the plasma membrane, and performed photobleaching experiments. In FRAP experiments, both classes of membrane-associated proteins exhibited free lateral diffusion in the PM. FLIP assays showed, however, that the lateral diffusion of these proteins was restricted to regions of PM over individual nuclei. Finally, when a PM pool was optically pulse-chased in photoactivation experiments, proteins could freely diffuse in the embryo PM, but only within the PM region over individual nuclei and not across the PM over adjacent nuclei. Altogether our findings suggest the presence of a plasma membrane diffusion barrier that restricts lateral diffusion of proteins in the PM over multiple nuclei. We are currently investigating the nature of such diffusion barriers using a genetic approach, as well as by performing ultrastructural studies. Implications of this plasma membrane compartmentalization in the development of the early embryo are discussed.

231C

The role of DRhoGEF2 during segmental groove formation in the Drosophila embryo. Shai Mulinari, Mojgan Padash Barmchi, Udo Häcker. Dept Exp Med Sci, Lund Strategic Research Center for Stem Cell Biology and Cell Therapy Lund Univ, Lund, SE.

Morphogenesis of the Drosophila embryo is associated with a dynamic rearrangement of the Actin-based cytoskeleton mediated by small GTPases of the Rho family. These GTPases act as molecular switches that are activated by guanine nucleotide exchange factors (RhoGEFs). One of these factors, DRhoGEF2 has been shown to play an important role in the constriction of Actin filaments during pole cell formation and blastoderm cellularization as well as in the generation of cell shape changes during gastrulation. Here we show that DRhoGEF2 is equally important during the formation of segment boundaries, which become morphologically distinguishable as tissue infoldings - termed segmental grooves - during late embryogenesis. Our analysis shows that DRhoGEF2, Actin and Myosin II co-localize and are dynamically re-distributed during segmental groove formation. Examination of embryos derived from DRhoGEF2 germ line clones indicates a role for DRhoGEF2 in the induction of cell shape changes that occur during segmental groove formation. Overexpression of DRhoGEF2 in the ectoderm induces cell shape changes in all expressing cells and a strong deepening of grooves at the segment boundaries that can be suppressed by Rho1 inactivation. DRhoGEF2-induced invagination of cells at the segment boundaries requires engrailed and hedgehog indicating a role for segment polarity genes in the regulation of DRhoGEF2 activity. Together our results show that DRhoGEF2 and Rho1 act downstream of segment polarity genes to regulate cytoskeletal rearrangement during segmental groove formation in the embryo. .

232A

Characterization of PTEN and skittles function during photoreceptor morphogenesis. Ella Palmer, Franck Pichaud. MRC LMCB, UCL, Gower Street, London. WC1E 6BT.

Previous work from our laboratory has shown that in the *Drosophila* epithelial photoreceptor, the conserved polarity gene bazooka (*baz*) interacts with the tumor-suppressor lipid phosphatase PTEN at the developing cell-cell junction (zonula adherens) where it controls PIP3 levels within the apical membrane domain (Pinal et al., Curr Biol 16:140-9). This work has demonstrated a direct link between a polarity gene and a tumor-suppressor gene mutated in more than 50 percent of cancers in humans. In order to further characterize PTEN's function in photoreceptor morphogenesis, we have initiated a structure-function analysis of this conserved lipid-protein phosphatase *in vivo*, in the *Drosophila* eye. Various transgenes, including protein/lipid phosphatase dead variants have been reintroduced into PTEN mutant eyes to test their ability to rescue the PTEN loss-of-function phenotype, as well as their sub-cellular localization. In addition we have broadened our study to include the role of phosphoinositol lipids during photoreceptor morphogenesis. Of particular interest, is the *Drosophila* type I phosphatidylinositol 4-phosphate 5 kinase (PIP5K) *skittles*, which is responsible for PIP2 biosynthesis, and appears to be involved in photoreceptor morphogenesis. We will present our data regarding PTEN's function in the developing photoreceptor as well as our characterization of the *skittles* phenotype in these cells.

233B

Characterization of mutants defective in salivary gland migration along the circular visceral mesoderm. Unisha B Patel. Cell and Developmental Biology, WMC of Cornell University, New York, NY.

The embryonic salivary gland is an elongated pair of epithelial tubes whose early development includes posterior migration along the circular visceral mesoderm (cVM). Proper formation of the cVM is required not only for initiation of posterior migration but also for detachment of the gland from the cVM later in migration. However, the precise role of the cVM in salivary gland migration is not known. To better understand the role of the cVM in salivary gland migration we analyzed a collection of gland migration mutants generated from a previous EMS mutagenesis screen (*M.M.Myat and D. J. Andrew, unpublished data*) for concomitant defects in cVM morphogenesis. Here, we report on characterization of one of these mutants, *jhu2388*. In *jhu2388* homozygous embryos salivary glands begin to migrate posteriorly; however, detachment of the gland from the cVM is delayed impeding further migration of the gland. Morphological analysis of the cVM showed that the cVM was disorganized and did not elongate in the dorsal-ventral axis to the same extent as in wild-type embryos. Furthermore, *jhu2388* genetically interacts with Rac1 GTPase, known to regulate E-cadherin-mediated cell-cell adhesion in migrating gland cells. We will present our efforts to identify the wild-type gene corresponding to *jhu2388* and further characterization of the salivary gland migration defect.

234C

Dissecting Rhodopsin 1 function in photoreceptor morphogenesis. Noelia Pinal, Franck Pichaud. Deot LMCB, MRC, London, GB.

G-protein coupled receptors (GPCR) such as the sensory visual pigment rhodopsin1 (Rh1) form a large conserved family of transmembrane receptors. In the fly eye, Rh1 is expressed in the outer photoreceptors (R1 to R6). Rh1 is involved in phototransduction in these cells, but is also required in late pupation for the morphogenesis and maintenance of the apical organelle called the rhabdomere that consists of a stack of microvilli. In addition, the Rho-GTPases Rac and Cdc42 have been shown to act downstream of Rh1 in apical organelle morphogenesis. However, beside the Rho-GTPases, the nature of the pathway involved in this process is not clear. To address this question, we have initiated a systematic structure-function study of Rh1 in vivo, using rescue assays. This study includes testing a role for the small G protein G?q know to be involved in phototransduction in the fly eye. In parallel, we have performed an RNAi screen in vivo, targeting the predicted fly guanine exchange factors (GEFs) and GTPase activating proteins (GAPs), in the hope of identifying the relevant factors needed for Rac1 and Cdc42 signalling in the context of Rh1 and apical organelle morphogenesis. We will be presenting the data from to this screen, as well as our analysis of the Rh1 function in apical membrane morphogenesis.

235A

Rac GTPase regulation of E-cadherin-mediated cell-cell adhesion in epithelial migration. Carolyn Pirraglia, Monn Monn Myat. Department of Cell & Developmental Biology, Weill Medical College of Cornell University, New York, NY 10021.

Members of the Rho family of small GTPases are key regulators of numerous cellular events, such as remodeling of the actin cytoskeleton and modulation of cell-cell adhesion during migration. We previously showed that the small GTPase Rac regulates E-cadherin-based cell-cell adhesion in migration of the embryonic salivary gland upon the circular visceral mesoderm (Pirraglia et al., 2006, Dev Biol., 290 p. 435-446). Inhibition of Rac function, either through loss of function mutations or dominant-negative mutations, disrupts salivary gland invagination and posterior migration. In contrast, constitutive activation of Rac induces motile behavior and subsequent cell death. Rac regulation of salivary gland migration occurs through modulation of E-cadherin/β-catenin-mediated cell-cell adhesion in a *shibire* (dynamin)-dependent manner. To identify the signaling pathway downstream of Rac involved in E-cadherin regulation we tested known downstream effectors of Rac for their role in salivary gland migration. Our data show that loss and gain of function mutants of p21 activated kinase (Pak) disrupt salivary gland migration in a manner similar to that of Rac mutants. Milder migration defects were also observed in embryos mutant for Pkn, another known downstream effector of Rac. Our data thus suggest that Pak and Pkn act downstream of Rac to regulate levels of E-cadherin-based cell-cell adhesion during gland migration.

236B

Actin capping proteins maintain epithelium integrity of *vestigial*-expressing cells in the wing blade epithelium. Sofia R. P. Rebelo, Florence Janody. Actin Dynamics Unit, Instituto Gulbenkian de Ciênca, Oeiras, PT.

In mosaic genetic screens, we identified loss of function mutations in the genes encoding Capping protein alpha (Cpa), the Cofilin homolog Twinstar (Tsr) and the Cyclase Associated Protein (CAP) Capulet. All restrict actin filaments polymerization but use different mechanisms to mediate this effect. Cofilin severs filaments and enhances dissociation of actin monomers from the pointed end; CAP sequesters actin monomers, preventing their incorporation into filaments; and Cpa restricts accessibility of the barbed end, inhibiting addition or loss of actin monomers. Interestingly, each has a different function in the development of epithelial tissues. We have found that Cpa, as well as its partner Capping protein beta (Cpb) prevent extrusion and death of cells in the wing blade epithelium, but are not required for this function in other regions of the wing disc. Although *cpa* and *tsr* mutations, both, increase actin filament polymerization throughout wing blade cells, only *cpa* is required to maintain *vestigial (vg)* expressing cells within the epithelium. In contrast, misexpression of *ultrabithorax (ubx)* prevents extrusion of *cpa* mutant cells in the wing blade epithelium. Furthermore, Vg enhances transcription of *cpa* in this region. This suggests that, in order to promote morphogenesis of the adult wing, one function of Vg is to modulate the expression or the recruitment of a cytoskeletal remodeling factor which weaken cell-cell contacts. The role of capping protein would be required to maintain the connection between cells in the epithelium. This implies that cytoskeletal properties of genetically defined epithelia are crucial to achieve distinct morphological outcomes.

237C

Myoblast fusion requires active remodeling of the actin cytoskeleton. Brian Richardson[1], Mary Baylies[2]. 1) Program in Biochemistry, Molecular and Cell Biology, Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Embryonic body wall muscles form from the fusion of two myoblast cell types: founder cells (FCs) and fusion-competent myoblasts (FCMs). Each FC seeds a specific muscle, while FCMs fuse to the FC and adopt a particular muscle program. After undergoing a stereotypic number of fusion events, individual syncytial myotubes undergo morphogenesis and acquire distinct size, shape and orientation. The molecular mechanisms underlying the fusion process, the number of fusion events and the acquisition of distinct cell shapes are not well understood. Using new methods for fixed and live imaging, we have identified an accumulation of F-actin at the site of fusion, which subsequently resolves, and is followed by, membrane breakdown between fusing myoblasts. Mutations in kette lead to defects in resolution of this F-actin accumulation, preventing myoblast fusion. Analyses of previously identified myoblast fusion mutations reveal distinct phenotypic classes with regard to these F-actin foci. These findings indicate that the known components of the fusion machinery do not converge solely on these foci, but instead separate into pathways that control several distinct cytoskeletal behaviors required for fusion. Our data provide insight to the roles and dynamics of the actin cytoskeleton and its regulators during cell fusion. Moreover, they lead to a revision of our existing model of myoblast fusion, providing a new paradigm for examining the basic mechanics underlying fusion in myoblasts and in other tissues.

238A

An RNAi screen for genes affecting the architecture and polarity of the *Drosophila* ovarian follicular epithelium. Emily C.N. Richardson, Franck Pichaud. MRC Laboratory for Molecular Cell Biology, University College London, London, GB.

We are interested in understanding the link between cell polarity and the cytoskeleton, and we would like to identify novel effectors of this process. In order to do this we are undertaking a screen for genes affecting epithelial cell architecture and polarity, using double stranded RNA interference (RNAi) in the ovarian follicular epithelium. For this screen we are focussing on predicted cytoskeleton-related genes representing a library of approximately 1000 genes in flies used by *Kiger et al. 2004*. The method of the screen is based on that described by *Zhu and Stein 2004*, which uses an *alphaTub84B* promoter to drive expression of RNAi in the follicular epithelium. In our assay, the cytoskeleton in the developing egg chambers can be visualized by staining with phalloidin to reveal F-actin and antibody staining against a-tubulin. To test the validity of our approach, we have run a pilot screen testing RNAi on known polarity genes as positive controls and setting-up the system so that it includes a temperature dependent control to switch-on the RNAi in adult females. We will be presenting the results we have obtained from a preliminary screen performed on a subset of cytoskeletal related genes.

239B

The Wiskott-Aldrich Syndrome Protein (WASP) is essential for *Drosophila* myoblast fusionä. Gritt Schäfer[1], Susanne Weber[2], Anne Holz[3], Sven Bogdan[4], Renate Renkawitz-Pohl[1], Susanne Filiz Önel[1]. 1) Dept. for Developmental Biology, Philipps-Universität, Karl-von-Frisch Str. 8, D-35043 Germany; 2) Institute for Moleculare Biology and Tumor Research, Philipps-Universität Marburg, Emil-Mannkopff-Str. 2, D-35033 Marburg, Germany; 3) Institute for Allgemeine und Spezielle Zoologie, Stephanstr. 24, Justus-Liebig- Universität Giessen, D-35390 Giessen, Germany; 4) Institute for Neurobiologie, Universität Münster, Badestr. 9, D-48149 Münster, Germany.

The somatic muscles of higher organisms arise through the fusion of mononucleated myoblasts. In the *Drosophila* embryo fusion is a dynamic process between two myoblast populations: founder cells (FCs) and fusion-competent myoblasts (FCMs). Cell-cell-recognition and adhesion between these two cell types is mediated through the Immunoglobulin super family (IgSF) domain proteins Dumbfounded/Kirre (Duf/Kirre) and Irregular optic chiasma C/Roughest (IrreC/Rst), which serve redundant functions in FCs. In contrast, FCMs express the IgSF member Sticks and Stones (Sns). Recent data have shown that Duf and Sns form a ring-shaped adhesion structure named FURMAS (Fusion-Restricted-Myogenic-Adhesive-Structure) at cell-cell contact points. This structure contains F-actin in high concentrations (Kesper et al, 2006). Formation of F-actin is regulated by the Arp2/3 complex, which becomes activated by the Wiskott-Aldrich syndrome protein (WASP) family. *Drosophila* WASP possesses the same conserved domains like mammalian WAS and N-WASP protein, consisting of four regulatory domains and an Arp2/3 activating domain. The latter is known as VCA-domain and is thought to bind monomeric G-actin (V-domain) as well as F-actin (CA-domain). Here we present the identification of a new *wasp* allele that lacks the Arp2/3 interacting CA domain. The loss of this domain seems to neutralize maternal WASP and thus disrupts myoblast fusion. Interestingly, WASP is present at the plasma membrane during the fusion process and is enriched at contact points between FC and FCMs.

240C

Activated myosin II is required for cell alignment in the epidermis. Robert P Simone. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

Epithelial sheets undergo coordinated cell shape changes that are vital to organogenesis and development in general. The ventral epidermis of the Drosophila embryo is an epithelial sheet that undergoes differential cell shape changes across the parasegment. This epidermis is an especially promising tissue in which to study this phenomenon because much is known about the signaling pathways that underlie the establishment and maintenance of differential cell fates across the parasegment. Initially, the epithelial cells of the ventral epidermis maintain a roughly hexagonal shape and are randomly packed. Over the course of several hours, cells within this epithelium change shape: their anterior and posterior edges lengthen and their dorsal and ventral edges shorten. Our quantitative measurements showed that each row of cells in one population align their anterior and posterior edges to form parallel lines while cells in another population align their edges to a lesser degree. We observed an enrichment of Actin, Myosin II (MyoII) and MyoII related proteins along the aligning edges of these cells and suspected that MyoII-mediated actomyosin contraction might be responsible for cell alignment. To test this, we exposed embryos at the onset of cell alignment to a Rho Kinase inhibitor (Y27632), which is known to prevent MyoII activation by blocking the activation of the myosin regulatory light chain. We observed the disruption of cell alignment and order, thus implicating MyoII in the alignment of cells within the ventral epidermal sheet. Discerning the roll of actomyosin contractility in cell alignment will contribute to the further description of Drosophila embryogenesis specifically and the understanding of epithelial morphogenesis generally.

241A

Investigating the role of *crinkled* (*ck*) Myosin VIIA in the morphogenesis of actin-rich cellular projections in Drosophila melanogaster. Vinay Singh, Jody Franke, Mark Chee, Daniel P. Kiehart. Department of Biology (DCMB), Duke University, Durham, NC.

Mutations in Myosin VIIA, an unconventional myosin, cause defects in actin-rich cellular projections across species. Myosin VIIA is implicated in orchestrating the morphology of the actin cytoskeleton in mouse and human hair cells and is required for hearing. Mutations in *crinkled*, which encodes the fly Myosin VIIA, causes deafness in fly and aberrant morphogenesis of actin-rich structures such as setae, microchaetae and marochaetae. To investigate the molecular and cellular basis of *ck*/myoVIIA function we initiated a screen for *ck*/myoVIIA enhancers and suppressors based on an RNAi induced phenotype. We made a UAS-*ck*/myoVIIA-RNAi construct and used it in a preliminary, “deficiency kit” screen for interactors. We have finished screening the 200 deficiencies that collectively uncover roughly 94% and 93.5% of the 2nd and the 3rd chromosome respectively. We identified 12 *ck*/myoVIIA-interacting deficiencies on the 2nd chromosome and 9 on the 3rd chromosome. Of the 9 interacting deficiencies on the 3rd chromosome, 2 are suppressors of the *ck*/myoVIIA mutant bristle phenotype (reduced bristle length) and one is a partial suppressor, 6 are enhancers. We are currently indentifying the genes responsible for the interactions at these deficiencies.

242B

A molecular analysis of *Src64* during cellularization. Taylor C Strong, Jeffrey Thomas. Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX.

Src64 encodes a non-receptor tyrosine kinase that is homologous to human Src family kinases. *Src64* has been shown to be involved in ring canal growth, dorsal closure, salivary gland invagination, as well as cell proliferation and apoptosis. Our lab is specifically looking at the role of *Src64* in microfilament ring constriction during cellularization. The *Src64*$^{\Delta 17}$ mutation deletes the first two exons of the gene and eliminates all but trace amounts of Src64 protein, suggesting that there is an alternate transcriptional start site that is unaffected by the *Src64*$^{\Delta 17}$ mutation. This alternate transcript may be responsible for the phenotypic differences observed between *Src64* deficient flies and *Src64* $^{\Delta 17}$ flies. Semi-quantitative RT-PCR confirmed the presence of low levels of *Src64* mRNA in *Src64*$^{\Delta 17}$ embryos. Using 5’ RACE analysis of *Src64*$^{\Delta 17}$ embryos we located the alternate transcriptional start site and identified a previously unknown exon located 14,570 upstream of the start codon. This exon lies 12,113 bp downstream of the two exons deleted in *Src64* $^{\Delta 17}$. To further investigate the role of *Src64* in development we identified missense mutations in the *Src64* coding region using the TILLING method. This approach has allowed us to isolate several mutations in an unbiased manner that disrupt the SH2 and tyrosine kinase domains. We will report on the cellularization phenotype of these mutants.

243C

The STE20 kinase *misshapen* acts with the SH2/SH3 adapter *dPOSH* in patterning the rhabdomere. Rhian Walther, Franck Pichaud. MRC LMCB, University College London, London, GB.

Understanding how epithelial cells achieve and maintain polarity are key questions in cell and developmental biology. To investigate these questions, we are studying photoreceptor morphogenesis in the genetically tractable *Drosophila* compound eye. Photoreceptors are polarized epithelial neurons. The development of the apical light-gathering organelle, or rhabdomere, of the photoreceptor is dependent on the specification of apical-basal (A/B) polarity and polarized cytoskeleton assembly. Loss of the STE20 kinase *misshapen* (*msn*) in the eye results in widened, elliptical rhabdomeres[1]. In addition to its role in patterning the rhabdomere, *msn* is required for photoreceptor axon guidance[2] and embryonic dorsal closure[3]. When Msn is over-expressed in photoreceptors, the rhabdomeres are narrowed. While the SH2/SH3 adapter *dreadlock* (*dock*) is required for *msn* function in the photoreceptor axon[2], previous results indicate that *msn* does not act with dock during rhabdomere morphogenesis. We have found that in patterning the rhabdomere the SH2/SH3 adapter *dPOSH* negatively phenocopies *msn*. Similar to *msn* loss of function, over expression of dPOSH results in widened rhabdomeres. Reducing *msn* activity enhances this phenotype. Reduction of *dPOSH* by RNAi results in narrowed rhabdomeres. Rhabdomere morphogenesis is not affected by loss of *basket* (*bsk*) and *hemipterous* (*hep*), which are JNK effectors needed for Msn-dependent dorsal closure[3], but is compromised by expressing RNAi against *licorne* (*lic*), an effector of signalling through the p38 stress kinases. Our results indicate that *msn* is a key modulator of the F-actin cytoskeleton and suggest that *msn* can act in a cell/tissue specific manner by using different adaptor molecules in different developmental contexts. 1. Treisman JE, Ito, N and Rubin GM (1997) Gene 186, 119-25. 2. Ruan, W, Pang, P and Rao, Y (1999) Neuron 24, 595-605. 3. Su YC, Maurel-Zaffran C, Treisman JE, and Skolnik EY. (2000). Mol Cell Biol. 20, 4736-44.

244A

Cloning and characterization of *E(br)165*, a mutation that dominantly enhances *br*^{1}. Xiaochen Wang, Elspeth Pearce, Robert Ward. Dept Molecular Biosciences, Univ Kansas, Lawrence, KS.

The elongation and eversion of leg imaginal discs during metamorphosis is an ideal system for studying hormone-regulated morphogenesis. *Broad* (*br*) is a key ecdysone-inducible early gene for leg morphogenesis. Previously we conducted a screen for dominant modifiers of *br*^{1}, and found that signaling through the Rho1 small GTPase is necessary to direct the cell shape changes that drive morphogenesis of the adult legs. In order to understand how ecdysone might be regulating Rho signaling, we have begun to clone *Enhancer of broad* (*E(br)*) mutations that also genetically interact with *Rho1*. One such mutation, *E(br)165*, is a completely penetrant embryonic lethal that displays defects in dorsal closure and cuticle deposition. Meiotic mapping placed the mutation in 26D and complementation tests indicated that it is a new allele of *Sec61*α, which encodes the main subunit of the translocon complex for co-translational import of proteins into the ER. Sequence analysis indicated that the *E(br)165* mutation truncates the protein in the amino-terminal half of the protein, suggesting that this is a strong loss of function mutation in *Sec61*α. We are currently attempting to rescue the mutation by ectopic expression of *Sec61*α. We will present our characterization of the role of *Sec61*α in dorsal closure. In addition, we are interested in the role of *Sec61*α during imaginal disc morphogenesis. Preliminary expression studies indicate that *Sec61*α is strongly expressed in leg imaginal discs during metamorphosis, but that its expression is not regulated by *broad*. We therefore suspect that a secreted or transmembrane protein regulated by the ecdysone pathway is playing a critical role in driving imaginal disc morphogenesis.

245B

An APC2-Diaphanous complex organizes actin in the Drosophila syncytial embryo. Rebecca L. Webb, Jasper S. Weinberg, Meng-Ning Zhou, Sarah E. Clark, Brooke M. McCartney. Dept Biological Sci, Carnegie Mellon Univ, Pittsburgh, PA.

Organization and rearrangement of the cytoskeleton is essential for many cellular processes. Adenomatous polyposis coli (APC) family proteins associate with actin and microtubules and influence their behavior, however the precise molecular mechanisms involved are poorly understood. Here we report a role for APC2 and the formin Diaphanous (Dia) in organizing actin in Drosophila syncytial embryos. We examined embryos null for Drosophila APC2 and observed incomplete actin rings and lack of actin furrow extension during syncytial mitoses. To determine the mechanism by which APC2 functions to organize actin, we evaluated the role of Dia, which functions to organize actin during Drosophila cellularization and is in a complex with APC and EB1 in migrating mammalian cultured cells. Similar to APC2 mutant embryos, dia mutants have defects in actin rings and furrow extension. We found that APC2 and Dia form a complex in syncytial embryos, and that localization of APC2 to actin rings and furrows is dependent on Dia. Further, we found that reduction of dia in APC2 mutant embryos enhances the severity of APC2 actin defects. We asked whether an APC2-Dia complex is downstream of Rho1, as predicted from other studies, by examining RhoGEF2 and Rho1 mutant syncytial embryos. The actin defects in Rho1 and RhoGEF2 mutants are overlapping but distinct from those of APC2 and dia, suggesting that they are functioning in parallel pathways. Based on their functions in other systems, APC2 and Dia may influence actin directly, or indirectly through microtubules. To distinguish between these possibilities we are using Total Internal Reflection Fluorescence microscopy to assess cortical microtubules in live wild type and mutant embryos. Furthermore, we are probing the relationship between APC2, Dia and EB1 in this system.

246C

Rho family GTPases in the Drosophila larval cellular immune response. Michael Williams, Dan Hultmark. UCMP, Umea Univ, Umea, SE.

Cell shape change and migration is an essential part of the motility required for circulating immune surveillance cells to function. One family of proteins known to be involved in actin cytoskeletal rearrangement and cell migration are the Rho GTPases. When an invading organism is recognized as foreign circulating hemocytes should remove it, either by phagocytosis and/or encapsulation. This reaction can be observed when the parasitoid wasp *Leptopilina boulardi* lays its eggs in the hemocoel of second instar *Drosophila* larvae. This invasion elicits a strong cellular response. Once the wasp egg is recognized, capsule formation ensues, requiring circulating plasmatocytes to change from a non-adhesive to adhesive enabling them to bind to the invader. The plasmatocytes attach and spread around the chorion of the wasp egg. After the cells have spread they form cellular junctions, thus separating the egg from the hemocoel. The last phases of capsule formation include lamellocyte adherence and melanization. From these encapsulation events it is obvious that adhesion and cell shape change are essential parts of the cellular response against parasitoid wasp eggs. Recently we have discovered that the Rho family members Rac1, Rac2, and Cdc42 are all necessary for a proper encapsulation response. Parasitized larvae mutant for any of these genes fail to properly encapsulate the wasp egg. In *Rac1* mutants hemocytes do not adhere well to the wasp egg. Hemocytes lacking Rac2 adhere well but fail to spread around the egg. While parasitized *Cdc42* mutant larvae properly encapsulate, but fail to melanize the wasp egg. Furthermore, both Rac2 and Cdc42 are required for filopodia formation in hemocytes, while Rac2 and Rac1 are involved in the formation of lamellipodia. Finally, Rac1 activation in hemocytes induces the activation of Rho1 via Basket. These data suggest that the Rho family GTPases are central regulators of the *Drosophila* cellular immune response.

247A

Reciprocal regulation of Rho GTPase and Crumbs in epithelial polarity and contraction. Na Xu, Benison Keung, Monn Myat. Dept Cell & Developmental Biol, Weill Medical Col of Cornell, New York, NY.

Rho GTPase is a small GTPase known to play multiple roles in epithelial morphogenesis. To understand how Rho regulates epithelial adhesion, polarity and cell movements, we have investigated its role in early development of the Drosophila embryonic salivary gland. The salivary gland is a pair of elongated epithelial tubes derived from the ventral ectoderm. In mid-embryogenesis, salivary gland cells invaginate from the ventral surface through a series of coordinated cell shape changes. Here, we report that Rho is required for proper invagination of salivary gland cells. In Rho mutant embryos, columnar epithelial morphology is lost, the actin cytoskeleton is disorganized and gland cells fail to invaginate. Although apical-basal polarity is established in Rho mutant cells during early stage of embryogenesis, polarity is not maintained and degenerates progressively. The earliest defect observed in Rho mutant cells is the loss of the apical determinant, Crumbs, from the apical-lateral membrane. In loss and gain-of-function studies, we provide evidence that Rho is required for the trafficking of Crumbs to the apical-lateral membrane and that Crumbs is required for cellular events downstream of Rho. Our studies provide the first link between Rho GTPase and Crumbs in maintenance of apical polarity and generation of cellular contraction during epithelial morphogenesis.

248B

Cloning and characterization of a dominant modifier of *broad*. Liang Zhang, Stefani Fontana, Ty Beaver, Kistie Patch, Robert Ward. Dept Molecular Biosciences, Univ Kansas, Lawrence, KS.

Our lab is interested in understanding the mechanisms of hormone dependent morphogenesis and we have been using leg eversion during metamorphosis as a model. In a screen for dominant modifiers of the malformed leg phenotype associated with br^{1}, we identified mutations in at least 15 different genes. One of these mutations, *Enhancer of broad 155* (*E(br)155*), shows complete embryonic lethality when homozygous, with poorly-differentiated cuticle and obvious defects in mid-embryonic morphogenesis. Specifically, 50% of the dead embryos have defects in dorsal closure, whereas 10% show defects in head involution. *E(br)155* shows strong genetic interaction with br^{1} and *Rho1*. We initially mapped the enhancing mutation to 30A-F. Subsequent P-element based meiotic mapping of the lethal mutation indicated that the original mutation contained two closely mapped mutations, one in 26B and the other in 27D. After recombining the mutations apart we discovered that both mutations are required for the embryonic phenotypes and for the interactions with br^{1} and *Rho1*. We recently conducted an F_2 mutagenesis screen and recovered 7 new alleles of the mutation in 26B and 3 new alleles of the mutation in 27D. We will present our characterization of these new alleles and our efforts in cloning the genes responsible for these mutations.

249C

The transcription factor Broad mediates hormone regulated cell shape change during *Drosophila* pupariation. Xiaofeng Zhou, Xiaoqun Zeng, Lynn M. Riddiford. Department of Biology, University of Washington, Seattle, WA.

Epithelial cell shape changes and cell rearrangement drive the transition of body shape. Pupariation is the first phase of body shape change during *Drosophila* metamorphosis. At this time, a fully developed larva shortens its body length by contracting muscles, and then constricts its epidermal cells to form a smooth barrel-shape puparium. Ecdysone triggers pupariation, but it is unclear how such signal interfaces with cytoskeletal components to control cell shape changes. Broad is an ecdysone-induced transcription factor that appears at the time of metamorphosis and is critical for specifying pupal development. The npr^3 null mutant of *broad* cannot form a normal smooth puparium due to the failure of epidermal cell constriction. F-actin is involved in cell shape changes, and phalloidin staining showed that cells driving *broad* RNAi contained much lower levels of F-actin than did normal cells at the time of pupariation. Moreover, *actin5C* mRNA in npr^3 mutants was about one-third the level in wild type larvae at this time. When *Rho1* RNAi was expressed in those cells, low levels of F-actin were also observed and smoothening of the puparium did not occur normally. Thus, Broad apparently is directing cell shape changes via the Rho pathway. Supported by NIH R01-GM60122.

250A

Car/Vps33A is necessary for SNARE-mediated trafficking to Lysosomes and Lysosome-related organelles (LROs). Mohammed Akbar, Sanchali Ray, Helmut Kramer. Ctr Basic Neuroscience, UT Southwestern Med Ctr, Dallas, TX.

Sec1/Munc18 (SM) proteins play a pivotal role in SNARE-mediated intracellular membrane fusion. In yeast the SM protein Vps33p, a component of the Vps-C/HOPS complex, is required for vacuolar biogenesis. In metazoa, two different HOPS-like complexes with distinct vps33 homologs have been implicated in trafficking to lysosomes or lysosome-related organelles (LROs). Hypomorphic alleles of vps33A cause pigmentation defects in *drosophila* and mouse whereas mutations in human vps33B cause a lethal multisystem disorder (ARC syndrome) suggesting that the two Vps33 homologues have distinct functions in trafficking to lysosomes (vps33B) and to LROs (vps33A). To test this hypothesis we generated a car null allele, $car^{\Delta 146}$. Larvae homozygous for $car^{\Delta 146}$ die during the second instar. car null cells in eye clones survived to adulthood but lacked almost all pigmentation consistent with a role of Car in trafficking to pigment granules in the eye. Furthermore $car^{\Delta 146}$ cells accumulated autophagosomes. This points to Car's requirement in the fusion of autophagosomes to lysosomes. Cells mutant for $car^{\Delta 146}$have no defects in the internalization of ligands (i.e., Boss, Delta). These ligands are, however, not delivered to lysosomes and instead accumulate in endosomes labeled by the late endosomal markers rab7 and gp150, but also partially positive for the early endosomal markers avl and hrs. Colocalization with rab11 (recycling endosomes) and lvl (Golgi) was negligible. Surprisingly, in the car null cells Rab7-positive vesicles were relocated from a cell-wide distribution to the sub-apical region but they remained distinct from close-by Rab5-positive structures. Interestingly, in car null mutants we found some Notch accumulation in endosomes but no signs of elevated Notch signaling as was observed in cells mutant for the early endosomal syntaxin Avalanche or the ESCRT proteins dVps25 or dVps23. Taken together, our results corroborate that Car/Vps33A function is necessary not only for trafficking to pigment granules but also for the fusion of late endosomes and autophagosomes to lysosomes.

251B

Characterization of the Apical Microtubule Association of Klarsicht in the Developing *Drosophila* Eye Disc. Susan Banks, Janice Fischer. ICMB, Univ Texas, Austin, Austin, TX.

The *Drosophila* eye is an ideal system for studying nuclear migration. Cell nuclei rise apically posterior to the morphogenetic furrow coordinately with recruitment into the ommatidium. As photoreceptors differentiate, the cytoskeleton reorganizes and a microtubule organizing center (MTOC) forms apically. The Klarsicht (Klar) protein is required to connect photoreceptor nuclei to the MTOC. The C-terminal KASH domain of Klar embeds in the outer nuclear envelope through indirect interactions with nuclear lamin. The N-terminus connects to microtubules, probably through dynein. In *klar* mutants, photoreceptor nuclei lose their connection to the apical MTOC, and some of them fall beneath the fenestrated membrane into the axons. Klar contains a KASH domain and an LD domain. The LD domain is expressed during embryogenesis and is necessary for proper lipid droplet migration. The LD domain is present only in an isoform of Klar (Klarβ), but is not present in another isoform of Klar (Klarα), which is required for proper nuclear migration in the developing eye disc. The forces generated during lipid droplet movement in the developing embryo suggest Klarβ associates with microtubules not only through dynein, but kinesin as well. Therefore, Klarα must have a domain in its N-terminus to bind dynein as well as other motors and regulatory proteins critical for apical microtubule association. The goal of this project is to determine what portion of Klar is required for association with apical microtubules and to identify the proteins through which this association occurs. The results may yield information regarding a protein binding domain in Klar as well as further information on proteins required for motor regulation during development. First, the minimal region necessary for apical microtubule association will be identified through expression of a series of deletion constructs. Then, the function of the region identified will be tested in a rescue assay. I will use the region as bait in a yeast 2-hybrid assay to identify proteins that bind to that region. Progress will be reported.

252C

The δ isoform of the transport regulator Klar plays a role in nuclear positioning within the eye disk and the ovary. Sean Cotton[1], Dae-Hwan Kim[1], Amanda Norvell[2], Michael Welte[1]. 1) Dept Biol, Brandeis Univ, Waltham, MA; 2) Dept Biol, The College of New Jersey, Ewing, NJ.

In Drosophila eye imaginal disks, nuclei undergo two stereotyped, motor-driven movements: a basal plunge at the morphogenetic furrow followed by an apical migration. The motor regulator Klarsicht plays a key role: eye disks express three Klar isoforms, α, γ, and δ. These isoforms share a common C-terminus—the KASH domain—which targets them to the nuclear envelope. When Klar α is disrupted, nuclei fail to migrate to the correct apical position. To address the role of Klar δ in the eye disk we generated a null allele. Eye disks from the Klar δ null are indistinguishable from the wild type; however, δ overexpression causes a basal displacement of nuclei. This effect is δ-specific since overexpression of either Klar α or γ does not cause a similar defect. We propose that Klar δ may have an ancillary role in the proper migration and anchorage of photoreceptor nuclei. Klar δ overexpression causes nuclear displacement in at least one other tissue: the oocyte. Although the initial migration of the nucleus seems unaffected, a significant number of late-stage oocytes from females overexpressing Klar δ have nuclei displaced from their stereotypic dorsal anterior corner. In contrast, overexpression of either Klar α or γ have no effect. Overexpressing Klar δ might provide a means to specifically disrupt the position of the nucleus without interfering with other important processes. Maternal overexpression of Klar δ also disrupts dorsal-ventral patterning of the eggshell; dorsal appendages are often fused, reduced in number, shifted posteriorly, or completely absent. Because similar defects arise when Grk signaling is disrupted, we examined Grk expression and found reduced levels and altered localization of Grk RNA and protein. We are currently determining whether the eggshell defects are a consequence of nuclear displacement or if the two phenotypes can be uncoupled. In the latter case, Klar δ may allow us to manipulate additional transport processes.

253A

***Hook-like* is a regulator of endocytic trafficking and pigment granule function in *Drosophila*.** Adam Haberman, Helmut Krämer. Cntr Basic Neurosci, UT Southwestern Med Cntr, Dallas, TX.

The trafficking of ligands and receptors to lysosomes and their subsequent degradation is an important aspect of their regulation. Many of the proteins involved in delivering proteins to lysosomes also participate in the biogenesis and regulation of lysosome related organelles (LROs), such as melanocytes and platelet dense granules. We performed an F1 genetic screen to identify mutants that affect trafficking to both lysosomes and to pigment granules, an eye specific LRO, in whole-eye mitotic clones. We identified 32 complementation groups that caused both a reduction of eye color and a defect in the trafficking of Boss protein to lysosomes. One of these complementation groups, which we have called *hook-like* (*hkl*), causes a mild reduction of eye color and perturbs trafficking of Boss to lysosomes. Surprisingly, hkl is homologous to Acinus, a mammalian protein involved in chromatin condensation and fragmentation during apoptosis. Acinus is activated by two cleavages that release a 106 amino acid domain, p17, which displays the highest similarity to Hkl (60% identical and 80% similar). We are investigating the possible role of *hkl* in chromatin destruction during apoptosis and the mechanism of its role in trafficking to lysosomes and pigment granules.

254B

***In Vivo* engineering of *klarsicht*: A tool kit to dissect a complex gene.** Ankit Jain, Michael Welte. Department of Biology, Brandeis University, Waltham, MA.

The transport regulator, Klar, is crucial for trafficking many intracellular organelles during *Drosophila* development e.g. lipid droplets, photoreceptor nuclei, secretory vesicles, and possibly many more. The *klarsicht* locus is large (106 kbp) and complex; there are several promoters and poly-A sites dispersed within the gene, and many alternate splicing events. None of the characterized lesions on *klar* disrupt all known Klar isoforms, and there is growing evidence of additional undiscovered isoforms. In order to generate new lesions that target specific regions of *klar*, we engineered this complex gene by adapting a technique previously used to make large, multigenic deletions. We deleted specific genomic regions from within *klar* by inducing recombination between FRT sites, which are carried on PiggyBac transposon insertions from the Exelixis collection.

This strategy has allowed us to overcome two problems in the analysis of *klar*. First, to determine the consequence of a complete lack of all Klar isoforms, we deleted the entire *klar* locus. These *klar* null animals are semi-viable and exhibit new phenotypes not seen in other *klar* alleles, most prominently an egg-laying defect. Second, to study how the N-terminal domains contribute to Klar's function, we deleted specific N-terminal regions of Klar while retaining the C-termini essential for targeting the protein to the correct intracellular location. Unlike a traditional structure-function analysis in which deletion constructs are expressed via transgenes, our approach is expected to preserve the endogenous regulation of *klar*.

In an alternative approach, we attempted to delete an internal exon of *klar* by screening for the loss of a *white*-bearing transposable element after X-ray mutagenesis. The resulting alleles have an intriguing *trans*-suppression property: They silence expression of a *white* transgene on the homologous chromosome, and this silencing depends on the position of the *white* transgene.

255C

Apical nuclear migration in the *D. melanogaster* 3rd instar eye imaginal disc requires the KASH protein Klarsicht and the SUN protein Klaroid. M. Kracklauer, S. Banks, J. Fischer. Dept MCD Biol, University of Texas at Austin, Austin, TX.

The compound eye of D. melanogaster develops from a monolayer epithelium called the eye disc. Towards the end of the third larval instar, a wave of apically constricted cells called the morphogenetic furrow moves across the eye disc, leaving differentiating cells in its wake. As cell fates become determined, nuclei rise apically, starting with the photoreceptor (R-) cells and followed by the non-neural cone cells. Apical nuclear migrations have been shown to depend on Klarsicht (Klar), a KASH protein. Mutants removing the KASH domain result in failure of R- and cone cell nuclei to migrate apically. The C-terminal KASH domain is ~60 amino acids in length and has one transmembrane (TM) domain. Studies in C. elegans and mammals suggest that KASH domain proteins reside in the outer nuclear membrane (ONM), remaining anchored there through interactions in the intermembrane space with SUN domain proteins in the inner nuclear membrane (INM). The D. melanogaster genome encodes two SUN domain proteins. We generated mutant stocks of both SUN genes using ends-out homologous recombination. Here, we describe the knockout phenotype of CG18584. Homozygous knockout individuals have adult eye and larval eye disc phenotypes indistinguishable from klar mutants. Based on these phenotypes, we call CG18584 klaroid (koi). Our studies suggest that Koi is found in the INM in all cells in the eye disc, and is retained there through interactions with the nuclear lamina protein Lam(Dm0). Klar in turn is found in the ONM of all determined cells in the eye disc. In koi mutants, ONM localization of Klar is abolished, with Klar localizing mostly to apical microtubules. In agreement with predictions from C. elegans studies, the klar, koi, and klar/koi double mutant nuclear migration phenotypes are indistinguishable from one another. Ongoing research is directed towards understanding the Klar/Koi interaction and determining the domain(s) of Koi that confer(s) INM localization.

256A

The endocytic regulator dRabenosyn is a novel neoplastic tumor suppressor. Holly A. Morrison, Heather Dionne, David Bilder. Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Correct localization of polarity regulators is essential for establishing and maintaining the organization of epithelial cells. Apically localized transmembrane regulators require not only targeted exocytic transport, but also endocytic removal to control their cell surface levels. The machinery that controls this removal in epithelia has been in part identified by recent genetic screens, which have uncovered a surprising link between systematic endocytosis of polarity regulators and the control of cell proliferation. Here we identify dRabenosyn, the *Drosophila* homolog of the human endocytic regulator Rabenosyn-5, as a novel tumor suppressor. Loss of dRabenosyn results in neoplastic transformation, characterized by the loss of apicobasal polarity and tissue overgrowth. dRabenosyn localizes to Rab5-positive endosomes, and mutant tissues accumulate the endocytic cargoes Notch and Crb at the plasma membrane. In mammalian cells, Rabenosyn-5 acts at the interface of the endocytic and recycling pathways via differential interactions with membrane trafficking machinery such as the Rabs and SNARE proteins. We will present data addressing the functions of dRabenosyn not only in the endocytic pathway but also in the recycling pathway.

257B

Distinct functions for the Kinesin-1 tail in oogenesis and axonal transport. Pangkong Moua, Debra J. Rose, Katherine M. Brendza, Rahul Warrior, William M. Saxton. Biology, Indiana University, Bloomington, IN.

Intracellular transport depends on complex interactions between motor proteins and their various cargoes. The long term goal in our lab is to understand the molecular mechanisms behind these interactions and how they contribute to cellular processes, normal development, and disease. Using Drosophila as a model system, we have focused on fast organelle transport in neurons, and on key transport processes in oogenesis. Kinesin-1 moves cargoes toward the plus ends of microtubules. It is commonly described as a heterotetramer composed of two identical heavy chains (khc) and two light chains (klc). Khc has a highly conserved N-terminal motor domain that binds microtubules, hydrolyzes ATP, and generates motion. A semi-conserved coiled-coil forming stalk contributes to Khc dimerization, Klc binding, and cargo binding. A globular “tail” at the C-terminus has a small conserved region that has been implicated in coordinating activation of the motor domain and cargo binding. We have recovered a large series of recessive lethal Khc alleles and ranked their severities by intra-allelic complementation. Most zygotic combinations cause posterior paralysis, or “tail flipping”, and axonal swellings with organelle accumulations. The severities of these classic axonal transport phenotypes parallel those determined by lethality analysis. However, tests for functions in oogenesis show a marked departure for one set of alleles. Missense changes in the Khc tail region that cause relatively strong larval paralysis and lethality have little or no effect on cytoplasmic streaming. Alleles of similar severity in the motor domain allow almost no streaming. This suggests unique molecular mechanisms of kinesin-1-cargo linkage and motor domain regulation during axonal transport in neurons and streaming in oocytes. One important implication is that while cargo-mediated motor regulation is critical in axonal transport, it is not important in oogenesis.

258C

Regulation of apical membrane architecture via Rab11-dependent intracellular trafficking. Jeremiah Roeth, Danny Willner, Mark Peifer. Department of Biology, UNC-CH, Chapel Hill, NC.

During development, epithelial cells are held together by cell-cell adherens junctions (AJs) that aid in the organization of an apical belt of actin filaments. AJs and the cytoskeleton must maintain a high level of plasticity to permit dramatic cell shape changes during morphogenesis and cell division. One mechanism to adjust adhesion is the regulation of surface levels of E-Cadherin. Rab11 is a small GTPase that regulates vesicle transport at the recycling endosome, facilitating both the recycling of internalized membrane proteins and transport from the secretory pathway. Studies in cultured mammalian cells suggest that intracellular trafficking of proteins such as E-Cadherin through the apical recycling compartment is critical for the proper maintenance of polarized cell architecture; however, the role of this compartment during epithelial morphogenesis is not well understood.

We hypothesize that the regulated trafficking of apical proteins is a mechanism to rapidly and reversibly modulate epithelial cell architecture and adhesion. Thus, we have perturbed Rab11 function and assessed the effects on epithelial morphogenesis. We have expressed a dominant negative Rab11 mutant (Rab11DN) in specific regions during embryogenesis and examined the consequences. Rab11DN expression resulted in the loss of Crumbs (Crb) from the apical region of epithelial cells, followed by the breakdown of apical AJs. Similarly, homozygosity for an allele of *rab11* (*rab11*^{j2D1}) induced loss of Crb and fragmented AJs in regions of the ventral epidermis. In addition, the amnioserosal cells were severely disrupted, often leading to a failure in germband retraction. Loss of Crb and AJs also correlated with increased apical actin polymerization, suggesting the coupling of adhesion and regulation of actin dynamics. We are currently investigating how Rab11-dependent trafficking of apical proteins is regulated, and how this process affects the apical cytoskeleton.

259A

Genetic Screens for Axonal and Dendritic Interactors of Kinesin Heavy Chain. Kristina Schimmelpfeng, Cheryl Herrera, Meike Roux, Lawrence S.B. Goldstein. Dept Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA 92093-0683.

Eukaryotic cells maintain order and function using motor proteins to transport molecules and organelles along cytoskeletal tracks. This task is particularly challenging in neurons since long distances between the site of protein synthesis, the cell body, and the site of protein interaction, the neurite terminals, have to be traveled in order to maintain the distinct functional domains that characterize the highly polarized neuronal architecture. Anterograde transport is mediated by members of the Kinesin motor protein family. In larval axons of heterozygote *kinesin heavy chain* (*khc*) mutants we frequently see a moderate amount of accumulations of synaptic markers such as cystein string protein, which is strongly enhanced in homozygous *khc* mutants. A dendritic marker, hTfR-GFP, localizes to cellbodies and dendrites in heterozygote *khc* mutants whereas we observe a mislocalization to axons in homozygous *khc* mutants. We took advantage of these phenotypes and performed screens to identify Khc interactors in axons - through enhancement of the clogging phenotype and in dendrites, through scoring mislocalization into axons. The characterization of some of the enhancers is currently being done.

260B

Determining the function of the intracellular domain of the cell adhesion molecule Echinoid. Grant W Simmons, Susan Spencer. Biology, Saint Louis University, Saint Louis, Missouri.

Echinoid (Ed) is an Ig-superfamily cell adhesion molecule important for regulating EGFR and Notch signaling during neurogenesis in the developing retina in *Drosophila* (Bai et al., 2001; Spencer and Cagan, 2003; Rawlins et al., 2003). Ed acts similar to DE-cadherin by mediating cell adhesion at adherens junctions and has been suggested to regulate endocytosis of cell surface proteins (Wei et al., 2005; Rawlins et al., 2003; Spencer and Cagan, 2003). Ed's extracellular domain contains 7 Ig repeats and one fibronectin-3 repeat, but its intracellular domain has no discernable domains except for a PDZ binding domain at its C-terminus. Ed's intracellular domain has been shown to be important. In the wing disc, overexpression of an *ed* construct lacking the intracellular domain resulted in extra wing vein material at the distal end of wing vein II and notching of the distal wing tip. This phenotype was similar to those observed with transheterozygous combinations of *ed* mutant alleles (Ahmed et al., 2003). Also, Ed's intracellular domain is phosphorylated in response to EGFR signaling (Spencer and Cagan, 2003). To further explore the role of Ed's intracellular domain, we have performed two types of yeast two-hybrids using the intracellular domain of Ed. We will show the results of these screens and discuss the implications of the binding proteins for Ed's function.

261C

Partners and dependencies in the modulation of surface protein level by β[Heavy]-spectrin. Graham Thomas, Mansi Khanna, Janice Williams, Elizabeth Klipfell, Krystal Sandilos. Depts Biol & BMB, Penn State Univ, University Park, PA.

The creation of an apicobasal axis is essential for the formation of an epithelium. During polarization, the subapical complex (SAC) induces the formation of the apical domain and organizes the *zonula adherens* (ZA) at the apical-lateral boundary. However, the stabilization of the ZA and the subsequent elaboration of the apical domain are still not well understood. Crumbs and the cytoskeletal scaffolding protein β[Heavy]-spectrin (βH) are part of a group of proteins involved in these downstream steps. Crumbs is required to establish apical polarity *and* the ZA, whereas βH is recruited by Crumbs and is required for ZA integrity in some tissues, but not for polarity. Together these proteins regulate apical membrane area in epithelia.

We have shown that expression of the C-terminal segment 33 of βH, (βH33) results in membrane expansion, probably due to sequestration of Dynamin. We interpret this dominant phenotype to indicate that the βH33 interacts with the endocytic machinery and thus normally acts as a 'tool' with which the Crumbs/βH complex modulates membrane area. We are testing this hypothesis in two ways. First, we are examining the dependence of surface protein levels on the presence of the βH membrane skeleton. Second, we are defining protein-interaction in the βH33 region. Current results will be reported, including an update on the interaction between βH and Annexin B9 (Anx B9), which was identified in the Curagen 2-hybrid screen.

βH33-induced membrane expansions have a distinctive morphology wherein the membrane self-associates *via* its inner leaflet. This 'adhesion' is characteristic of some annexins and we have shown that Anx B9 accumulates in βH33-induced membrane extensions. We have also made an inducible RNAi construct that efficiently knocks down Anx B9 protein and eliminates this self-association. Vertebrate Annexins have widespread roles in the endomembrane system and so we propose the existence of a Crumbs - βH - Anx B9 pathway in modulating membrane area and the ZA.

262A

Plus-end Transport of Lipid Droplets in the *Drosophila* Embryo is Driven by Kinesin I and Influenced by Levels of Halo and Dynein. Susan Tran, Michael Welte. Dept Biol, Brandeis Univ, Waltham, MA.

Many cargoes and organelles move bidirectionally along microtubules, yet net displacement still occurs. It is well established that motors, such as dynein and kinesins, drive intracellular transport, but little is known about how directionality is determined. In our model system, lipid droplets (LDs) in the early *Drosophila* embryo undergo a global switch in distribution during cellularization; LDs go from a uniform distribution to a net inward relocalization towards the plus ends of microtubule tracks. A crucial factor required for this switch is the novel protein Halo. Its overexpression enhances, reduction decreases, and complete removal abolishes LD plus-end transport. Halo is, thus, a limiting factor specifically required for upregulating plus-end transport.

To better understand how Halo functions as a transacting factor that modifies the transport machinery, we sought to identify its downstream targets and discovered that Kinesin I is the plus-end motor. Embryos that lack Kinesin I do not undergo LD plus-end transport, and two hypomorphic *Khc* (*Kinesin heavy chain*) alleles show a dominant negative effect in reduced transport. In addition, Kinesin I is immediately and acutely inhibited upon injection of an antibody against Khc in wildtype embryos, suggesting that Kinesin I physically interacts with LDs. Surprisingly, reducing or increasing Khc causes unexpected effects; Khc reduction induces hyper-plus-end transport (similar to Halo overexpression), while Khc overexpression reduces plus-end transport (similar to Halo reduction). When both Kinesin I and Halo are simultaneously reduced, plus-end transport is once again normal. Intriguingly, decreasing the opposite motor, Dynein, reduces rather than enhances plus-end transport; this effect is rescued by overexpressing Halo. These observations suggest that stochiometry is important and implicate a dosage effect where relative levels of Halo and motors influence LD directionality.

263B

A genetic screen for regulators of Amyloid Precursor Protein in axonal transport. Carole Weaver, Kristina Schimmelpfeng, Cheryl Herrera, Lawrence S.B. Goldstein. Department of Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA.

Mutations in Amyloid Precursor Protein (APP) are one of the few known causes of Alzheimer's disease, yet the normal cellular functions of APP remain unclear. One important function of this protein was revealed by the discovery that APP joins a complex with the microtubule motor protein kinesin-I, suggesting that APP, a type I transmembrane protein, could link kinesin to vesicular cargo during axonal transport. APP has since been shown to be required for axonal transport in vivo, and alterations in APP and kinesin-I function lead to Alzheimer's-like phenotypes in the mouse brain, including axonal swellings, amyloid plaque deposition, and neurodegeneration. Therefore, further characterization of APP function in transport should reveal important information about how defects in APP lead to neurodegeneration and disease. We are conducting a genetic screen in Drosophila melanogaster to identify genes that are involved in the regulation of APP axonal transport. Gain- or loss-of-function of the Drosophila homologue APPL, as well as overexpression of human APP, in neurons of the fly larva causes locomotor defects and organelle accumulations within axons, phenotypes that are characteristic of transport mutations in the fly. We have screened chromosomal deficiencies (covering about 75 percent of the Drosophila genome) for loci that enhance the phenotypes caused by panneuronal overexpression of human APP, and have identified 16 chromosomal regions containing strong enhancers. Characterization of these loci is currently underway.

264C

Exploring a potential function of *Drosophila* Msp-300 in Nuclear Positioning. Xuanhua Xie, Janice Fischer. 2500 Speedway, MBB1 312, Austin, TX.

KASH domain-containing proteins function in nuclear positioning by attaching nuclear with their C-terminal KASH domain to cytoskeleton. Msp-300 is one of the two KASH proteins in *Drosophila* that is believed to play a role for nuclear anchorage in cytoplasmic dumping during oogenesis. To investigate a potential role of a KASH form Msp-300 during eye development, we prepared an msp-300-KASH knock-out fly using homologous recombination method. Surprisingly, the knock-out fly doesn't show any defect in photoreceptor or cone cell nuclei migration although RT-PCR and in situ immunostaining results indicate expression of a KASH form Msp-300 in eye discs from third instar larva. Similar results were observed when we homozygosed msp-300^{SZ75} allele in eye discs with GMR-hid method. Further analysis demonstrated that Msp-300-KASH domain is dispensible during oogenesis although immunostaining result implies requirement of a full-length KASH form Msp-300 for oocyte-nuclear positioning. After cleaning up the msp-300^{SZ75} allele, we found out that this stop-codon allele is not homozygous lethal, which is claimed in published work. And the duplication Dp19 cannot rescue the dumpless phenotype of x msp-300^{SZ75} allele. Combined with other two partial msp-300 deletions, we found out that the peri-nuclear signals in oocytes are gone in all kinds of combinations of msp-300$^{KASH-KO}$, msp-300^{SZ75}, Df8674 and Df7497, but no dumpless phenotype was observed. Signal in apical area of eye disc is gone in msp-300$^{KASH-KO}$ homozygotes, but not in msp-300^{SZ75} homozygotes, which indicate an alternative KASH form Msp-300 is expressed in eye discs. It is likely that Msp-300 plays a redundant role in nuclear positioning during oogenesis and eye development.

265A

Identification of protein interactors of Dribble— a single KH domain nucleolar protein in Drosophila. Ching-Gee Choi[1,2], H. Y. Edwin Chan[1,2,3]. 1) Laboratory of Drosophila Research; 2) Molecular Biotechnology Programme; 3) Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, China.

Ribosome biogenesis starts with the synthesis of rRNA precursors (pre-rRNAs), which undergo a series of processing steps including base modifications and ribonucleolytic cleavages to generate mature rRNA— a component of functional ribosomal subunits. Maturation of pre-rRNAs depends on various processing factors including ribosomal and non-ribosomal proteins. Dribble (DBE) is a Drosophila non-ribosomal nucleolar protein which contains a RNA binding motif— K homology (KH) domain. The involvement of DBE in pre-rRNA processing had been demonstrated by the pre-rRNA cleavage defect in its mutants. Since only a RNA binding domain but no other functional domain is predicted in DBE, the mechanistic role of DBE in pre-rRNA cleavage is speculated to act through other proteins like endo- or exonuclease. The objective of this study is to identify proteins that associate with DBE by affinity pull-down experiments. Through mass spectrometric identification of proteins that bound specifically to DBE, the interaction profile of DBE will be established and provide crucial hints on the functional role of DBE in ribosome biogenesis.

266B

Polyhomeotic functions in the maintenance of epithelial integrity in the Drosophila melanogaster wing and ovarian follicle. Pierre Gandille[1], Karine Narbonne-Reveau[2], Elisabeth Boissonneau[1], Denise Busson[3], Anne-Marie Pret[1]. 1) CGM-CNRS-UPR 2167, 91 198 Gif-sur-Yvette, France; 2) Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, USA; 3) Institut Jacques Monod (UMR 7592), Laboratoire de Génétique du Développement et Evolution, 75 251 Paris Cedex 05, France.

The development of multicellular organisms and homeostasis in the adult requires organization of cells into layers or epithelia. In this process, cell-cell adhesion is mediated by formation of specialized junctions, in particular adherens junctions composed of E-cadherin, which is localized at the cell membrane and forms direct homophilic bonds, and the intracellular α- and β-catenins. In the course of a screen to identify genes implicated in follicular epithelium formation in *Drosophila* ovaries, we isolated mutations in *polyhomeotic* (*ph*), a member of the Polycomb group (Pc-G) genes. Pc-G proteins are required for the maintenance of a repressed state of target gene transcription (notably that of homeotic genes during development). We conducted clonal analysis of homozygous amorphic mutations for *ph*, using the FLP/FRT system, in both the follicular and wing disc epithelia. We found that cell clones mutant for *ph* function induced in both systems reduce contacts with neighboring wild-type cells and are expulsed from the epithelium. Expulsion is accompanied by specific reorganisation of adherens junctions. Moreover, in the wing disc, transcription of *shotgun* encoding DE-Cadherin is compromised in ph mutant cells. In addition, upregulation of Talin, a protein known for its role in linking integrins to the actin cytoskeleton, is also observed in wing disc cells mutant for ph. Interestingly, a recent study demonstrates a novel integrins-independent role for Talin as a transcriptional repressor of *shotgun*. Therefore, we hypothesize that, in a wild-type context, Ph acts as a repressor of *talin* and allows higher expression of *shotgun*.

267C

Analysis of proteins required for CDM-meditated myoblast fusion in *Drosophila*. Erika R Geisbrecht[1], Mei-Hui Chen[1], Lakshmi Balagopalan[2], Susan M Abmayr[1]. 1) Stowers Institute, Kansas City, MO; 2) NIH, Bethesda, MD.

Myoblast city (mbc) is a member of the CDM (CED-5, DOCK 180, Myoblast city) superfamily required for myoblast fusion in *Drosophila*. These proteins are characterized by an N-terminal SH3 domain, two internal dock homology (DHR1 and DHR2, or Docker) domains, and a C-terminal proline-rich region. We have found that the adapter protein Crk binds to the proline-rich sites in MBC, but surprisingly is not required in the CDM pathway for myoblast fusion as mutations which abolish Crk/MBC binding are still able to rescue the *mbc* fusion defect. In contrast, the SH3 and Docker domains are required as mutations that disrupt conserved amino acids abrogate *mbc* function.

To identify proteins that interact with MBC in myoblast fusion, two independent methods were utilized: 1) the SH3 domain of MBC was used in a yeast-2-hybrid screen and 2) tagged MBC expressed in the embryonic musculature was immunoprecipitated and the associated proteins identified my mass spectroscopy. Both techniques revealed interaction with CED-12/ELMO, an ~82 kD protein with both pleckstrin homology (PH) and proline-rich domains in the C-terminus. In *C. elegans* and vertebrate studies, a ternary CED-12/MBC/Rac complex is required for cell migration and apoptotic cell clearance. Using mutants isolated from an EMS screen, we are characterizing the role of *ced-12* in *Drosophila* embryonic muscle development, including it's role in the CDM pathway.

268A

Identifying Interactors of Invadolysin - a novel metalloprotease required for mitosis and migration. Shubha Gururaja Rao, Bryce Nelson, Margarete Heck. Queen's Medical Research Institute, Centre for Cardiovascular Science, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK.

The regulation of the cell cycle is mediated by networks of phosphorylations and ubiquitin-directed proteolysis. Invadolysin is a novel zinc metalloprotease whose function is essential for mitosis. It has homology to Leishmanolysin/GP63, a metalloprotease of the M8 family conserved in all higher eukaryotes. The original Drosophila allele, *l(3)IX-14* was generated in a chemical mutagenesis screen using ICR-170 (Shearn et al. 1971), while the gene was cloned by inverse PCR of a P-element transposon allele generated in our laboratory. Early characterization of the phenotype showed a mitotic defect (Gatti and Baker 1989). The important phenotypes of the mutation include metaphase delay, chromosome condensation defects, abnormal spindle morphology, and increased levels of nuclear envelope proteins. Invadolysin has a cytoplasmic localization in the form of unusual ring-like structures (resembling invadopodia, hence the name Invadolysin) in human cells and localizes to the leading edge of migrating macrophages (McHugh et al. 2004). In this study, we also showed that Invadolysin was important for germ cell migration. We have carried out a second site non-complementation screen with the Drosdel deficiency collection in order to identify genetic interactors of *.invadolysin*. The results of this interaction screen will be presented.

269B

Genetic analysis of an aseptic wound response pathway in epidermis. Michelle T. Juarez, Joesph C. Pearson, William J. McGinnis. Biological Sciences, UC San Diego, La Jolla, CA.

As some chordate and arthropod animals moved from aquatic to terrestrial environments 400 million years ago, they required the development of an impermeable barrier to protect the organism and maintain homeostasis of internal fluids. The contrasts between the monolayer of cuticle-secreting epidermis found in *Drosophila* and the multilayer keratinized epidermal tissue found in mammals suggested independent evolution of the two protective barriers. However, both mammal and arthropod epidermal barriers depend on the cross-linking of proteins and lipids to form an impermeable barrier, and recent evidence suggests that the development and repair of this protective barrier from epidermal cells evolved using a similar genetic system in both animal systems. Recent work in *Drosophila* showed that the transcriptional activation of the cross-linking enzyme genes *Dopa decarboxylase (Ddc)* and *tyrosine hydroxylase (pale)*, associated with an embryonic aseptic wound in the epidermal barrier depends on the function of the Grainy head (Grh) transcription factor. Similar research on a mouse Grh ortholog indicates it is also required for mammalian epidermal barrier function and regulation of cross-linking enzymes. We have fused wound response enhancers (WRE) from a variety of *Drosophila* genes activated by aseptic epidermal wounds to reporter genes and are using these constructs to screen *Drosophila* deficiency lines. The targeted FRT mediated deficiencies from *Exelixis* provide mapped endpoints, smaller (~140kb) deletions, and currently delete ~56% of the genome. Identification of several conserved sequence motifs in the WRE suggests a role for other known transcription factors, including AP-1. For example, mutations in a specific isoform of *Drosophila* Fos are currently being tested for activation of the epidermal wound response. The goal of this genetic analysis is to uncover additional components that act along with Grh to sense aseptic wounds, signal nearby epidermal cells of an epidermal breach, and activate the gene battery required to repair the breach and reseal a protective layer.

270C

Novel roles for the actin-binding protein Quail. Dorothy A. Lerit, and Elizabeth R. Gavis. Dept. of Molecular Biology, Princeton University, Princeton, NJ.

The posterior pole of the *Drosophila* oocyte marks the site of assembly of the germ plasm, a specialized cytoplasm enriched with RNA and protein. Proper germ plasm assembly is required for the formation of pole cells, the germ-line progenitors, and is also necessary for the localization of maternal mRNAs to the embryonic posterior pole. We have identified the actin-binding protein Quail as a component of the germ plasm. Previous studies of Quail have focused on its role in the ovarian nurse cells[1], but little is known about its role in the oocyte or early embryo. We are currently characterizing Quail function in the pole plasm through both genetic and molecular studies. [1]Mahajan-Miklos and Cooley. 1994 *Cell*, 78:291-301.

271A

Function of Zona Pellucida proteins in epidermal cell morphogenesis. Francois Payre[1], Isabelle Fernandes[1], Helene Chanaut-Delalande[1,2], Philippe Valenti[1], Serge Plaza[1]. 1) Centre de Biologie du Developpement, Universite P. Sabatier, Toulouse, FR; 2) Biozentrum der Universitat Basel, Basel, Switzerland.

Drosophila embryonic epidermal cells undergo a stereotyped reorganization of their apical shape during differentiation. While a subset of cells adopt a smooth apical surface, each segment displays several rows of cells forming actin-rich apical extensions, which eventually give rise to the larval denticles and dorsal hairs (collectively referred to as trichomes). The formation of epidermal trichomes is triggered by the activity of Shavenbaby, a transcription factor acting cell autonomously. We recently identified *miniature*, as a direct downstream target of Shavenbaby, and shown that *miniature* is required for the proper formation of trichomes. Miniature belongs to the evolutionarily conserved family of Zona Pellucida proteins that are components of the Extra Cellular Matrix. Zona Pellucida proteins are known to form extracellular fibers through the physical interaction of different members of the Zona Pellucida family. Consistent with this idea, we found that Shavenbaby also directs the expression of additional genes encoding Zona Pellucida proteins. Among the 16 Zona Pellucida genes present in Drosophila, we found that 8 of them are coregulated by Shavenbaby to be expressed in trichome cells. Characterization of the individual function of these Zona Pellucida proteins reveals that, while they contribute to the same differentiation process, they display specific roles in the formation of apical extensions. These data shed new light on the role of Zona Pellucida proteins in the control of subcellular cell shape remodeling during development. In addition, our studies allowing a dissection of the individual contribution of each of these proteins in the control of epidermal cell shape provide novel insights into the functional diversification of members of the Zona Pellucida family.

272B

Systematic analysis of phosphoinositide phosphates during morphogenesis. Inês Ribeiro, Amy Kiger. Division of Biological Sciences, UCSD, La Jolla, CA.

Cell shape changes at the basis of morphogenesis require coordinated subcellular changes in the cytoskeleton, membrane and adhesion. Phosphoinositide phosphates (PIPs), the phosphorylated forms of phosphatidylinositol, control localized and dynamic cellular processes through the recruitment of specific PIP-binding proteins. The association of mutations in PIP regulators with human diseases demonstrate that PIP regulation is crucial. However, little is known about the mechanisms for PIP cell spatial regulation, the identity of PIP responses and developmental PIP requirements *in vivo*. We are taking two initial approaches to address the roles for PIPs in morphogenesis, by (1) generating a complete collection of reporters to analyze PIP distribution during embryonic development, and (2) by altering PIP distributions through disruption of specific PIP regulators. We took nine well-characterized PIP-binding domains to make a non-overlapping set of fluorescently labeled protein fusion reporters to detect six PIP forms. The reporters were verified for PIP specificity by functional assays in *Drosophila* cells. We are creating transgenic *Drosophila* lines carrying one or more reporters for simultaneous detection in live cells during morphogenesis. We will present analysis of PI(3)P dynamics using the existing reporter, GFP-2xFYVE, in live wildtype embryos during dorsal closure. Embryonic dorsal closure involves coordinated morphogenesis of two cell types, requiring cell shape changes in the contracting amnioserosa cells and the stretching overlying epidermis. To assess the role of PI(3)P in this process, we are altering PI(3)P levels by pharmacological disruption of PI3-kinases and overexpression of the *myotubularin* phosphatase. We are also using the new reporters to reveal the identity of the functional PIPs affected in dorsal closure mutants, including mutants for the *sac1* phosphoinositide phosphatase. The combination of a unique collection of PIP reporters and live cell analysis provides an important entryway to understanding phosphoinositide-mediated mechanisms during morphogenesis.

273C

Dissecting Muscle and Neuronal Disorders in Drosophila Muscular Dystrophy. Halyna Shcherbata[1], Andriy Yatsenko[1,2], Maria Kucherenko[1,2], Uri Nudel[3], David Yaffe[3], David Baker[1], Hannele Ruohola-Baker[1]. 1) Dept Biochemistry, Univ Washington, Seattle, WA; 2) Ivan Franko Lviv University, Ukraine; 3) The Weizmann Institute of Science, Rehovolt, Izrael.

The transmembrane protein Dystroglycan is part of a complex that links the extracellular matrix to cytoskeletal actin via the cytoplasmic protein Dystrophin. The Dystrophin contains an actin binding domain on its N-terminus and the Dystroglycan interacting WW+EFhand-domain on its C-terminus. These linkages are vital and disruption of any component or the interaction between them can cause muscular dystrophy in humans. The phenotypes caused by Drosophila Dg and Dys mutations are remarkably similar to phenotypes observed in human muscular dystrophy patients: decreased mobility, age-dependent muscle degeneration and brain defects, which suggest that functional dissection of Dg-Dys complex in Drosophila should provide new insights into the origin and potential treatment of these fatal neuromuscular diseases. We have now shown that perturbation of Dg-Dys complex causes phenotypes that resemble Nck/Dock-Pak-Trio phenotypes in photoreceptor path-finding, suggesting that Dg may be one of the key players in Nck/Dock signaling pathway for axon guidance and target recognition in Drosophila. Interestingly, Insulin receptor-tyrosine kinase (InR) mutants also show similar phenotypes to those of Nck/Dock signaling in photoreceptor axon path-finding and these two proteins show genetic and biochemical interactions. Our data now add Dg-Dys complex to this pathway. Furthermore, biochemical interaction between Nck/Dock and Dg has been reported supporting the hypothesis that InR, Dg and Nck/Dock interaction regulates Dg-Dys complex. Furthermore, we have now shown that Dg interacts genetically with InR and Dock in photoreceptor axon path-finding. Since Dys interacts with Dg but not with InR and Dock, it is tempting to speculate that Dg can selectively interact with either Dys or InR and Dock. The work presented in this study is the first demonstration of genetic interaction between Dg and InR. Future biochemical studies should unravel the molecular mechanism of this interaction.

274A

Identification of substrates for the *Drosophila* ABC-transporter DMRP. Jolene Tarnay[1], Steven Robinow[2]. 1) Cell & Molec Biol, Univ Hawaii - Manoa, Honolulu, HI; 2) Department of Zoology, University of Hawaii, Honolulu, HI.

The *Drosophila dMRP* gene is orthologous to the human MRP1, MRP2 and MRP3 genes. These multiple drug resistance associated proteins (MRP) are members of the ATP-binding cassette (ABC) transporter superfamily and are involved in several integral cellular processes such as ion transport, signal transduction and detoxification. High expression levels of human MRP1, MRP2 and MRP3 have been associated with chemotherapeutic drug resistance in many cancers. Xenobiotic exposure has also been found to cause transcriptional up-regulation of human MRP genes. We have generated mutations in the *dMRP* gene using a transposase-mediated double P-excision strategy. *dMRP* mutants are homozygous viable. To determine a physiological function for *dMRP*, we have evaluated the sensitivity of mutant and control flies to various contact pesticides. Each pesticide belongs to one of the four main groups of organic pesticides: organochlorines (DDT), organophosphates (fenitrothione), carbamates (aldicarb) and pyrethroids (allethrin). Compared to control animals, *dMRP* mutants show no difference in response to fenitrothione or allethrin, but show increased sensitivity to DDT and aldicarb. Based on these results we will be testing the hypotheses that *dMRP* mutant animals have increased sensitivity to organochlorines and carbamates and that DMRP functions in the transport of these specific classes of pesticides. Identification of ABC-transporter substrates has proven difficult and is often left to guesswork. We are testing the hypothesis that compounds that cause *dMRP* transcriptional up-regulation are actual DMRP substrates. RT-PCR analysis has shown that larval exposure to the known verterbrate MRP substrates chlorpromazine, phenobarbital and dexamethasone causes *dMRP* up-regulation. We are currently in the process of testing DMRPs ability to transport these drugs. If a correlation is found between DMRP substrates and *dMRP* transcriptional regulation, then *Drosophila* may provide a simple and useful model to help identify substances that may result in the transcriptional up-regulation of it's human counterparts.

275B

Roles for Myotubularin Phosphoinositide Phosphatase in Membrane Homeostasis and Cellular Morphogenesis. Michaella Velichkova, Amy Kiger. Division of Biological Sciences, UCSD, La Jolla, CA.

Accumulation of specific phosphoinositide phosphates (PIPs) at distinct membrane domains provides spatial control for diverse cellular processes, including membrane trafficking, protein sorting and cytoskeletal organization. However, little is known about *in vivo* mechanisms for PIP regulation and cellular responses in multicellular organisms. We are employing complementary functional approaches in *Drosophila* cell cultures and in development to characterize PIP-dependent processes required for cellular morphogenesis. Using RNAi, we identified *myotubularin (mtm)*, a PI 3-phosphate phosphatase, as important for an induced cell shape change. The *in vitro* substrates of myotubularin phosphatases, PI(3)P and PI(3,5)P_2, have been localized throughout the endolysosomal system. Therefore, we examined *mtm*-depleted cells for defects in related compartment identities and functions. Whereas no defects were detected in early endosomes, *mtm* RNAi knockdown resulted in 3x enlargement of late endosome-lysosome compartments and a delay in late fluid phase transport, suggesting a role for *mtm* in membrane homeostasis. Cells depleted for *mtm* also exhibited an accumulation of the PI(3)P reporter, GFP-2xFYVE, supporting that PI(3)P is a *Drosophila* Mtm substrate. We generated the first *mtm* mutants that we used to identify an essential *mtm* function for metamorphosis and similar *in vivo* roles for PI(3)P and endolysosomal regulation. To identify functional pools of Mtm substrates, we modulated PIP levels by knockdown of opposing PI3-kinase functions. Of the three *Drosophila* PI3-kinases, we found that only co-RNAi of the class II PI3-kinase, *Pi3K68D*, resulted in suppression of both *mtm* -related defects in cell shape and endolysosomes. Together, these data suggest that Mtm mediates turnover of a specific pool of Pi3K68D-generated PI(3)P, with functions important for endolysosome homeostasis and cellular morphogenesis. Currently, we are conducting RNAi screens to identify novel modifiers of *mtm* phenotypes and testing roles for differential spatial regulation of Mtm protein *in vivo*.

276C

***awd*, the homologue of the human *Nm23* metastasis suppressor gene, regulates epithelial integrity of follicle cells.** Julie Ann Woolworth[1], Tien Hsu[2]. 1) Dept Molecular & Cellular Biology, Medical Univ South Carolina, Charleston, SC; 2) Dept Pathology & Laboratory Medicine, Medical Univ South Carolina, Charleston, SC.

Nm23 is a putative tumor metastasis suppressor and has been implicated in the suppression of cell motility, but its *in vivo* function remains unclear. In particular, Nm23 has been implicated in normal epithelial homeostasis but its exact role is not known. Since in humans, up to 80% of solid tumors are of epithelial origin, the epithelial function of Nm23 may also be critical in tumorigenesis. Nm23 is a highly conserved enzyme containing nucleoside diphosphate kinase activity. During our studies in the follicular epithelium of *Drosophila melanogaster*, we made a number of interesting observations regarding *awd*, the *Drosophila* homologue to *Nm23H1/H2*. First, *Nm23/awd* is down regulated during border cell migration. Second, in normal follicular epithelium, *Nm23/awd* is critical for establishing and maintaining epithelial polarity by down-regulating membrane accumulation of the E-Cadherin/β-catenin complex (adherens junction, AJ). Third, the epithelial function of *Nm23/awd* depends on both its expression level and its subcellular localization. Lastly, both up-regulation and down-regulation can disrupt the epithelium. We hypothesize that proper expression of Nm23/Awd is critical for maintaining epithelial homeostasis and that the disruption of normal expression (either up or down regulation) can contribute to different aspects of tumorigenesis. These results explain the somewhat contradictory clinical findings that either high or low Nm23 expression levels can be correlated with unfavorable prognosis.

277A

APC2, Armadillo and α-catenin form a complex in *Drosophila* syncytial embryos. Meng-Ning Zhou, Andrea Blitzer, Brooke McCartney. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

The cytoskeleton enables cells to shape themselves for various physiological activities such as division, polarization and migration. How the cytoskeletal network is assembled and regulated within a cell is of great interest in cell biology. The organization of cortical actin cycles between caps during interphase and rings and furrows during mitosis in cycle 10-13 *Drosophila* syncytial embryos. This provides an excellent model system to investigate the functions of proteins involved in actin organization. We have shown that APC2, a *Drosophila* homologue of APC (Adenomatous polyposis coli), localizes to syncytial cortical actin structures and is required for the normal development of actin rings and furrows. We are examining the mechanisms by which APC2 influences actin organization and have shown that APC2 forms a complex with Armadillo (Arm) and α-catenin (αCat) in early *Drosophila* embryos. Based on what is known about a homologous complex in vertebrate cells, we predicted that the interaction between APC2 and αCat would be mediated by Arm. Surprisingly, using GST pull-down assay, we have shown that the C-terminus of APC2 is able to interact with αCat independent of Arm. Consistent with the notion that APC2 and αCat form a complex independent of Arm, we have identified cortical sites in the syncytial embryo where APC2 and αCat colocalize in the apparent absence of Arm. We are mapping the minimum domains in APC2 and in αCat that are responsible for this interaction in embryo lysate. Furthermore, we are developing live imaging tools to probe these interactions using Fluorescence Resonance Energy Transfer (FRET) in S2 cells and live embryos.

278B

Interaction of Drosophila rab GDI with Gint3, a ubiquitin-domain-containing protein. Clarissa Cheney, Palak Amin, Alyssa Cope, Michael Lawson, Brian Richardson, Alex Chen, Katherine Ayres, Alexis Moore, Naveen Sangji, Michelle Keese. Dept Biol, Pomona Col, Claremont, CA.

Rab GTPases play multiple roles in vesicle transport. Rab GDP dissociation inhibitor (GDI) regulates rab placement by retrieving rabs from target membranes and delivering rabs through the cytoplasm to donor membranes. GDI plays a crucial role in ensuring that the correct rab is placed in the correct membrane. To identify proteins that interact with GDI, we have carried out screens for GDI interactors, using Drosophila as the model system, since the fly genome has a single GDI, but multiple rabs. In a yeast two-hybrid (Y2H) screen, a protein with a ubiquitin-like UBX domain was identified as a GDI interactor. This protein was named Gint3, for GDI interacting protein 3. Gint3 has a PUG N-glycanase-associated domain and a UBX ubiquitin-like domain, but no obvious transmembrane domain. Gint3 did not interact with L319 mutant GDI, which has a mutation that alters the lipid-binding pocket of Domain II of GDI (Ricard et al, Genesis 31:17). Gint3 also did not interact with AK307 GDI, which has E364K mutation. Phylogenetic analysis of Gint3 and UBX domain proteins shows that Gint3 forms a distinct subfamily of UBX domain proteins with the highest bootstrap value. The region between the PUG and UBX domains also shows a high degree of conservation. Supported by NSF-RUI grant 0212730 to CMC.

279C

Orchestrating secretory machinery in the larval salivary gland. Benjamin FB Costantino, Daniel Bricker, Andrew Andres. Biological Sciences, UNLV, Las Vegas, NV.

The foundations of multicellular life are built upon a variety of efficient cell signaling pathways. Our attention centers on the developmental signal, 20-hydroxyecdysone (ecdysone). Ecdysone is a steroid that directly activates target genes of responding cells in a tissue specific manner. In the larval salivary gland, ecdysone triggers the synthesis and secretion of exocytotic granules containing glycoproteins to the lumen of the gland. This physiological response cannot occur if core exocytotic machinery is not active or is mislocalized. What is not understood, however, is how this machinery responds to the ecdysone signal on a molecular level. We have evidence that calmodulin, actin, and myosin VI work together to secrete granules at the plasma membrane. Confocal imaging of calmodulin tagged with GFP taken before and after in vivo ecdysone exposure show a dynamic localization from the nucleus to the cytoplasm. This is known to coincide with an increase in calcium levels. Presumably, this increase in calcium concentration allow cytoplasmic calmodulin to associate with myosin VI. Furthermore, we have experimentally shown that myosin VI and actin colocalize at the plasma membrane when granules are being secreted. This is compelling evidence that ecdysone conducts the direct activation of calmodulin and in-turn influences the behavior of myosin VI and actin to complete proper granule exocytosis. Our current model of ecdysone directed granule exocytosis will be presented.*Drosophila melanogasterin vivo*.

280A

The role of an Arf-like GTPase in plasma membrane remodeling required for tracheal tube connection. Ken Kakihara[1,2], Kagayaki Kato[1], Hosei Wada[1], Shigeo Hayashi[1,2]. 1) Morphogenetic Signaling, Riken CDB, Kobe, Hyogo, JP; 2) Dept. Sci. Tech., Kobe Univ. Kobe, Hyogo, JP.

Tubular organs, such as the vascular system of vertebrates and tracheal system of insects, are seen in various organisms. During development, tubes are connected to each other and form continuous tubular networks. Previous studies suggested the involvement of intracellular vesicles in the tube connection process, but the role of intracellular vesicle traffic and the molecular mechanism controlling the tube fusion process are largely unknown. In the development of Drosophila tracheal system, tracheal branches are connected to each other at their tips. The cells at the tip of tracheal branches, called the fusion cells, play pivotal roles during tube connection by converting into doughnut shape. It was shown that intracellular vesicles are assembled prior to this unique cell shape transformation event. In order to gain further understanding of the molecular mechanism of fusion cell shape conversion and tube connection, we focused on previously reported ADP-ribosylation factor-like 3 (Arl3), which is exclusively expressed in fusion cells (BDGP in situ project). We found that Arl3 co-localized with the intracellular vesicles within fusion cells. Arl is closely related to ADP-ribosylation factor (Arf), which was shown to be involved in vesicle trafficking, but both Arl and Arf's functions during development remain to be discovered. We found that in Arl3 mutants, conversion of fusion cells into doughnut shape failed and the tracheal system was not connected. No apparent defects in cytoskeletal organization or cell adhesion were observed in these mutants. These data suggest that in fusion cells, Arl3 controls intracellular vesicles that promotes juxtaposition of inner plasma membranes and their fusion to support the tracheal tube connection.

281B

In vivo motor protein interaction revealed in spatial and temporal dynamics of vesicle transport in Drosophila segmental nerve axons. Gerald F Reis[1], Ge Yang[2], Sameer Shah[3], Lukasz Szpankowski[4], Gaudenz Danuser[2], Lawerence Goldstein[5]. 1) Neuroscience, UCSD, La Jolla, CA; 2) The Scripps Research Institute, La Jolla, CA; 3) Bioengineering, University of Maryland; 4) Bioinformatics, UCSD, La Jolla; 5) Cellular and Molecular Medicine, UCSD, La Jolla.

Proper functioning of microtubule-based bidirectional axonal transport is crucial for neuronal function and survival. We studied molecular mechanisms of neuronal transport by imaging and measuring high-resolution trajectories of axonal cargo motion in vivo. Until recently, a major hurdle in obtaining an accurate quantitative analysis of single axon cargo trafficking hinged on development of reliable and efficient computational tools to track vesicle motion and generate complete spatial and temporal readouts for large datasets. The computational challenge lies in the physiology of vesicle transport, which is often comprised of dense anti-parallel motion with apparent particle crossing, merging, and splitting. We have developed reliable and efficient computational tools to recover complete trajectories of large numbers of cargoes as well as characterize the spatial and temporal dynamics of vesicle transport. These tools include a novel multiple-hypothesis-testing (MHT) algorithm for resolving particle superposition. Human-machine interaction is used to complement automation, ensuring complete and accurate trajectory readouts. We are able to recover complete tracks of large numbers of cargoes, thereby validating an approach suitable for high-throughput processing. We imaged movement of cargoes containing YFP fused to the human amyloid precursor protein in a subpopulation of Drosophila segmental nerve axons. We imaged transport in wild type and mutants expressing a 50% genetic reduction in components of kinesin, dynein, and dynactin motor complexes, enabling comparative analyses of several transport parameters across 8 genotypes and over 600 movies. Our results reveal mutant-dependent changes in cargo populations and motility parameters, suggesting distinct modes of coordination and regulation for kinesin and dynein.

282C

Epigenetic regulation of centromeres. Sylvia Erhardt[1], Barbara Mellone[1], Craig Betts[2], Aaron Straight[2], Gary Karpen[1]. 1) Dept Genome Biol, Lawrence Berkeley National Lab, Berkeley, CA; 2) Beckman Center, Dept. of Biochemistry, Stanford University, Stanford, CA.

The centromere is the single chromatin region on every chromosome that is necessary for chromosome segregation during mitosis and meiosis, by providing an anchor point for kinetochore formation. The kinetochore is a multi-protein structure that mediates chromosome-spindle interactions and the mitotic checkpoint in eukaryotes. Centromeric DNA is not conserved and epigenetic mechanisms are thought to regulate centromere identity. Centromeric chromatin exhibits distinct combination of epigenetic modifications from euchromatin or heterochromatin. Furthermore, the histone H3-variant CenpA (CID in Drosophila) is exclusively and constitutively present in centromeric chromatin, making it an excellent candidate for an epigenetic mark. CenpA/CID is absolutely required for kinetochore formation and misexpression of CenpA/CID causes the formation of functional ectopic kinetochores. We utilized co-immunoprecipitation assays with CenpA/CID containing chromatin and a genome-wide RNAi screen to identify genes that are physically or functionally interacting with CenpA/CID. We have identified new and known epigenetic factors that are associated with centromeric chromatin. We are studying their functions in centromere biology by analyzing their cellular localizations, interacting partners, mutant phenotypes, and effects on kinetochore components and chromatin structure in Drosophila.

283A

Centromere formation and function in Drosophila. Barbara Mellone[1,2], Sylvia Erhardt[1,2], Craig Betts[3], Aaron Straight[3], Gary Karpen[1,2]. 1) Dept. of Genome Sciences, Lawrence Berkeley Laboratory, Berkeley, CA; 2) MCB Dept., University of California at Berkeley, Berkeley, CA; 3) Dept. of Biochemistry, Stanford University School of Medicine, Stanford, CA.

The kinetochore is a multi-protein structure essential for chromosome segregation during eukaryotic cell division, and is assembled at specific, heritable chromosome regions called centromeres. Malfuction of the kinetochore/centromere complex is associated with chromosomal abnormalities, a hallmark of many human cancers. Surprisingly, most eukaryotes do not rely on a DNA sequence-dependent mechanism to mediate kinetochore assembly and centromere inheritance. Instead, these processes are regulated by an epigenetic mechanism involving the incorporation of specialized nucleosomes containing the histone H3-like protein CID into centromeric chromatin. Currently, one major challenge in the field is to identify the mechanism(s) responsible for targeting CID specifically to centromeres. We have isolated novel genes required for CID centromeric localization (CDLs- CID DeLocalization) in a genome-wide RNAi screen using Drosophila Kc cells. CDL-1 is a novel centromeric protein. We are using combinatorial experimental approaches to determine the role of CDLs in centromere formation and function.

284B

Lethal 6/neverland is an essential 3L heterochromatic gene involved in the ecdysone biosynthetic pathway in D. melanogaster. Monika A Syrzycka, Don AR Sinclair, Kathleen A Fitzpatrick, Barry Honda. Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

We are interested in the structure, function and regulation of heterochromatic genes in D. melanogaster. To this end, we have undertaken a number of genetic screens and identified roughly one dozen essential loci in the centric heterochromatin of the left arm of the third chromosome. One of these, lethal 6, encodes a novel protein with homology to oxygenases. It contains a conserved Rieske electron carrier domain and a non-heme iron binding domain, as well as two additional conserved domains with no known function. Recently, Yoshiyama et al. (Development 133:2565, 2006) presented RNAi and other data indicating that this gene, named neverland, is involved in one of the early steps of the ecdysone biosynthetic pathway. We are currently working with them to investigate the expression and developmental regulation of lethal 6/neverland.

285C

The role of MT2 in *Drosophila melanogaster*. Catalina Alfonso, Keith Maggert. Dept Biochemistry/Biophysics, Texas A&M Univ, College Station, TX.

DNA methylation is present in vertebrates, plants, fungi, invertebrates, and many bacterial species. The majority of methylated cytosines are in transposons, repetitive sequences, and are associated with imprinted genes and genes on the inactive X chromosome in mammals. Methylated CpG in promoter regions causes those genes to be strongly and heritably repressed. DNA methylation is mediated by a conserved group of proteins, called DNA methyltransferases. In *Drosophila*, Mt2 is the only DNA methyltransferase homologue. Though every detail of sequence and structure indicates that Mt2 should be a DNA cytosine methyltransferase, in vitro assays have failed to detect reliable methyltransferase activity, although some laboratories have reported low levels of DNA methylation, and others show RNA methyltransferase activity. We have generated the only amorphic allele of *Drosophila* Mt2 ($Mt2^{r2}$). In order to verify and expand the knowledge of the role of Mt2 in *Drosophila*, *in situ* hybridization, immunohistochemistry, RT-PCR and methylation *in vitro* assays were performed using wild type, $Mt2^{r2}$, and MT2-overexpressing embryos. We detected the presence of Mt2 mRNA in embryos, and adult females and males, ruling out a role restricted to early development. MT2 may be involved in centromere function since Mt2 is found in organisms that have regional centromeres, such as *S. pombe*, but absent from organisms that have alternative centromere structure such as *S. cerevisiae*. Flies that carry the $Mt2^{r2}$ allele, have a small increase in the frequency of lost maternal X chromosomes. Our data indicates that Mt2 may play a role in centromere structure. However, the fact that there are not obvious phenotypes in the mutant flies, and that the increase in frequency in chromosome lost is small, make these results difficult to follow up. We are investigating the level of expression of Mt2 in different tissues using RT-PCR and quantitative PCR. We are also testing different substrates that have been proposed to be methylated by MT2, using sensitive methods, such as *in vitro* methylation assays and HPLC-TLC.

286A

CHD1: A broadly expressed chromatin remodeling factor with a potential role in wing development. Jennifer A. Armstrong, Ivy E. McDaniel, Jennifer M. Lee, Parimal A. Deodhar. Joint Sci Dept, The Claremont Colleges, Claremont, CA.

The CHD1 chromodomain-containing protein is a member of the SWI2-like family of ATP-dependent chromatin remodeling factors. This family includes the trithorax group proteins Brahma and Kismet. While the localization patterns of Brahma and Kismet strongly overlap all forms of RNA Polymerase II (Pol II) on larval salivary gland polytene chromosomes, CHD1 precisely co-localizes with the elongating form of Pol II. This unusual chromosomal localization pattern suggests that CHD1 may be required to directly facilitate the passage of elongating Pol II through chromatin. It is unclear whether CHD1 is absolutely required for gene activity at all Pol II genes, or whether CHD1 function may be redundant with that of other chromatin remodeling factors. Consistent with a global role in gene expression, *in situ* RNA hybridization reveals that *chd1* is broadly expressed throughout embryonic development and is expressed in all imaginal discs of third instar larvae. To understand the function of CHD1 we have generated two distinct deletions within the *chd1* gene using imprecise excision of a P element located in the *chd1* promoter. Analyses of these deletions suggest that CHD1 is required for wing development and oogenesis. Over-expression studies using the GAL4 driver system similarly support a role for CHD1 in wing development.

287B

Requirements for HP1-mediated silent chromatin spreading. Diane E. Cryderman, Karrie A. Hines, Lori L. Wallrath. Department of Biochemistry, University of Iowa, Iowa City, IA.

Heterochromatin Protein 1 (HP1) is enriched in centric regions of the genome and plays a role in gene silencing. To determine the mechanism of silencing and spreading, we have tethered HP1 to sites 1.9 and 3.7 kb upstream of two euchromatic reporter genes and observed changes in histone modification, altered nucleosome packaging, and gene silencing. Silencing at 3.7 kb was dependent on SU(VAR)3-9, consistent with current models of heterochromatin spreading. To determine the domains of HP1 required for silent chromatin spreading, mutant forms of HP1 were used in the tethering assay. These mutants possess amino acid substitutions that disrupt association with methylated histones, dimerization of HP1, and interactions with PxVxL partner proteins. Association of the mutant forms of HP1 caused K9 histone H3 methylation and silencing of the reporter gene at 1.9 kb, but not at 3.7 kb, suggesting distance-dependent mechanisms for HP1 silencing. Unlike heterochromatin in *S. pombe*, silencing and spreading in Drosophila is insensitive to mutations in the RNAi pathway and Trichostatin A. Taken together, these data demonstrate a requirement for HP1 dimerization and partner protein interactions for long distance spreading and silencing, and implicate a histone methyltransferase other than SU(VAR)3-9 in short distance silencing.

288C

The histone-acetylase dGcn5 is involved in modulation of high order chromatin structures. Caroline Jacquier[1], Clément Carré[1], Anita Ciurciu[2], Orban Komonyi[2], Delphine Fagegaltier[1], Josette Pidoux[1], Hervé Tricoire[3], Laszlo Tora[4], Imre Boros[2], Christophe Antoniewski[1]. 1) Developmental Biology, CNRS / Institut Pasteur, Paris, FR; 2) Department of Genetics and Molecular Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary; 3) Institut Jacques Monod 2 place Jussieu 75251 Paris, FR; 4) IGBMC/UMR 7104 CNRS, Parc d'Innovation,1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex,FR.

Gene expression in eukaryotes has to accommodate the presence of nucleosomes and the packaging of DNA into higher order chromatin structures. Two major families of complexes regulate chromatin structure. Remodeling complexes with ATPase activity remodel nucleosomal arrays. Histone modifiers catalyze covalent modifications of histone tails. It has been proposed that these modifications act as marks to recruit various proteins to chromatin. As part of the multiprotein complex SAGA, the Histone Acetylase dGcn5 is the major HAT responsible for acetylation of lysine 9 and 14 of histone H3. dGcn5 is also the catalytic subunit of ATAC, another HAT multiprotein complex which acetylates lysine 5 and 12 of histone H4. Unexpectedly we found that acetylation of residues H3-K9 and K14 is also lost in the absence of CBP, another major HAT. Our results suggest that CBP may be involved in deposition of histone marks necessary for targeting the dGcn5-containing SAGA complex to chromatin. We recently observed that Gcn5 male mutants display an abnormal X chromosome structure similar to that one found in mutants of the ISWI and Nurf301 proteins, two subunits of the NURF remodeling complex. Male X decondensation was also found in a strain mutated in the ATAC specific component Ada2A. We further demonstrated that ISWI activity is required for normal H4-K12 acetylation by ATAC, suggesting that ISWI is involved in the recruitment of this complex to chromatin. Altogether, these data illustrate how an interplay between nucleosome remodeling and histone modifications can specify high order chromatin states.

289A

Genetic Interactions between RNAi components and RNA Polymerase II subunit in Drosophila. Harsh Kavi, James Birchler. Dept Biological Sci, Univ Missouri, Columbia, Columbia, MO.

Heterochromatin is thought to be formed by transcription via RNA Pol II of aberrant/repeated regions such as those found in the centromeric regions that enter into the RNAi pathway and guide chromatin modification for gene silencing. Recent evidence in S. pombe indicates that mutations in RNA Pol II second largest subunit subunit affects the production of siRNA and the subsequent histone modifications associated with centromeric heterochromatin. It has also been postulated that the C-Terminal Domain of the largest subunit of RNA PolII provides the scaffold for the assembly of RNAi components in S. pombe. We examined this issue in Drosophila. Our initial results indicate a genetic interaction between RNAi components in Drosophila (dcr2,aub, ago2, hls and piwi) and the second largest subunit of RNA Pol II. The interaction between mutations in the RNAi machinery genes and RNA pol II results in a synergistic suppression of PEV (Position Effect Variegation) indicating a possible effect on heterochromatin formation in Drosophila. The analysis of polytene chromosomes revealed a decrease in the H3-mK9 modification at the chromocenter in the double heterozygous (hlsE616, hls 125, dcr2 and RNA Pol II A5) mutants when compared with normal. This study suggests a link between RNA Pol II and RNAi mediated heterochromatin assembly in higher metazoan cells.*S. pombeDrosophiladcr2,aub, ago2, hls and piwihls 125, dcr2 and RNA Pol II A5*.

290B

High-Resolution Mapping of histone modifications in Drosophila Stage 5 embryos. Sasha Langley, Gary Karpen. Dept MCB, Univ California Berkeley/LBNL, Berkeley, CA.

The distribution of histone proteins, their variants, and post-translational modifications reflect the crucial roles they play in epigenetic regulation of gene expression and chromosome functions in eukaryotic organisms. Fine-scale mapping of the genomic locations of these elements of chromatin organization in stage 5 embryos reveals a spectrum of complex features present during early embryonic development. Together with transcriptional profiles, these maps allow identification of new functional or structural roles for the chromosomal sub-domains defined by particular epigenetic marks. We will report on Native ChIP-array mapping of H3K4Me2, H3K4Me3, H3K9Me2, and the histone variant, H2Av, in mononucleosomal chromatin purified from stage 5 embryos of the genomic sequence reference stock y; cn bw; sp. High-density oligonucleotide probe arrays (25mers spaced every 35 base pairs; manufactured by Affymetrix) spanning all annotated coding and noncoding genomic regions, as well as many of the known heterochromatic gene regions, afford high-resolution and quantitative views of the variations and covariation in the relative densities of target chromatin proteins. In addition to analyses of covariation of our data with stage-specific expression data and a systematic comparison of modification patterns on X and autosomes, we will present analysis of the differences and similarities in modification patterns in heterochromatic and euchromatic regions. H2Av exhibits particularly interesting genomic distribution in the stage 5 embryo, mapping across expressed and unexpressed genes in distinct patterns, which differ significantly from those reported for the H2A variant, H2AZ, S. cerevisiae. Finally, we have identified multiple patterns of covariation of H3K4 methylation and H2Av consistent with distinct functional roles not only for the individual modifications and H2Av or their combination, but also their variation across different gene regions.

291C

Chromatin structure of genes silenced by heterochromatin in *trans*. Parul Nisha, Amy K. Csink. Dept Biological Sci, Carnegie Mellon Univ, Pittsburgh, PA.

Heterochromatic silencing in *cis* (along the same DNA molecule) entails nucleosomal compaction, histone modifications and recruitment of specific proteins. Heterochromatin can also silence genes on a separate but adjacent DNA molecule (in *trans*). Here we use the bw^D system to compare chromatin structure changes by heterochromatin in *trans* and *cis*. An insertion of heterochromatin in the euchromatic *bw* coding region results in the bw^D allele that also inactivates bw^+ or a transgene inserted nearby on the *trans* homolog. This *trans* silencing is caused by the relocation of the wild type copy to the centric heterochromatin compartment. To first analyze the region in *cis* to bw^D, ChIP was carried out. While ^{2}MeH3K9 levels were higher within and/or adjoining the heterochromatic insertion, no difference was observed 3 to 10 Kb distal. In contrast, increased HP1 levels were found as far as 10 Kb distal of the bw^D insertion in *cis*. A high local concentration of HP1, due to the relocation of the chromosomal regions to the heterochromatic compartment, may promote the diffusion to and interaction of HP1 with weaker binding sites, allowing its direct or indirect interaction with DNA even in the absence of ^{2}MeH3K9. To study chromatin structure in *trans*, restriction enzyme (RE) accessibility assays were performed on the *hsp26* regulatory region of the P*{ hsp26-Pt}* transgene inserted 5 kb from *bw* on the wild type homolog. The transgene showed reduced accessibility of the RE sites in the presence of bw^D indicating a more compact structure. In contrast, ChIP for histone modifications on the regulatory and transcribed regions of P*{ hsp26-Pt}* transgene and the regulatory region of P*{lacW}*, another transgene in the same location, did not show enrichment of heterochromatic signatures in adults. ChIP of HP1 found increased levels on the coding region of the P*{ hsp26-Pt}* transgene but not over the regulatory region or for P*{lacW}*. Overall these experiments suggest that the compaction of nucleosomes, in the absence of stereotypical histone modifications, is a feature of heterochromatic silencing in *trans*.

292A

Deciphering the roles of histone demethylases in chromatin-based mechanisms during development. Neetu Singh, Felice Elefant. Dept Bioscience/Biotechnology, Drexel Univ, Philadelphia, PA.

Histone methylation was once thought to be a permanent mark in forming active and silent states of chromatin. However through the identification of LSD1, a lysine-specific histone demethylase (HDM), it is now known that histone methylation is reversible. Histone acetyltransferases (HAT) are epigenetic regulators that typically modify chromatin to an active state, thereby facilitating gene expression. ELP3 is a characterized HAT which has been shown to contain a second catalytic domain harboring potential HDM activity. The histone modifications regulated by LSD1 and ELP3 have been studied at a molecular level; however their role during development remains unclear. To investigate HDM developmental function, we are using the highly characterized Drosophila model system. We are focusing our studies on the Drosophila HAT (Dmel/HAT) homolog of human Elp3, which plays an essential role in transcriptional activation and elongation, and contains a putative HDM domain, and the Drosophila HDM (Dmel/HDM) human homolog of Lsd1, which was the first HDM to be identified, and acts as a coactivator for transcriptional activation by the androgen receptor. To decipher Dmel/ELP3 and Dmel/LSD1 function, we identified Drosophila sequences that demonstrated high homology to human ELP3 and LSD1 genes using BLAST searches at Flybase and cloned the cDNAs encoding these genes into the pUAST vector. We are currently using an inducible GAL4/RNAi based system in Drosophila to induce RNA knockdown of each of these genes in a variety of tissues and developmental stages of choice. We are also creating transgenic flies carrying a non-inverted repeat control construct as well as over-expression constructs for each of these genes. Assays will be performed to test the levels of acetylation and/or demethylation in the fly after altering endogenous Dmel/ELP3 and Dmel/LSD1 levels. Aberrant phenotypes resulting from the under- and/or over-expression of Dmel/ELP3 or Dmel/LSD1 in specific tissues will be correlated to known developmental pathways to aid in deciphering their cellular function during development. *Drosophila*.

293B

Deciphering the effects of the amyloid precursor protein (APP) on the regulation of the histone acetyltransferase Tip60's target genes. Meridith Toth, Felice Elefant. Dept Bioscience/Biotechnology, Drexel Univ, Philadelphia, PA.

The majority of DNA is packaged around histone protein cores into highly condensed chromatin. In order to allow gene expression to proceed in these regions, post-transcriptional modifications are required to decondense the chromatin, thus allowing transcriptional machinery to access the genes. Targeted gene expression, which is critical in differentiation and development, is made possible by one such modification, acetylation, which is associated with active gene domains. Acetylation is catalyzed by histone acetyltransferases (HATs), proteins that transfer the acetyl group from acetyl co-A to conserved lysine residues on histone protein tails. Misregulation of HATs has implicated them in various neurodegenerative diseases and cancers. The goal of this study is to consider how misregulation of the HAT TIP60 may lead to disease using a Drosophila model system. Tip60 has been implicated in Alzheimer's Disease (AD) through its interaction with the C-terminal domain of the amyloid precursor protein (APP-CT), which is released upon cleavage by gamma-secretase. This complex has been proposed to regulate downstream target genes at the transcriptional level. To determine whether Dmel/TIP60 is involved in the detrimental effects that result from APP overexpression in the fly, we will use dominant negative mutations that disrupt the HAT activity of Dmel/Tip60 and express these proteins in specific tissues using the GAL4/UAS inducible system. These fly lines will be used to determine whether disruption of the chromatin modulating activity of Dmel/TIP60 can rescue the detrimental phenotypic effects shown by overexpression of APP in the wing disc as well as in neuronal tissues. Further, we will consider the changes in Tip60 endogenous target gene regulation associated with APP over-expression through chromatin assays. These studies may be the first to implicate the epigenetic function of Tip60 in the pathogenesis of AD in vivo in a multicellular organism.

294C

Poly ADP-ribose Polymerase in chromatin and transcriptional regulation. Alexei Tulin, Natasha Naumova, Elena Kotova, Aaron Pinnola. Dept Basic Sci, Fox Chase Cancer Ctr, Philadelphia, PA.

Our goal is to investigate the mechanisms by which poly(ADP-ribosyl)ation of proteins regulates gene expression. The level of protein poly(ADP-ribosyl)ation (pADPr) reflects the relative activities of the poly(ADP-ribose) polymerase (PARP) enzyme, which utilizes NAD to create pADPr-modified proteins, and the poly(ADP-Ribose) glycohydrolase (PARG) enzyme, which removes pADPr moieties. Our studies in Drosophila first revealed vital roles for PARP protein in the establishment of silent chromatin domains as well as in the chromatin loosening and transcriptional activation of a subset of inducible chromosomal loci. Subsequently, increased expression of inactive PARP1 protein has been implicated in the formation of condensed and silent chromatin domains, whereas upon the stimulation of PARP enzymatic activity, chromatin decondenses and becomes transcriptionally active. At present, the main gaps in our understanding of the PARP1-dependent transcriptional regulation are (1) the mechanism of PARP protein targeting to specific chromatin domains, and (2) the mechanism of local PARP activation. Recently, we have successfully identified novel chromatin-associated PARP1 partners by use of a Tandem Affinity Purification (TAP) strategy together with sucrose gradient purification and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Candidate interactors arising from this approach were functionally analyzed for influence on PARP1, using standard genetic approaches combined with immunostaining and confocal microscopy. Based on this work, the variant histone H2Av (an H2Az, H2Ax homolog in Drosophila) has been identified as a protein that promotes targeting of PARP1 to chromatin. Ser137-phosphorylated H2Av has been shown to co-localize with foci of local PARP1 activation in vivo.

295A

Histone Acetyltransferase Human Homolog Dmel/TIP60 is Essential for Multicellular Development in Drosophila. Xianmin Zhu, Felice Elefant. Dept Bioscience/Biotechnology, Drexel Univ, Philadelphia, PA.

Chromatin packaging directly influences gene programming as it permits only certain portions of the genome to be activated in any given developmental stage, cell, and tissue-type. Histone acetyltransferases (HATs) are a key class of chromatin regulatory proteins that mediate such developmental chromatin control, however their specific roles during multicellular development remain unclear. Here, we report the first isolation and developmental characterization of a Drosophila HAT gene (Dmel/TIP60) that is the homolog of the human HAT gene TIP60. We show that Dmel/TIP60 is differentially expressed during Drosophila development, with transcript levels significantly peaking during embryogenesis. We further demonstrate that reducing endogenous Dmel/TIP60 expression in Drosophila embryonic cells by RNAi results in cellular defects and lethality. Finally, using a GAL4 targeted RNAi system in Drosophila, we show that ubiquitous and cell/tissue specific reduction of Dmel/TIP60 expression results in lethality and/or cell/tissue specific phenotypes during fly development. Our results suggest a mechanism for HAT regulation involving developmental control of HAT expression profiles, and show that Dmel/TIP60 is essential for multicellular development. Significantly, our inducible and targeted HAT knockdown system in Drosophila now provides a powerful tool to effectively study the roles of these chromatin mediators in specific tissues and cell types during development.

296B

Centrosomal localization is required for proper chromosomal distribution of a chromatin insulator component. Omar Akbari, Daniel Oliver, Chi-Yun Pai. Dept Biol, Univ Nevada, Reno, Reno, NV.

Chromatin insulators or boundary elements are important players for the organization of the chromatin structure in the cell nucleus. Insulator elements have been found in many organisms from yeast to mammals. However, the distribution of chromatin insulators in the genome is not well characterized and the molecular mechanisms remain unclear. The gypsy chromatin insulator of Drosophila melanogaster is one of the best understood chromatin insulators. It is comprised of a DNA sequence bound by a complex of at least four characterized proteins. We recently discovered that CP190, a centrosomal protein, is a component of gypsy insulator complex. CP190 colocalizes with Su(Hw) and Mod22, two other gypsy insulator components, on the polytene chromosome. In addition, it is also present in complexes independent of Su(Hw) and Mod22. CP190 localizes to the centrosome during mitosis. It is relocated to the nucleus and is associated with chromatin during interphase. This unique localization during cell cycle has led us to investigate whether CP190's centrosomal localization may contribute to gypsy insulator function. To understand how the function of CP190 on the chromatin is regulated during cell cycle, we studied a truncated CP190 protein (CP190dCen) which does not localize to the centrosome. We analyzed localization of CP190dCen on the polytene chromosome at both Su(Hw)-Mod22 sites, and non-Su(Hw)/Mod22 sites. The distribution of CP190dCen on polytene chromosomes is more diffused at the non-Su(Hw)/Mod22 sites but still localizes clearly to the Su(Hw)/Mod22 sites. On the other hand, the CP190 mutant that has deletion in the C-terminal charged domain (CP190H4-1) has the same distribution as wildtype. While CP190H4-1 is partially functional, the CP190dCen protein restores the defective gypsy chromatin insulator functions completely. Our results suggest that the centrosomal localization is not required for the localization of CP190 to the Su(Hw)/Mod22 insulator complex, but may affect the localization of the non-Su(Hw)/Mod22 CP190 insulator complex .

297C

Insulating activity analysis of the interbands of D. melanogaster polytene chromosomes. Maria B. Berkaeva, Sergey A. Demakov, Igor F. Zhimulev. Institute of cytology and genetics SB RAS, Novosibirsk, RU.

The subdivision of D. melanogaster polytene chromosomes into bands and interbands suggests a structural chromatin organization that is related to the formation of functional domains of gene expression. Interbands appear as a regular decompacted structures, but in spite of many efforts their genetic and molecular organization remains unclear. Interbands are difficult for studying due to their small size and uncertain limits. It was proposed that there should be insulator elements which confine interbands and probably delimit chromosomal domains. In the present work we assessed the insulating activity of the 61C7/C8 and 85D9/D10 interbands in a position effect assay. The DNA fragments tested were thought to contain full-size interbands. DNA sequence of the 61C7/C8 interband was found to possess putative binding sites for the insulator protein BEAF and the transcriptional factor Adf-1. Moreover, we found that this interband bound both proteins in salivary gland. However, none of the DNA fragments tested revealed insulator properties. Probably, interbands contain several activities which can interfere with each other, so to uncover them one should work with smaller DNA fragments. Experiments to test the insulating activity of different parts of the interbands DNA are in progress and will help us to define the interbands limits and to understand their molecular organization.

298A

Study of an endogenous insulator found downstream of the Drosophila mini-white gene. Pavel G Georgiev, Darya Chetverina. Institute of Gene Biology RAS, Moscow, RU.

Insulators are a class of elements that define independent domains of gene function. Specialized DNA sequences that ensure proper regulation of gene expression - enhancers, silencers and insulators - have been identified in higher eukaryotes. Enhancers activate, whereas silencers inhibit gene expression if they are placed either upstream or downstream from the gene promoter. Insulators are thought to isolate independent transcriptional units from cross-reaction with neighboring regulatory sequences by specifically blocking the activity of an enhancer and/or a silencer. We have identified a novel endogenous insulator located at the 3' end of the white gene. In transgenic constructs, interaction between mw insulators flanking the yellow and white genes results in effective blocking of upstream enhancers. Two mw insulators placed between the enhancers and promoters allow bypass only when they are inserted in opposed orientations, suggesting co-directional pairing of the insulators. The mw insulator can partially neutralize the activity of the gypsy insulator, demonstrating functional interaction between unrelated insulators. It is likely that many properties of insulators previously described with the aid of transgenic lines containing the mini-white gene could have been partially influenced by the presence of the mw insulator in conventional constructs.

299B

Dual functional activity of the Mcp insulator from the Drosophila bithorax complex and effects of insulator pairing on gene expression. Pavel G Georgiev, Olga Kyrchanova, Stepan Toshchakov. Institute of Gene Biology RAS, Moscow, RU.

Boundary elements have been found in the Abd-B 3' cis-regulatory region that is subdivided into a series of iab domains. Each iab domain appears to contain at least one enhancer that initiates Abd-B expression in the early embryo, as well as a PRE silencer element that maintains the expression pattern throughout development. It has been proposed that insulators flank each iab region and organize the Abd-B regulatory DNA into a series of separate chromatin loop domains. Previously, a 340-bp insulator-like element was identified in one such 755-bp Mcp fragment linked to the PcG-dependent silencer. In this study, we have divided the insulator into two functional parts: a 210-bp core was sufficient for pairing of sequence-remote Mcp elements, while the rest was essential for fencing off the silencer. In transgenic two-gene constructs with two Mcp insulators (or their cores) surrounding yellow, the upstream yeast GAL4 sites could activate the distal white only if the insulators were in opposed orientations (head-to-head or tail-to-tail), consistent with the looping/bypass model/idea. The same was true for the efficiency of the cognate eye enhancer, while yellow thus isolated in the loop from its enhancers experienced deeper blocking. These results provide further evidence that (i) the barrier activity and the enhancer-blocking activity of insulator-like elements are indeed two distinct functions, and (ii) the mutual placing and orientation of the insulator-like elements can determine the proper enhancer-promoter communication.

300C

tRNA genes: a potential role as boundary elements in Drosophila melanogaster. Paola Guerrero, Keith Maggert. Dept Biology, Texas A & M Univ, College Station, TX.

Boundary elements are regulators of gene expression. They break enhancer-promoter communication when they are placed between an enhancer and a promoter. Likewise, these regulatory elements prevent the spread of heterochromatin and the consequent silencing of transgenes. Hence, they may define the transition between repressive and active chromatin. Boundary elements have been identified in many organisms such as yeast, human, mouse, chicken, fruit fly, frog and sea urchin. Two tRNAs ($tRNA^{Ala}$ and $tRNA^{Thr}$) have been found to act as boundaries in two yeast species. At the centromere of chromosomes two and three in *S. pombe*, tRNAs are located at the transition between methylated Lysine 4 and Lysine 9 of histone H3 which are marks for active and repressed chromatin, respectively. We are currently investigating whether a subset of active tRNAs ($tRNA^{Ala}$, $tRNA^{Asp}$, $tRNA^{Arg}$, $tRNA^{Thr}$, $tRNA^{Ser}$, $tRNA^{Sec}$, $tRNA^{Lys}$, $tRNA^{Ile}$) have heterochromatin-barrier and/or enhancer-blocking activity in *Drosophila melanogaster* and how these activities may be regulated. Our preliminary results indicate that $tRNA^{Sec}$ might act as boundary element, and we are in the process of confirming this hypothesis. $tRNA^{Asp}$ and $tRNA^{Lys,\ Ile}$ lacking pol III control sequences do not have boundary activity, suggesting that active transcription may be required for this activity.

301A

Characterization of knockout mutations in the BEAF gene generated by homologous recombination. Craig M. Hart, Matthew K. Gilbert, Swarnava Roy. Dept Biological Sci, Louisiana State Univ, Baton Rouge, LA.

Many models have attempted to describe the relationship between chromatin structure and gene regulation. Chromatin domain insulators (or boundary elements) are likely to play a role in this relationship. One well studied insulator is the scs' sequence located at the 87A7 hsp70 locus in Drosophila. Two 32 kDa Boundary Element Associated Factors, BEAF-32A and BEAF-32B, bind to this sequence. Insulator binding proteins such as BEAF block communication between an enhancer and promoter when present between the enhancer and promoter, and protect a transgene from chromosomal position effects when they bracket the transgene. Because of these properties, insulators are thought to divide chromosomes into independently functioning genetic domains. By dividing chromosomes into domains, they are thought to link function to nuclear organization and chromatin structure. Using homologous recombination, we have isolated two mutations in the single gene encoding both BEAF isoforms. One mutation eliminates the ability to produce the 32A protein (A-KO), and the other mutation knocks out the ability to produce both 32A and 32B (AB-KO). Characterization of the effects of these mutations will be presented.

302B

Regulation of the Fab-8 Insulator of the Abd-B Gene Requires the Activities of dCTCF and Mod(mdg4). Sheryl Smith, Qi Chen, Ian Thomas, Jamie Planck, Lan Lin, Jumin Zhou. Gene Expression Program, The Wistar Institute, Philadelphia, PA.

The Abd-B gene of the Bithorax Complex (BX-C) contains an extended 3' regulatory region that is comprised of segment-specific enhancers that are separated by insulator/boundary elements. Previously, we have identified an element that is required for enhancer-promoter communication. This element, The Promoter Targeting Sequence (PTS) lies just adjacent to the Fab-8 insulator and functions in insulator bypass, but its initial activity depends on the presence of an insulator. In an effort to better understand enhancer-promoter communication at the PTS/Fab-8 insulator, we have employed DNA affinity chromatography to detect proteins associated with these elements. We have found that the PTS element associates with the protein Mod(mdg4)-67.2, a protein previously implicated in both insulator function and insulator bypass. We have also shown that the Fab-8 insulator is associated with the protein dCTCF, and that both Mod(mdg4)-67.2 and CTCF are required for proper Fab-8 function. dCTCF and Mod(mdg4)-67.2 are associated at many loci on polytene chromosomes and potentially act as co-regulators at a significant number of target elements. Currently, we are using a ChIP-chip strategy to identify other targets of dCTCF within the BX-C, and within the genome as a whole in an effort to better understand the role of dCTCF in insulator activity and insulator bypass.

303C

Zygotic gene activity is dispensable for the initiation of somatic homolog pairing. Jack R. Bateman, Ting Wu. Department of Genetics, Harvard Medical School, Boston, MA.

It has been nearly 100 years since Nettie Stevens first reported that mitotic figures from Drosophila show intimate pairing between homologous chromosomes. Today, we recognize the importance of somatic homolog pairing in both invertebrates and vertebrates, with significant roles in DNA damage repair, transvection, X-inactivation, and parental imprinting. However, our understanding of how homologous sequences are recognized and brought together is incomplete. In Drosophila, previous analyses have shown that homologs first associate during the syncitial blastoderm stage, when the rapid cell cycles of early embryogenesis elongate and the zygotic genome begins transcription. At least two hypotheses could account for this timing of pairing onset: first, that the rapidity of cell cycles earlier in development prevents homologs from forming stable contacts, and second, that specific zygotic gene products must be expressed to initiate pairing. We have addressed the latter hypothesis by using compound chromosomes to generate embryos lacking large segments of the genome, a strategy similar to one used by Eric Wieschaus for the study of cellularization. For example, in a cross of attached-X females to normal males, ¼ of the progeny lack an X chromosome; such embryos develop normally until zygotic transcription begins, permitting an analysis of pairing in the absence of all X-linked genes. Using fluorescence in situ hybridization (FISH) to specific chromosomal loci, we find that somatic homolog pairing can occur in the absence of any of the major chromosome arms, implying that specific zygotic genes are dispensable for the initiation of pairing. We are currently addressing the role of the cell cycle in modulating pairing by assessing the initiation of pairing under conditions that disrupt cell cycle timing.

304A

Comprehensive analysis of the *Minute* loci in the Drosophila genome. Kevin Cook[1], John Roote[2], Andrew Lambertsson[3], Gunter Reuter[4], Michael Ashburner[2], Gillian Millburn[2], Paul Harrison[5], Zhan Yu[5], Thomas Kaufman[1], Naoya Kenmochi[6], Sally Leevers[7], Steven Marygold[2,7]. 1) Bloomington Stock Center, Indiana University; 2) University of Cambridge; 3) University of Oslo; 4) Martin Luther University, Halle; 5) McGill University; 6) Miyazaki University; 7) Cancer Research UK, London Research Institute.

Minutes are the largest class of haploinsufficient genes in Drosophila. Since they are frequently haplosterile or essentially haplolethal, *Minute* loci often prevent the recovery of deletions. Indeed, they are the largest biological obstacle to attaining full deletion coverage of the genome.

We have conducted a comprehensive, genome-wide analysis of all the *Minute* genes of *Drosophila melanogaster*. To do this, we took advantage of the recent improvements in deletion coverage generated by systematic screens at the Bloomington Stock Center, Exelixis Inc., and the DrosDel Project, and the increased number of transposable element insertions that are now available. We combined the examination of these mutant fly strains with a survey of published data in the *Minute* field and the identification of all ribosomal protein-encoding genes in the genome.

We can now conclude that *D. melanogaster* possesses 63 *Minute* loci and that they all correspond to a single class of genes; namely, those that encode protein components of the cytoplasmic ribosome. This proves the longstanding idea that *Minutes* correspond to ribosomal protein genes and that *Minute* phenotypes arise from disrupted protein synthesis. Interestingly, many cytoribosomal genes are not *Minutes*, and none of the genes encoding mitochondrial ribosomal proteins are *Minutes*. Taken together, our data will greatly aid further efforts to maximize genomic deletion coverage in *D. melanogaster* and will prove valuable to further genetic studies of ribosomal proteins and haploinsufficiency in Drosophila and other organisms.

305B

A model of segmental duplication formation in Drosophila melanogaster: impact of transposable elements. Anna-sophie A.S Fiston[1], Dominique D. Anxolabéhère[2], Hadi H. Quesneville[1]. 1) Bioinformatics and genomics, Jacques Monod Institut, Paris, FR; 2) Dynamics of the Genome and Evolution, Jacques Monod Institut, Paris, FR.

Segmental duplications (SDs) are low-copy repeats of DNA segments that have long been recognized to be strongly involved in genome organization and evolution. But to date, their formation mechanism still remains obscure. Developping a computational pipeline, we have detected SDs in the Drosophila melanogaster genome. They represent 1.7% of the genome, with lengths ranging from 21 bp to 139 kb. Our analysis indicates SD enrichment in transposable elements (TEs) and a high SD density in heterochromatic regions. We propose a model for SD formation based on the "Synthesis-Dependent-Strand-Annealing" model (SDSA), a double-strand breaks (DSB) homologous repair model. Our model that we call: "Duplication-Dependent-Strand-Annealing" (DDSA) predicts, after a DSB, the search for an ectopic homologous region, here a repeat, to initiate the repair. Indeed, a repeat near one of the single-strand tails generated by the DSB could choose a homologous repeat at an ectopic site as template. At the end, the template is duplicated in the gap created by the DSB. DDSA as for SDSA model also predicts dissociations of the strand during synthesis. If re-annealing follows the dissociation, the synthesis could be continued. The traces left by this process that we have observed support the DDSA model as SD formation model in D. melanogaster genome and suggest a containment of the dissociated strand in the repair complex. According to the model DDSA, we expect to find repeat regions at the SD ends. Indeed, SD ends are enriched in TEs compared to random sequences located in the same genomic environment. Thus, our study supports that TEs are involved in SD formation.

306C

Trans-regulatory effects at the *Drosophila apterous* locus. Daryl Gohl[1], Martin Müller[2], Paul Schedl[1]. 1) Dept. of Molecular Biology, Princeton University, Princeton, NJ; 2) Dept. of Cell Biology, University of Basel, Basel, Switzerland.

In *Drosophila*, somatic pairing of homologous chromosomes allows for cross-talk between genes and regulatory elements on the two homologs. To date, a number of trans-regulatory effects have been reported. One such effect is the phenomenon of transvection, in which regulatory elements on one homolog can affect the expression of a gene in trans.

We report a new instance of transvection at the *apterous* (*ap*) locus. Two different insertions of boundary elements were identified in the *ap* regulatory domain. The boundary elements are inserted between the *ap* wing enhancer and the *ap* promoter and have highly penetrant wing defects when homozygous or when crossed to a deficiency for the *ap* locus. However, when the boundary insertions are crossed to a deletion of the *ap* promoter region and first exon (ap^{UGO35}), strong interallelic complementation characteristic of transvection is observed. A deletion of the *ap* wing enhancer was generated by FRT-mediated recombination. When the wing enhancer deletion is crossed to ap^{UGO35}, interallelic complementation is also observed, suggesting that the wing enhancer on the ap^{UGO35} chromosome is activating the intact *ap* gene in trans.

In addition to the trans-activation seen with the *ap* wing enhancer, we have observed a trans-silencing effect that occurs at the *ap* locus in tissues where *ap* is not expressed. This trans-repression effect seems to be mediated by a novel PRE in the *ap* regulatory region. Interestingly, the trans-silencing extends for approximately 50kb on either side of the PRE and may define the limits of a distinct *ap* regulatory domain. The silencing in trans also shows a sequence specificity suggesting that within the *ap* regulatory domain the silenced chromatin performs a homology search in trans to silence homologous sequences. Thus pairing-dependent regulation can be detected at the *ap* locus both in tissues where *ap* is active and in tissues where *ap* is inactivated by PRE-mediated silencing.

307A

The Drosophila Heterochromatin Genome Project. Roger Hoskins, Christopher Smith, Cameron Kennedy, David Acevedo, Joseph Carlson, Susan Celniker, Gary Karpen. Department of Genome Biology, Lawrence Berkeley National Laboratory, Berkeley, CA.

The DHGP is defining the sequence, organization, and functional elements in *D. melanogaster* heterochromatin. Heterochromatin has presented major challenges for genome projects due to the difficulties of cloning, mapping, assembling and annotating regions rich in repetitive elements. A comprehensive description of the heterochromatin is nevertheless necessary for understanding expression of heterochromatic genes, gene silencing, and the functions and evolution of essential chromosomal elements. We have focused on regions that contain genes and are rich in middle repetitive DNAs (e.g. transposons), and have not yet extensively analyzed simple sequence DNAs (e.g. satellites).

We used STS content to construct a BAC-based physical map of most heterochromatic whole genome shotgun (WGS) scaffolds larger than 15 kb. The map joins 119 scaffolds into 29 BAC contigs. We localized 19 of the larger BAC contigs on the cytogenetic map of the heterochromatin using FISH of BACs, cDNAs and *P* element insertions. Using the WGS scaffolds and 10-kb genomic clones, we produced 15 Mb of improved heterochromatic sequence (Release 5) by filling 777 gaps, sizing 23 gaps, sequencing extending clones, and resolving misassembled regions. We used custom software to align cDNAs, comparative genome sequence data and gene predictions to annotate protein-coding and non-coding gene models. Complex nests of transposon fragments comprise 80% of the sequence. Finally, we have identified orthologs in the genome sequences of 15 other insect species to study the evolution of heterochromatin.

308B

The involvement of Drosophila error-prone DNA polymerases in DNA double-strand break repair. Daniel P. Kane, Sarah Rubin, Justine Liepkalns, Mitch McVey. Tufts University, Medford, MA.

The double-strand break is a type of DNA damage that threatens genomic stability and cell survival. Cells have developed repair mechanisms to minimize any threats from breakage, including homology-directed repair (HDR) and non-homologous end-joining (NHEJ). One type of HDR, termed synthesis-dependent strand annealing, involves resection of the 5' ends at the site of the break, invasion of a homologous template to form a displacement loop (D-loop) structure, and synthesis across the break site. In contrast, NHEJ involves the direct rejoining of DNA ends. NHEJ can occur independently of HDR or after aborted HDR. In Drosophila, error-prone end-joining frequently proceeds through annealing at small microhomologies, and occasionally involves the addition of extra nucleotides at the break site. Templates for these inserted nucleotides can often be identified near the break site. Recent evidence suggests that vertebrate polymerase eta (pol η), an error-prone polymerase, is involved in DNA synthesis at D-loop structures and functions in double-strand break repair. We are investigating whether Drosophila error-prone polymerases are involved in HDR and/or NHEJ. Mutants of polymerase eta were obtained through an imprecise P element excision screen. We have shown that Pol η mutants are sensitive to the DNA damaging agents methyl methanesulfonate and camptothecin. We are currently testing gamma ray sensitivity. To measure repair capacity of pol η mutants in the context of a large, double-stranded gap, we used a previously characterized P-element excision assay. Interestingly, we observed a reduction in both HDR and NHEJ repair. Preliminary sequence analysis of repair junctions from aberrant repair products suggests that neither the use of microhomologies nor nucleotide insertion was affected by the loss of pol η. This suggests that other error-prone polymerases may play partially redundant roles during double-strand break repair. Future studies will investigate the exact role of pol η in break repair and will examine potential roles of other error-prone DNA polymerases, particularly pol zeta.

309C

SUUR protein follows replication during the S-phase in *Drosophila melanogaster* salivary gland polytene chromosomes. Tatiana D. Kolesnikova, Eugenia N. Andreyeva. Institute Of Cytology And Genetics, Russian Academy of Sciences, Novosibirsk, RU.

The *Suppressor of UnderReplication (SuUR)* gene controls the DNA underreplication in intercalary and pericentric heterochromatin of *D. melanogaster* salivary gland polytene chromosomes. It is known that in salivary gland polytene chromosomes of late third instar larvae SUUR binds to pericentric (PH) and intercalary (IH) heterochromatin regions. In the present work we investigated dynamics of binding of the SUUR protein during DNA replication. In the earliest stage of the S-phase, SUUR binds mostly to the chromocenter, the SUUR and IdU incorporation signals completing each other. Later SUUR is observed in PH and the majority of bands of the polytene arms so that no conspicuous signals are observed in IH. Confined intensive signals appear only in IH and PH in the late stage of replication. At the end of S-phase, the signal corresponding to IdU incorporation becomes granular that evidence for replication of those regions which were underreplicated or compactized inside PH and IH. It is important that the signal corresponding to SUUR incorporation coincides completely with the distribution of the IdU signal. Therefore, the SUUR localization follow replication since the stage of the discrete labeling, so that in the late 3rd larval instar we reveal SUUR in places where it appeared at the end of S-phase. Thus we may suppose that SUUR is able of either immediate interaction with the replication machinery or of interaction with chromatin in all chromosomes and so retards replication.

310A

Mapping the Y-to-autosome translocation in Drosophila pseudoobscura. Amanda M. Larracuente[1], Mohamed A.F. Noor[2], Andrew G. Clark[1]. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) DCMB Group/Biology,Duke University,Durham, NC.

In a search for Y-linked genes in D. pseudoobscura, Carvalho and Clark (2005) discovered that there has been a translocation of the “ancestral” Y chromosome to an autosome in the D. pseudoobscura lineage. Using male-parent backcrosses, all lines of evidence to date point to a translocation of these formerly Y-linked genes to the dot chromosome (also known as Muller F or the 5th chromosome) in D. pseudoobscura. The relation between the dot and sex chromosomes has been a recurring theme in Drosophila evolution. Parallels between the MSL complex involved in dosage compensation and the painting of fourth (POF) dot-specific protein, together with evidence that the dot tends to pair with the X during meiosis in triplo-dot flies and the biasing intersexes towards females when dot dosage is increased in 2X:3A flies indicate that the dot is more like an X chromosome than an autosome in many ways (reviewed in Larsson and Meller 2006). The dot is less heterochromatic than the Y chromosome, and while recombination is negligible, it occurs more frequently than on the Y. Translocation of male fertility factors onto the dot may mean that they may now face selection pressures due to possible mis-expression in females. After the translocation in D. pseudoobscura, both the introns of these genes and intergenic regions underwent massive deletions of repetitive material. Re-sequencing of portions of the Y-to-autosome translocated region in D. pseudoobscura and D. persimilis reveal exceptionally low levels of nucleotide diversity, consistent with several evolutionary scenarios, including a simple model of recurrent selective sweeps.

311B

Identifying Regulators of Chromosome Territories in Drosophila Melanogaster. Akiko Minoda, Gary Karpen. Dept Genome Biol, Lawrence Berkeley Natl Lab, Berkeley, CA.

Chromosomes and sub-chromosomal regions are not randomly positioned in interphase nuclei, but instead are organized into separate domains called chromosome territories (CTs). This aspect of nuclear architecture is important for proper gene expression and other genome functions. In addition, defective nuclear organization is associated with cancers and other diseases, and may affect the rate of translocations between certain chromosomes that are associated with leukemia and other cancers. However, the mechanisms and molecules that regulate CT formation and the causes of defective organization in diseased cells are unknown.

CTs in Drosophila cells and how I aim to identify proteins that are required for establishing and/or maintaining CTs will be presented.

312C

***Tarsal-less* defines a new family of polycistronic genes in eukaryotes containing small ORFs with key functions during development.** Jose Pueyo-Marques, Maximo Galindo, Sylvaine Fouix, Juan Pablo Couso. School of Life Sciences, Univ Sussex, Brighton, GB.

The work of the last decades has seen a breakthrough in our understanding of the genetic and molecular mechanisms of development. Classical genetic approaches have been complemented by systematic searches for new genes and their functions, resulting in an exponential increase of information. However, the number of known key regulatory genes and signalling proteins remains small. One exciting possibility is that new types of genes with new coding arrangements await discovery and characterisation. Here we present the characterisation of a new type of non-canonical gene, *tarsal-less* (*tal*) that had been previously classified as a putative non-coding RNA (Tupy et al., 2005). *tal* mutants show defects in embryonic and post-embryonic development. The *tal* single messenger contains several small ORFs, which we named 1A, 2A, 3A, AA and B. Our rescue and ectopic experiments with full-length and mutated *tal* cDNAs indicate that the functional element of this gene is a 33-nucleotide-long ORF which is translated into an 11 aminoacid peptide. Using *in vitro* translation essays and *in vivo* experiments we show that 1A, 2A, AA, but not B, can be translated, demonstrating the polycistronic nature of the gene. Therefore *tal* defines two novel paradigms in eukaryotic coding genes: the existence of unprocessed short peptides in with key biological functions, and its arrangement in polycistronic messengers. In addition, the identification of *tal* homologues in other species defines a family which represents a new class of genes.

313A

Aberrant mono-methylation of histone H4 lysine 20 activates the DNA damage checkpoint in *Drosophila*. Ayako Sakaguchi, Ruth Steward. Waksman Institute, Rutgers University, Piscataway, NJ.

PR-Set7 is a histone methyltransferase that specifically mono-methylates histone H4 lysine 20 (H4K20). In *Drosophila* larvae, tissues with higher rates of cell divisions, such as imaginal discs, are severely affected by the depletion of PR-Set7, suggesting that PR-Set7-dependent mono-methylation plays an important role in cell proliferation.

We investigated the *in vivo* function of PR-Set7-dependent methylation by studying the cell cycle in mutant neuroblasts. Neuroblasts are diploid and their cell cycle progression has been well documented. We found that in *PR-Set7* mutants, both the mitotic and the S phase indexes were reduced, progression through early mitosis was delayed, and cyclin B was downregulated by APC/C proteolysis. In a double mutant of *PR-Set7* and *mei-41* (the fly *ATR* ortholog), the abnormalities of cell cycle progression and protein level of cyclin B were restored, indicating that in *PR-Set7* the DNA damage checkpoint is activated, and that this activation results in mitotic abnormalities. We also observed abnormal chromosome condensation in *PR-Set7* that was enhanced when the checkpoint-arrest was abolished in the double mutant, suggesting that the defect in chromosome condensation is independent of the checkpoint activation.

Most histone methylations have generally been considered to be regulators of transcription. However, since the expression of genes involved in the DNA damage checkpoint is normal in *PR-Set7*, control of gene expression is not involved in activation of the checkpoint. We therefore propose that mono-methylated H4K20 is involved in the maintenance of proper higher order structure of DNA and is consequently essential for chromosome condensation. In addition to gene expression at specific sites, histone methylation may control the organization of chromatin structure more globally.

314B

Overexpression of the *D1* gene affects chromosome morphology and viability. Marissa B Smith, Karen S Weiler. Department of Biology, West Virginia University, Morgantown, WV.

The D1 protein is the Drosophila homologue of mammalian HMGA, having multiple copies of a motif that effects DNA-binding to the minor groove of AT-rich sequences, called an AT-hook. D1 has been shown to bind to the AATAT and 359 bp repeat satellites within the heterochromatin, as well as several euchromatic sites on polytene chromosomes. We are using the GAL4-UAS system to ectopically express *D1*, as a means to elucidate its function. Ubiquitous *D1* overexpression causes lethality over several developmental stages, dependent upon the *GAL4* driver. The predominant developmental arrest resulting from *tub-GAL4* mediated *D1* overexpression is at the second to third instar molt. Further investigation of this lethality is in progress. When *D1* overexpression is limited to the third instar salivary gland, the polytene chromosomes are refractory to spreading due to numerous ectopic contacts. By modulating the GAL4 level, we were able to obtain chromosome squashes where the ectopic contacts could be mapped. A discussion of the sites of D1-induced ectopic association, in light of previously observed D1 binding sites and the Drosophila genome sequence, will be presented.

315C

The Role of *Drosophila* Ligase III in DNA Break Repair. Ilana Traynis, Mitch McVey. Biology Department, Tufts University, Medford, MA.

Two known pathways for repair of DNA double-strand breaks (DSBs) are homologous recombination and non-homologous end joining. Accurate end joining in yeast and mammalian systems requires the DNA ligase IV protein. In flies, *MUS309* encodes the DmBlm DNA helicase, which acts during homologous recombination. Previous studies using either *Drosophila lig4* or *mus309* mutants suggest the existence of an end-joining mechanism that is error-prone and frequently results in large deletions. To further characterize this repair pathway, we measured adult viability and DSB repair capacity in *lig4 mus309* double mutants. We observed reduced viability and an increased rate of developmental defects in the double mutants relative to the single mutants. However, repair of a DSB induced by P-element excision in the male germline was similar to that observed in *mus309* mutants. Together, these data suggest that ligase IV and DmBlm are involved in separate repair pathways during development and that a ligase IV-independent pathway operates robustly in the male germline.

To further study this alternative pathway we examined the role of DNA ligase III in DSB repair. Previous experiments using mammalian cell culture indicate that ligase III may function in alternative end-joining repair. Using BLAST, we identified a *Drosophila* LIG3 homolog with 51% identity to human LIG3. To assess possible repair roles of LIG3 we have used *lig3* mutants with a piggyBac transposon inserted in the coding region of *LIG3*. Mutagen studies with methylmethane sulfonate and camptothecin have demonstrated an extreme sensitivity in homozygous *lig3* mutants. Interestingly, *lig3* males have increased sensitivity relative to females. To determine if LIG3 has a role in the ligase IV-independent DSB repair pathway, we are currently comparing viability differences and mutagen sensitivities in *lig3 lig4* double mutants. Additionally, we will use the previously described P-element assay to assess the efficacy and fidelity of DSB repair in both *lig3* and *lig3 lig4* mutants.

316A

Endogenous interbands of D. melanogaster polytene chromosomes contain non-coding regions of genome and form ectopic interbands. T. Yu. Vatolina, S. A. Demakov, V. F. Semeshin, V. V. Shloma, I. F. Zhimulev. Russian Academy of Sciences, Institute of Cytology and Genetics, Novosibirsk, RU.

An electron microscopic analysis (EM) of polytene chromosomes of transgenic flies revealed that P transposons are inserted predominantly into interbands (IBs). To characterize more IBs to identify the DNA sequences necessary and sufficient for IBs formation two constructs were created. The transposons pICon-3C and pICon-61C (Ideal Construct) contain the 1,5 kb and 4,7 kb long DNA fragments from the IBs 3C6/C7 and 61C7/C8 accordingly, placed between two FRT sites. EM analysis of salivary gland polytene chromosomes demonstrated that both types of constructs formed distinct yet morphologically similar IBs. Following FRT/FLP recombination, 7 stocks were established where the transposons that differed only in the absense of the IBs DNA, were present in exactly the same genomic position. Consistently, in all of these stocks a disappearance of the ectopic IB from transposon insertion site was observed. Thus, accurate mapping of DNA sequences which are necessary for IB formation is now possible. EM analysis of 7 integration sites showed that the transposons integrated in IBs. Using “plasmid rescue” assay we have cloned DNA of IBs 1A8/1B1-4, 5F3(4)/F5-6, 8E9/8E10-11, 67B9-B13, 79D1-2/D3, 84F10/F11-12 and 87C8/C9. The IB sequences spanning 2 kb in both directions from insertion sites were analyzed. Comparative analysis of the DNA sequences demonstrated that: 1) IBs are unique sequences of Drosophila genome; 2) in most cases insertion sites map to the non-coding regions (introns and intergenic spacers); 3) virtually all IBs studied display high potential of binding to nuclear matrix proteins; 4) all IBs contain short, high conservative sites with unknown functions. Furthermore, the transfer of IB DNA into novel genetic environment does not restrict IB formation. The experiments performed do not support the idea on the existence of distinct cytogenetic “band + interband” units and suggest the autonomy of the decompacted state of IBs.

317B

Tissue-specific roles of Drosophila DNA ligase 4 in suppressing large deletions during repair of complementary-ended DNA double strand breaks. Amy M. Yu, Mitch McVey. Department of Biology, Tufts University, Medford, MA.

Inaccurate repair of DNA double strand breaks (DSBs) by end-joining pathways can cause genome-destabilizing events. The predominant end-joining repair pathway in vertebrates depends on DNA ligase 4 (Lig4). Lig4-independent end joining is slower and less accurate than Lig4-dependent end joining. Thus, Lig4-independent end joining may contribute substantially to genomic instability, but it is not well studied in vivo, as deletion of Lig4 in vertebrates is embryonic lethal.

Flies repair DSBs by both Lig4-dependent and independent end-joining pathways. Lig4-null flies are viable and fertile. Fly requirements for Lig4 vary with tissue and developmental context. Prior investigations of fly Lig4-independent end-joining repair have studied its role in repairing radiation or P-element induced DSBs, which both require processing before re-ligation. The role of Lig4-independent end joining in repairing a directly re-ligatable DSB is not known. Therefore, we have undertaken studies to ask if in flies, abrogation of Lig4-dependent end joining affects repair of the complementary-ended DSBs produced by the I-SceI endonuclease.

Preliminary results show that relative to wild-type flies, repair of I-SceI induced DSBs in eye cells of lig4 mutant flies is more likely to cause large deletions. Repair products containing large deletions are rarely recovered from the pre-meiotic germline of both wild-type and lig4 male flies, though germline repair events in lig4 mutant flies often contain sequence alterations characteristic of end joining. Thus, a less error prone Lig4-independent end-joining pathway may operate in the male pre-meiotic germline. These results are consistent with tissue-specific regulation of end-joining repair fidelity. Experiments are underway to characterize the variation in repair products produced by Lig4-independent DSB repair.

318C

ESC-like forms the ESCL/E(Z) complex and functions during embryonic and postembryonic stages. Feng Tie, Rebeccah Kurzhals, Carl Stratton, Jayashree Prasad-Sinha, Peter Harte. Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

The Drosophila Polycomb-Group (PcG) protein ESC-like (ESCL) has a high degree of sequence similarity to ESC in both its WD domain and amino (N)-terminal region. In contrast to ESC, which is present mainly during embryogenesis, ESCL is continuously present from early embryo to adults. Although ESCL can replace ESC in the ESC/E(Z) complex when ESC disappears, it is unclear whether ESCL plays a role during embryogenesis when both ESC and ESCL are present. Like ESC, ESCL binds directly to E(Z) via its WD region and to histone H3 via its N-terminus, and mediates E(Z)-dependent methylation of histone H3 lysine 27. ESCL also binds, via its N-terminus, to itself and to ESC. The binding of ESCL to histone H3 is enhanced in the presence of ESC. ESCL/E(Z) complexes are present not only at postembryonic stages but also during embryogenesis when the ESC/E(Z) complexes are also present. ESCL binds to chromosomes and Polycomb Response Element (PRE) in embryos. Our data suggest that ESCL functions not only in larval and adult stages when ESC is absent, but also during embryogenesis when ESC is relatively abundant. We also present genetic analysis of escl that indicates it is required during both embryonic and postembryonic stages.

319A

Repetitious Element *1360* as a Target for Heterochromatin Formation in *Drosophila melanogaster*. Kathryn L. Huisinga, Alejandra Figueroa-Clarevega, Stephen McDaniel, Shachar Shimonovich, Amy Wu, Jo Wuller, Sarah C.R. Elgin. Department of Biology, Washington University, Saint Louis, MO.

Position effect variegation (PEV) occurs in Drosophila when normally euchromatic genes are relocated to a heterochromatic domain, either by rearrangement or transposition. This variegating phenotype, which results from silencing of gene expression in some of the cells where it is normally active, is indicative of heterochromatin formation. Earlier work suggested that the repetitious element *1360* can serve as an initiation site for heterochromatin formation in the fourth chromosome (Sun et al. 2004. Mol Cell Biol. 24:8210). To directly test if *1360* is capable of initiating heterochromatin formation, *P* element constructs containing either one or four copies of *1360* upstream of an *hsp70-white* reporter were constructed and transformants carrying the transgenes were recovered (Haynes et al. 2006 Curr Biol. in press). Additional lines were generated by mobilization of X-chromosome *P* element insertions. For each of the *1360*-containing *P* elements 2500 males were screened for mobilization. With one copy of *1360*, 4% of recovered lines exhibit PEV and with four copies of *1360*, 12% of recovered lines exhibit PEV. Previous mobilizations of an *hsp70-white P* element reporter lacking the *1360* element resulted in a 1% recovery rate of flies exhibiting PEV (Wallrath & Elgin. 1995. Genes Dev. 9:1263). These results suggest that an increasing number of copies of *1360* increase the likelihood of initiating heterochromatin formation. Interestingly, the percentage of variegating lines recovered on the fourth chromosome has not increased. Rather, a higher fraction of the variegating lines are now on the second and third chromosomes. Precise sites of insertion are being mapped to determine if an increase in *1360* copy number allows ectopic heterochromatin formation outside of the regions normally considered heterochromatic.

320B

Studies of the Preferential Male Lethality of Mutants for the HOAP Protein. Hui Li, Michelle Collins, Rebecca Kellum. Biology, University of Kentucky, Lexington, KY.

The telomere capping HOAP (HP1/ORC Associated Protein) protein was originally identified as a component of a multiprotein complex containing heterochromatin protein 1 (HP1) and subunits of the origin recognition complex (ORC) that was isolated from the maternally loaded cytoplasm of early Drosophila embryos. HOAP immunostaining of polytene chromosomes showed prominent enrichment of HOAP in telomeres, with weaker staining also observed in regions of pericentric heterochromatin and numerous euchromatic bands. To examine the possible role of HOAP at these euchromatic sites, we have used Affymetrix Drosophila Genome Arrays to undertake a microarray expression profiling study of mutants for HOAP (cav). These experiments identified 105 genes that are down-regulated and 21 genes that are up-regulated in cav mutant larvae when using a log 2r cut-off value of 3.5 and pvalue <0.01. Analyses of the gene representation in tissue-specific EST libraries revealed 77% of the down-regulated genes to be represented only or primarily in adult testes-derived EST libraries. We used an RT-PCR assay with sex-specific primers for amplification of alternatively spliced tra transcripts to test the possibility that the observed testes bias reflects preferential male lethality of cav mutant larvae, as has been observed in animals depleted for the HP1 or Su(var)3-7 heterochromatin proteins. A bias for the female-specific tra transcript was, indeed, observed in cav mutant RNA. We also observed a two- to three-fold reduction in viability of males expressing cav interference RNA through a transgenic Gal4-induced RNA interference system. We are currently using a variety of experimental approaches to examine the possible role of the dosage compensation machinery, presence of a Y chromosome, and male-specific regulatory roles for HOAP and HP1 in the preferential male lethality.

321C

Short and long-range trans-regulatory interactions mediated by reciprocal translocations with a heterochromatic break point on the fourth chromosome. Martin Muller[1], Daryl Gohl[2], Henrik Gyurkovics[3], Olivier Cuvier[4], Markus Affolter[1], Paul Schedl[2]. 1) Dept. of Cellbiology Biozentrum CH-4056 Basel; 2) Dept. of Molecular Biology Princeton University Princeton NJ 08544; 3) Institute Of Genetics BRC H-6701 Szeged; 4) Institute of Human Genetics CNRS F-34396 Montpellier.

Transposons carrying the Polycomb responsive element (PRE) Mcp from the Bithorax complex can show pairing-dependent silencing of an associated mini-white (mw) reporter in the adult eye. This effect is position dependent. Work from the Kassis lab has indicated that the position dependence might be explained by the presence of other PREs in the vicinity of a P{mw, Mcp} insert. For instance, a P{mw, Mcp} insert showing normal dosage-dependent expression of the mw reporter may become pairing-sensitive if it is juxtaposed into the vicinity of another PRE following to a genomic rearrangement. In order to see if such rearrangements could be isolated, an X-ray screen was initiated using a non-pairing-dependent insert on the 3rd chromosome at 69B. One candidate rearrangement was recovered. Flies trans-heterozygous for the rearranged P{mw, Mcp}-bearing chromosome and the original P{mw, Mcp} insert have clearly lighter eye color than P{mw, Mcp}/+ controls. The cytological analysis of the rearranged chromosome showed that it is associated with a reciprocal T(3;4) translocation, where one break is at 69B, and the other is at the base of the 4th chromosome. Additional genetic characterization of the pairing-dependent interaction revealed that it is dependent on heterochromatin components and not on proteins involved in Polycomb-mediated silencing. Further investigations of this phenomenon have shown that (a) the pairing-dependent silencing effect is independent of the Mcp element (b) trans-silencing of mw reporters can be detected on both sides of the 3rd chromosomal break (c) trans silencing can be effective over more than 100 kb and can be discontinuous. In analogy to the model for bwD, we propose that reciprocal heterochromatic T(3;4) translocations mediate trans silencing by relocating the sister chromosome into a heterochromatic environment.

322A

Investigating the Role of *rDNA* in Genomic Imprinting. Silvana Paredes, Keith Maggert. Dept Biol, Texas A&M Univ, College Station, TX.

Genomic imprinting is the differential behavior that a gene or chromosome can exhibit according to the sex of the parent from which it has been inherited. The disruption of this differential behavior may lead to inappropriate gene regulation and several diseases. Surprisingly little is known about this epigenetic phenomenon. How it is established and maintained during adult life is not well understood. Imprinting has been observed in some mammals, plants, and insects such as *Drosophila melanogaster*. In *Drosophila*, only the sex chromosomes show imprinting. In addition to imprinting, the *X* and *Y* chromosomes exhibit other epigenetic phenomena. Since they are the only chromosomes that harbor *rDNA*, epigenetic phenomena linked to *rDNA*, such as Nucleolar Dominance and epigenetic silencing, are also exclusive to this chromosomes. These features about the *X* and *Y* chromosomes of *Drosophila* led us to believe that there may be a relationship between imprinting and the *rDNA*. In order to address the role of the *rDNA* in imprinting, we used the *I-CreI* homing endonuclease to create fly lines with *rDNA* deletions and evaluate the effect of the deletions on imprinting. In deficient lines, we estimated the *rDNA* amount by Real Time PCR and genetically analyzed the imprinting pattern using an imprinted *white*$^{+}$ gene, inserted into the *Y* chromosome. After the induced expression of the *I-CreI* endonuclease, we see increased expression of the *white*$^{+}$ gene. As we expected, according to what it is know about *rDNA* magnification, this increased expression is lost in the next generation. Our preliminary data suggest that there is a relationship between *rDNA* and the expression of the *white*$^{+}$ gene. We are addressing whether the observed effect of the *rDNA* deletions is related to imprinting, Position Effect Variegation (PEV), or some other phenomenon.

323B

Studies on the function of MU2. Raghuvar G Dronamraju, James M Mason. Laboratory of Molecular Genetics National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Telomeres play an important role in the stabilization of chromosome ends. A large number of proteins are known to be involved in telomere maintenance. A mutation in one of these proteins leads to telomere fusions. Mutations in *mu2*, however, decrease the rate of DNA repair and potentiate the recovery of terminal deficiencies. Previously, we proposed that *mu2* plays an important role in the repair of DSBs by modulating chromatin structure. This conclusion is supported by the observations that *mu2* mutations are recessive suppressors of PEV, and that MU2 interacts in two-hybrid experiments with HP1, which is an important component of chromatin. We used eGFP as a tag in our studies on the function of MU2 protein. As expected, MU2 localized to the nucleus after transient transfection of S2 cells. We plan to use the eGFP tag for co-Immunoprecipitation with HP1 to verify the yeast two hybrid results and to study the biochemical properties of the protein and the effects of radiation. In addition, over-expression of *mu2* in flies under the control of the Actin 5C (ubiquitous) and nos (ovary-specific) promoters may help to elucidate the function in specific organs. We also plan to overexpress *mu2* in repair deficient backgrounds to ask whether it has any effects on the efficiency of DNA repair. Since the distribution of MU2 protein is as yet unknown, we intend to express *mu2* under its own promoter with an eGFP tag and ascertain the developmental expression pattern. A stable cell line is being generated. In addition, N-terminal 6X His tagged MU2 was over-expressed in bacteria and purified for the generation of polyclonal antibodies. Generating polyclonal antibodies to MU2 would give a more realistic picture of the expression during development. These studies may throw light on to the as yet unknown but important functions of MU2, which may help in the better understanding of the chromatin dynamics during DNA repair.

324C

Rapid evolution of Drosophila telomere proteins. Nels C. Elde, Harmit S. Malik. Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

The telomeres of Drosophila are remarkable evolutionary variants, which do not employ telomerase to replenish chromosomal ends. Instead, specific retrotransposons function as telomeres. Despite this fundamental difference, telomeres of all species share many common features. Numerous factors interact with telomeres to protect these vital genomic regions. This includes telomere-capping proteins, some of which are DNA repair factors. The involvement of such proteins in the regulation of telomeres highlights the relationship between telomeres and chromosomal stability. Defects in telomeres result in abnormalities associated with aging and nearly all forms of cancer. A novel, fast evolving Drosophila gene, named *caravaggio*, has an essential role in telomere capping. Disruptions of *caravaggio* are known to cause extensive telomere fusions. This was unexpected, because essential genes are commonly under strong evolutionary constraints to maintain vital functions. A clue, which may explain this paradox, comes from the observation that many rapidly evolving genes co-evolve in relationships promoting adaptive changes. Examples include genes engaged in antagonistic interactions, such as host-pathogen conflicts. Drosophila telomeres appear similar because cells co-opted otherwise autonomous, or "selfish," retrotransposons to maintain chromosomal ends. Therefore, a hypothesis accounting for the rapid evolution of *caravaggio* is that it has been subject to opposing selective forces promoting co-evolution with fast evolving telomeric retrotransposons, while also maintaining an essential function. Supporting this view, we detected evolutionary signatures consistent with positive selection in a defined region of *caravaggio* in independent comparisons between Drosophila species. Experiments testing the consequences of rapid evolution in *caravaggio* will provide insight toward understanding the role of such fast evolving genes in telomere function.

325A

Recycling the genome: Umbrea, a telomere-associated protein in *Drosophila*, arose from a duplicated HP1 protein. Mary Alice Hiatt, Nels Elde, Danielle Vermaak, Harmit Malik. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Heterochromatin makes up a large portion of eukaryotic genomes, but due to the repetitive nature of heterochromatic sequences and paucity of genes, studying heterochromatic sequence is difficult. An alternative to directly studying heterochromatin is to study the proteins that interact with it, such as Heterochromatin Protein 1A (HP1A), a constitutive component of heterochromatin and *Drosophila* telomeres. *Drosophila* maintain telomeres by transposition of retrotransposons to chromosome ends. HP1A contains two conserved domains, chromo and chromo shadow. The chromo domain is important for binding histones and controlling telomere elongation. The chromo shadow domain is necessary for protein interactions and dimerization, and telomere protection. There are five known paralogs of HP1A in *Drosophila* that contain both conserved domains, but we recently identified a paralog, Umbrea, with only a chromo shadow domain. *Umbrea* is the result of a duplication of *Heterochromatin Protein 1B (HP1B)*. While many duplicate genes become pseudogenes or sub-functionalize, we propose that *Umbrea* has undergone neofunctionalization, thereby acquiring a novel function. We have identified several factors that support neofunctionalization of *Umbrea*. *Umbrea* and *HP1B* are under different selective constraints; *Umbrea* is undergoing positive selection, while *HP1B* is highly conserved. Umbrea also has a distinct localization pattern from HP1B in *D. melanogaster* cells; HP1B has been shown to localize to both het- and eu- chromatin, while Umbrea forms discrete dots in heterochromatin. Umbrea also interacts a known telomere protein, Caravaggio, which is important for telomere protection. *Umbrea* is an example of a duplication that has diverged and acquired a novel function. The mechanisms of telomere protection and elongation in *Drosophila* are not well understood. Studying *Umbrea* will contribute to our understanding of *Drosophila* telomeres, and the evolutionary processes that shape new genes.

326B

Mutator2: A possible chromatin modulator at telomeres. Sudha Prasad, James M Mason. Lab Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC.27709.

The two important functions of telomeres, maintenance of terminal sequences and, distinction of a chromosome end from a double strand break, can be separated in Drosophila. Previous work from the lab has shown that mutants for mu2 exhibit delayed repair of γ-ray-induced lesions in oocytes, as well as reduced meiotic recombination and increased spontaneous somatic recombination. Induced oocyte lesions that are not repaired by the time of fertilization are stabilized in the zygote as terminal deficiencies. We hypothesize that mu2 controls some aspect of the oocyte nucleus, possibly chromatin structure or association of the chromosomes with the matrix, that modulates both DNA repair and meiotic recombination. Several heteroallelic combinations of mu2 mutations, suppressed PEV, suggesting that mu2 might render its effects by chromatin remodeling. Such chromatin-mediated effects can be achieved by protein-protein interactions. To identify such interactions we looked for binding partners by yeast two-hybrid technology. The cDNA corresponding to MU2 protein was screened against available Drosophila egg and S2 libraries. We found that a region of the MU2 protein close to the BRCT domain interacts with the chromo shadow domain of Su(var)205. We are in the process of narrowing down the MU2 region that might be associated with such interaction. To localize in-vivo and in-vitro MU2 protein, we have identified amino acid regions near the N- and C-termini unique to MU2 for expression in a bacterial system and are in the process of purification of these fragments for further experimentation.*mu2Su(var)205*.

327C

Epigenetic *trans*-silencing and *P* element repression in *Drosophila melanogaster*. Stéphane Ronsseray, Anne-Laure Todeschini, Thibaut Josse, Laure Teysset, Augustin de Vanssay, Dominique Anxolabéhère. Dynamique du Génome, Inst. Jacques Monod, Paris, France.

P transposable element is a recent invader of the *Drosophila melanogaster* genome. *P* copies inserted at the *X* chromosome telomere, in heterochromatic "Telomeric Associated Sequences" (TAS), have strong *P* element regulatory properties which are highly sensitive to mutations in genes involved in heterochromatin formation (*Su(var)205* encoding HP1) and in RNA interference (*aubergine*) (Ronsseray et al. 1996, Reiss et al. 2004). *P-lacZ* transgenes or defective *P* elements inserted in TAS can also repress in *trans* an euchromatic *P-lacZ* in the germline (Roche and Rio 1998, Marin et al. 2000). This last phenomenon has been called "*Trans*-Silencing Effect" (TSE). It requires a certain length of sequence homology between the telomeric insertion and the euchromatic transgene (Marin et al. 2000, Ronsseray et al. 2001, 2003). We will present the properties of TSE. Our analysis reveals that: (1) TSE is restricted to the female germline; (2) TSE shows a maternal effect: it is epigenetically transmitted since maternal inheritance can be detected for 5 generations; (3) only telomeric insertions located in TAS can induce TSE (a single transgene in euchromatin or in pericentric heterochromatin does not induce TSE); (4) all euchromatic *P-lacZ* insertions tested, located on all major chromosomes, are sensitive to TSE; (5) an incomplete TSE leads to variegation; (6) TSE is sensitive to the dose of HP1 and of SU(VAR)3-7, two proteins which are components of heterochromatin; (7) TSE is sensitive to mutations affecting *aubergine, homeless* and *piwi*, three genes involved in RNA interference. These results allow us to propose that TSE involves a small RNA-dependent heterochromatin formation pathway which was coopted by the *P* element to establish its own transposition repression after its recent invasion of the *D. melanogaster* genome.

328A

The multiplicity of *Drosophila* telomeric retrotransposons unveils extensive and rapid evolution of *Drosophila* telomeres. Alfredo Villasante[1], Rosario Planelló[1], María Méndez-Lago[1], Susan Celniker[2], José P. Abad[1]. 1) Centro de Biologia Molecular, CSIC-Univ Autonoma, Madrid, Spain; 2) Department of Genome Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA.

Drosophila telomeres do not have arrays of simple telomerase-generated G-rich repeats. Instead, *Drosophila* maintains its telomeres by occasional transposition of specific non-long terminal repeat (non-LTR) retrotransposons to chromosome ends. The genus *Drosophila* provides a superb model system for comparative telomere analysis. PCR and in silico surveys in the sibling species of *Drosophila melanogaster* and in more distantly related species show that multiple telomeric retrotransposons (*TRs*) maintain telomeres in *Drosophila* and that the recurrent loss of most of its ORF2 gives rise to half-telomeric-retrotransposons (*HTRs*) during *Drosophila* evolution. The phylogenetic relationship among these telomeric elements is congruent with the phylogeny of the species, suggesting that they have been vertical inherited from a common ancestor. Our results indicate that an existing non-LTR retrotransposons was recruited to perform the cellular function of telomere maintenance. This extensive and rapid evolution of *Drosophila TRs* may recapitulate the ancestral mechanism that led to the common origin of telomerases.

329B

Nerfin-1: A novel binding partner of Scalloped. Ankush Garg[1], Alexander Kuzin[2], Thomas Brody[2], Ward Odenwald[2], John Bell[1]. 1) Dept Biological Sci, University of Alberta, Edmonton, AB, CA; 2) Neural Cell-Fate Determinants Section, National Institutes of Health, Bethesda, MD, USA.

Scalloped (SD), a TEA/ATTS domain containing protein, is required for the proper development of *Drosophila melanogaster*. Despite being expressed in a variety of tissues, most of the work on SD has been restricted to understanding its role and function in patterning the adult wing. In the wing SD interacts with a co-factor, Vestigial (VG). Previous experiments have demonstrated that SD cannot activate transcription on its own and requires VG to form a functional transcriptional complex. In the mammalian system the SD homolog, TEF-1, is known to bind to several different co-factors in a tissue specific manner. These observations led to the idea that SD requires co-factors to activate transcription. Using a series of *in vivo* and *in vitro* experiments, we were able to identify a novel binging partner of SD - Nervous finger-1 (nerfin-1). Nerfin-1 is a Zn finger transcription factor that is expressed in neural precursor cells and in the eye imaginal disc.

330C

Ash1, Pc and Pho influence embryonic transcriptional activation by Myc via a bivalent chromatin domain. Julie Goodliffe[1], Michael Cole[2], Eric Wieschaus[3]. 1) Biology Department, UNC Charlotte, Charlotte, NC; 2) Dartmouth Medical School, Lebanon, NH; 3) HHMI, Princeton University, Princeton, NJ.

Myc is a transcriptional regulator whose function is essential for normal development. An excess of its function, however, leads to tumorigenesis and is present in 50% of human tumors. Transcriptional activation by Myc requires its binding to DNA for activation of target genes, and 11% of cellular loci in the human genome contain high-affinity binding sites for Myc. Repression by Myc, however, is not mediated by binding to its canonical binding site, and therefore Myc must be recruited to sites by other factors. Many important aspects of Myc biology remain unclear, including: how do normal Myc-expressing cells utilize Myc's essential functions while avoiding Myc's ability to induce tumorigenesis? To identify chromatin-binding proteins with a potential role in controlling Myc activity, we conducted a genome-wide screen using a mini-white reporter for Myc activity, and identified the Trithorax Group protein Ash1 as a modifier of dMyc activity. Ash1 is a histone methyltransferase known for its role in opposing Polycomb repression of homeotic genes. Using RNAi in the embryo and Affymetrix microarrays, we show that ash1 RNAi causes the increased expression of many genes, suggesting that it works as a repressor in the embryo in contrast to its known role in maintenance of activation. Many of the Ash1 repression targets are also repressed by Pc and Pho, as determined by concurrent microarray analysis of Pc and pho RNAi embryos, suggesting that the three have common repression targets. Further, many of these overlapping targets are also activated by Myc overexpression. Genetic, genomic and chromatin immunoprecipitation data suggest a model in which Myc's embryonic targets are within a reversible bivalent chromatin domain maintained by Ash1, Pc and Pho, allowing for controlled activation or repression upon differentiation.

331A

Cell cycle regulation by retinal determination genes. Jennifer Jemc[1], Ilaria Rebay[2]. 1) Dept. Biology MIT, Cambridge, MA; 2) Ben May Inst, Univ Chicago, Chicago, IL.

Cell cycle regulation is vital throughout the life of an organism, from early development when aberrant regulation can lead to defects in cell and tissue specification to later in life, when misregulation can lead to diseases like cancer. Multiple signaling pathways have been implicated in regulating the proper spatial and temporal expression of cell cycle genes. Recent work in vertebrates has suggested that members of the retinal determination gene network (RDGN) regulate cell cycle genes during development and may themselves be upregulated in multiple cancers. Using a microarray approach to identify transcriptional targets of the Drosophila retinal determination proteins Eyes Absent (EYA) and Sine Oculis (SO), we identified the cell cycle regulator *string* (*stg*) as an EYA-SO target. In addition to previous data suggesting regulation of this gene by the EGFR transcription factor, Pointed, and the transcriptional repressor Tramtrack, EYA and SO are also able to induce *stg* expression. Examination of the *stg* genomic locus has revealed potential binding sites for SO as well as additional transcription factors, including Su(H), suggesting multiple layers of transcriptional control are required to regulate *stg* expression. We are investigating the control of *stg* expression by these factors in coordination with EYA and SO. Given the recent characterization of EYA as a phosphatase, the possibility of differential regulation of targets by EYA phosphatase activity is also being examined. Regulation of *stg* by multiple transcription factors, including members of the RDGN, emphasizes the complexity underlying proliferation control during development and suggests a mechanism for how the eye-specific program of cell division may be regulated. Through identification of EYA-SO transcriptional targets and examination of additional factors impinging on the expression of these targets we aim to understand how EYA and SO are integrated with multiple signaling pathways during eye development.

332B

Identifying the activator of early zen expression during early Drosophila embryos. Chung-Yi Nien, Hsiao-Yun Liu, Nikolai Kirov, Christine Rushlow. Department of Biology, New York University, New York, NY.

zen has a dynamic expression pattern in early embryonic development. zen transcripts appear at cell cycle 10 in a broad dorsal-on/ventral-off pattern that is replaced by a dorsal stripe by the end of cellularization. The transcription of the early broad pattern is repressed by Dorsal protein, but the activator has remained elusive. During our studies on the zen ventral repression element we found a small enhancer that could drive activation of a lacZ reporter gene in early embryos. Within this fragment are four copies of the sequence C/TAGGT/CAG, which was recently reported by ten Bosch et al. (2006) to mediate the early transcription of not only zen, but also three of the four XSEs (X-chromosome signal elements), sisA, sc, sisC, but not runt. They termed these sequences TAG-team sites. We performed a yeast one-hybrid screen using a 0-6 hr embryonic cDNA library of GAD-tagged cDNAs (GAL4 Activation Domain; Yu et al., 1999), and a target-reporter construct containing a 90 bp fragment from the zen enhancer with four TAG-team sites. We identified a putative zinc-finger containing protein as the best candidate for the zen activator. We designated this gene zexless (zex) for zen/Sxl-less, for the time being. We showed that a Zex-GST fusion protein containing four of the six zinc fingers binds to oligonucleotides containing TAG sites in gel mobility shift assays. in situ hybridization showed that zex transcripts are present in mid-stage egg chambers in germ line cells but not follicle cells. Transcripts are also ubiquitous in very young embryos. These results indicate that zex is an excellent candidate for the transcriptional activator of zen. We are in the process of creating a null mutation in zex and will then determine if zen and XSE transcripts are absent in zex germ line clones.*zen, zex, sisA, sc, sisC, runtzen/Sxl-lesslacZzexless*.

333C

Whole genome analysis of Bcd dependent transcriptional regulation in the early fly embryo. Amanda Ochoa-Espinosa, Stephen Small. Department of Biology, New York University, New York, NY.

bicoid (*bcd*) is a major patterning gene during early *Drosophila* development; it encodes a transcriptional activator that forms a morphogenetic gradient along the anterior-posterior (AP) axis of the embryo. The threshold model of Bcd function proposes that the enhancers of Bcd target genes contain binding sites that make them differentially sensitive to Bcd concentration, and that sensitivity controls where they are expressed along the embryo length. One of the aims of my current research is to re-visit the Bcd morphogen hypothesis. Even though it is known that the interaction of several factors is necessary to achieve the final expression domains of individual target genes, it is still relevant to ask whether Bcd target genes are activated via a threshold dependent mechanism. The second goal is to identify novel Bcd regulated genes. Our strategy consists of expressing varying uniform levels of bcd all over the embryo and performing microarray experiments from the RNA of these embryos. In doing so, we aim to learn about the transcriptional regulation of genes involved in the segmentation cascade, specifically in terms of the response to Bcd levels, as well as to identify novel genes functioning in the early embryo. Preliminary *in situ* experiments of diagnostic genes in embryos with different uniform levels of Bcd show that in some cases specific Bcd target genes are either uniformly on or completely off in these embryos. Quantitative real time PCR experiments suggest that the different levels of expressions observed by *in situ* are accurately reflected using this technique. Microarray gene expression results from embryos with different uniform levels of Bcd the will be discussed.

334A

RNAi effects on Domino. Jim Zhong, Wooly Pierre, Barry Yedvobnick. Biology Department, Emory University, Atlanta, GA.

Our lab has been screening the genome for EP induced modifiers of a dominant negative loss of Notch pathway signaling. The screen is based on GAL4-UAS mediated overexpression of truncated Mastermind (Mam) across the wing margin. Previously, we found that an EP insertion upstream of the domino locus enhanced the wing phenotype associated with expression of the Mam truncation (Hall et al 2004). Domino is related to the human SRCAP protein that functions in chromatin remodeling, and it was recently reported that Domino functions as a transcriptional activator of Notch targets necessary for proper wing margin formation (Eissenberg et al 2005). We are investigating the role of Domino in Notch signaling via RNA interference. Exonic domino sequence has been cloned into the SympUAST vector, which allows GAL4-UAS directed expression of double stranded RNA (Giordano et al 2002). Strains containing multiple copies of Symp-Domino transgenes elicit wing margin defects when crossed to wing margin GAL4 drivers. We are currently testing the strains for effects in other tissues.

335B

Virtual embryos as tools for 3D gene expression analyses. Cris L. Luengo Hendriks[1], Charless C. Fowlkes[2], Soile V.E. Keränen[3], Lisa Simirenko[3], Gunther H. Weber[4], Oliver Rübel[4], Min-Yu Huang[4], Angela H. DePace[3], Clara N. Henriquez[3], Xiao-Yong Li[3], Hou C. Chu[3], David W. Kaszuba[1], Amy Beaton[1], Susan E. Celniker[1], Bernd Hamann[4], Michael B. Eisen[3], Jitendra Malik[2], David W. Knowles[1], Mark D. Biggin[3]. 1) Life Sci. Div., Lawrence Berkeley Lab, Berkeley, CA; 2) Computer Sci. Div., UC Berkeley, CA; 3) Genomics Div., Lawrence Berkeley Lab, Berkeley, CA; 4) Inst. for Data Analysis and Visualization, UC Davis, CA.

The Berkeley *Drosophila* Transcription Network Project (BDTNP) is a multidisciplinary collaboration for studying the developmental regulatory network of *Drosophila* blastoderm embryos. One component of this project maps the blastoderm expression patterns of 37 principal developmental regulatory genes and hundreds of their targets at cellular resolution, and uses these data to model potential regulatory interactions. We have now generated such 3D data for 24 of the principal regulators and over 80 putative target genes, the latter selected using BDTNP ChIP-chip binding data and BDGP expression data. Gene expression data in regulatory factor mutant embryos and other *Drosophila* species is also being collected. Because each imaged embryo contains expression information of only two genes, expression data from hundreds of embryos is mapped onto a virtual embryo to allow many genes' expression to be compared and modeled within each cohort. These virtual embryos contain nuclei placed to match the average density pattern and embryo shape for each cohort. This allows temporal comparison within each nucleus between earlier expression of regulators in one cohort to the later expression of target gene patterns in another cohort, as well as better estimates of the developmental increase in complexity. Gene expression in such virtual embryos can be viewed with our tool called PointCloudXplore, which provides realistic interactive exploration of the 3D expression data as well as abstract views for analyzing the correlation between expression patterns within the N-dimensional gene expression space.

336C

Elucidation of molecular processes involved in enhancer blocking by the Su(Hw) insulator protein. Brian McCluskey[1], David Gilmour[2], Pamela Geyer[1]. 1) Biochemistry, University of Iowa, Iowa City, IA 52242; 2) Biochemistry, Pennsylvania State University, University Park, PA 16802.

Insulators are specialized DNA sequences that define domains within chromosomes to allow appropriate levels of gene expression. Insulators block enhancer and silencer activity when placed between these regulatory elements and a promoter. The mechanism used by insulators to prevent enhancer-activated transcription is poorly understood. To understand these processes, we study the Drosophila Suppressor of Hairy-wing [Su(Hw)] insulator protein that forms a chromosomal complex with two BTB/POZ domain proteins, Mod(mdg4)67.2 and Centrosomal Protein (CP) 190. We have developed a reporter gene system, wherein enhancer-activated transcription depends on the binding of the defined Tet(on)-VP16 fusion protein to five Tet(on) binding sites upstream of a minimal *heat shock protein (hsp70)* promoter. We find that insertion of Su(Hw) binding sites, but not equivalently-sized spacer DNA, between the Tet(on) binding sites and the *hsp70* promoter blocks enhancer activation in a Su(Hw) protein-dependent manner. Using potassium permanganate footprinting, we demonstrate that the Su(Hw) protein prevents recruitment of RNA Pol II to the reporter gene. These studies are being extended to define how recruitment of RNA Pol II by Tet(on)-VP16 is disrupted. Our studies will provide insights into mechanisms used by insulators to block enhancer signaling.

337A

***Bhringi*, a highly conserved regulator of Twist transcription factor activity.** Scott J. Nowak[1], Katie Gonzalez[2], Mary K. Baylies[1]. 1) Baylies Laboratory/RRL 1065, Sloan Kettering Inst, New York, NY; 2) Scripps Research Institute, La Jolla, CA.

A modified yeast two-hybrid screen exploiting the differential regulation of the *tinman* and *Dmef2* enhancers revealed CG8580, a gene we have named *bhringi* (*bhr*), as a novel Twist interaction partner on Twist-dependent enhancers. The phenotypes of *bhringi* mutants reveal a role for *bhr* during muscle development: loss of *bhr* during embryogenesis results in muscle loss, severely altered muscle morphology, and defective muscle attachments. *bhr* encodes a novel 201 residue protein with no identifiable features, yet regions of the Bhringi protein are highly conserved among across numerous species, including *C. elegans*, *Xenopus*, *ticks*, mice and humans. The *bhringi* transcript and protein is expressed uniformly throughout the embryo during all phases of embryogenesis. Bhringi is detected via immunohistochemistry in the nucleus, where it co-localizes with Twist. Additionally, Bhringi interacts with Twist both genetically and physically by GST-pulldown and co-immunoprecipitation *in vitro*. Further, loss of specific Twist-dependent target gene expression is also observed in *bhringi* mutants. Bhringi is also capable of interaction with subunits of the Brahma chromatin remodeling complex, thereby indicating a possible mechanism for Bhringi-dependent remodeling of chromatin for the proper expression of Twist-dependent genes during *Drosophila* development.

338B

Identification and characterization of Wingless Response Elements from a direct Wg-target gene, naked cuticle (nkd) in Drosophila. Jinhee Chang[1], Mikyung Chang[1], Scott Barolo[2], Kenneth M. Cadigan[1]. 1) Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109; 2) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor MI 48109.

Secreted proteins of the Wg/Wnt family act through a highly conserved signaling cascade to regulate transcription of target genes. In the current model, TCF, a sequence specific DNA-binding protein, is bound to regulatory cis-elements in the target gene locus and controls transcription, through interacting with other cofactors. In order to learn more about Wg-directed transcriptional regulation, we studied naked cuticle (nkd), a direct target gene of the Wg pathway in Drosophila. The nkd locus contains 20 clusters that at least three or more potential TCF binding sites (consensus STTTGW; S= G or C and W= A or T) within 150 bp. Chromatin Immunoprecipitation (ChIP) with TCF antibody revealed that only 2 of these potential TCF clusters are bound by TCF in Kc cells. To test whether these 2 clusters that are bound by TCF can act as Wingless Response Elements (WREs), we used a reporter gene assay in fly cultured cells. Each WRE can activate reporter gene expression upon Wg signaling in fly Kc, S2R+ and Clone8 cells. Consistent with being directly regulated by Wg signaling, each WRE loses Wg responsiveness when putative TCF sites were mutated. The nkd WREs identified in cell culture are also active in a variety of fly tissues in partially overlapping patterns consistent with activation by Wg signaling. The sum of the WREs covers the entire pattern of nkd expression in wing and leg imaginal discs but the pattern in embryos is incomplete. We are currently testing the sufficiency of the identified WREs in recapitulating nkd expression by using the endogenous nkd promoter instead of the heterologous hsp70 minimal promoter. Also we are going to test the physiological importance of the WREs on nkd expression by deleting WREs from the nkd locus and measuring nkd expression.*Drosophilanaked cuticlenkdcis*.

339C

3-Dimensional quantitative analysis of gene expression in multiple Drosophila species. Angela H. DePace[1], Stewart MacArthur[2], Daniel Pollard[1], Venky Iyer[1], Soile Keränen[2], Clara N. Henriquez[2], Cris Luengo Hendriks[2], Charless Fowlkes[3], Lisa Simirenko[2], Jitendra Malik[3], David W. Knowles[2], Mark D. Biggin[2], Michael B. Eisen[1,2]. 1) Molecular and Cellular Biology, UC Berkeley, CA; 2) Genome Sciences, Lawrence Berkeley National Lab, CA; 3) Computer Science, UC Berkeley, CA.

Understanding how transcriptional regulatory sequences evolve requires us to link changes in sequence with changes in function. The Berkeley Drosophila Transcription Network Project (BDTNP http://bdtnp.lbl.gov) is characterizing the transcriptional regulatory elements that control early development in, *Drosophila melanogaster* and quantitatively describing their function, i.e., the patterns in which the corresponding genes are expressed. A novel set of computational tools converts confocal images of fluorescently stained blastoderm embryos into a composite 3D map where averaged expression patterns for many genes are present in the same cellular resolution morphological framework. We are now applying these high-resolution imaging methods to a closely related Drosophila species, *D. pseudoobscura*. We are determining the expression patterns of key transcriptional regulators and a subset of their targets, including genes adjacent to potential regulatory regions with interesting binding site dynamics, such as overall changes in composition and lineage specific gains and losses. Because we can detect subtle quantitative and spatial changes in expression patterns, this imaging approach is particularly well suited to discovering whether even small sequence changes alter gene regulation. By including many genes in our models, we can interpret these changes in the context of the regulatory network as a whole. Computational analysis of the spatial relationship between gene expression patterns can glean candidate regulatory relationships and allow us to hunt for regulatory novelty at the level of transcriptional network architecture. This type of detailed functional characterization of the output of regulatory elements will allow us to interpret the abundant regulatory sequence variation across the recently sequenced Drosophila species.

340A

Phylogenetic Footprinting Analysis in the Regulatory Regions of the *Drosophila Enhancer of split* Genes. Deborah Eastman, Morgan Maeder, Bryanne Robson, Benjamin Polansky. Dept Biol, Connecticut College, New London, CT.

The Notch signaling pathway regulates cell fate decisions during development. During *Drosophila* neurogenesis Su(H) dependent Notch activation upregulates transcription of the *Enhancer of split-Complex (E(spl)-C)* genes. Seven of these genes code for bHLH transcriptional repressors that function to down-regulate proneural gene activity. All seven genes contain upstream binding sites for Su(H), proneural proteins, and E(spl) bHLH proteins resulting in overlapping expression patterns during embryonic development. However, their expression patterns are distinct during later embryonic stages and in larval imaginal discs. We are interested in determining the regulators that are responsible for this variation of expression. Toward this end we are using phylogenetic footprinting to identify conserved sequences in the promoters of these genes. We have compared the *E(spl)* promoters from nine different Drosophila species and found the highest level of conservation in *mgamma*, *mbeta* and *m4*. Fine analysis of Su(H) sites shows that high affinity paired sites are completely conserved in *m3*, *m4*, *m7*, *m8*, *mgamma*, and *mdelta* as is the Su(H) paired site/proneural site (SPS plus A) architecture conferring expression in proneural clusters. Low affinity Su(H) sites are not well conserved, suggesting that they may be degenerate high affinity sites and may not play a functional role during development. We have identified additional transcription factor binding site consensus sequences upstream of the *E(spl)* genes and compared these sites in nine species of *Drosophila*. Many of these sites are conserved and may provide us with information about how these genes are differentially regulated during development. We have also identified novel sequences that are upstream of multiple *E(spl)* genes and their conservation across *Drosophila* species suggests that they may play a role in the expression of these genes.

341B

Coupling between SxlPe and SxlPm reinforces the female developmental switch. Alejandra Gonzalez, James Erickson. Dept Biol, Texas A&M Univ, College Station, TX.

Sex-lethal is the master regulatory gene that determines sex in Drosophila. Transcription of Sxl is controlled by two different promoters. The establishment promoter, SxlPe, responds to the female dose of two X chromosomes to produce an early form of SXL protein females. In contrast, the maintenance promoter, SxlPm is active in both sexes, but pre-mRNAs from SxlPm are spliced into functional Sxl messages only in the presence of SXL proteins. The transition from the establishment phase to the maintenance phase of sex determination, depends on SxlPe-derived protein products acting on SxlPm-derived mRNAs. Thereafter, sex is maintained by positive autoregulation by, and on, SxlPm-derived products. We have found that the initial activation of SxlPm is also sensitive to X chromosome dose. Some, but not all, of the transcription factors needed to activate SxlPe in XX embryos also cause SxlPm to come on earlier in females than in males. We propose that the use of common regulators at SxlPe and SxlPm helps speed the build up SxlPm transcripts in females thus reinforcing the transition to the stable maintenance mode of Sxl expression.

342C

Evolution of *cis*-regulatory sequences in acalyptrate Cyclorrhapha: sequencing and analysis of 200 developmental loci in *Drosophilidae*, *Sepsidae*, and *Tephritidae*. Emily Hare[1], Brant Peterson[1], Venky Iyer[1], Rudolf Meier[2], Rick Kurashima[3], Eric Jang[3], Brian Wiegmann[4], Michael Eisen[1,5]. 1) Dept Molecular & Cell Biol, Univ of California, Berkeley, Berkeley, CA; 2) Department of Biological Sciences, National University of Singapore, Singapore; 3) Pacific Basin Agricultural Research Center, UDSA Agricultural Research Service, Honolulu, HI; 4) Department of Entomology, North Carolina State University, Raleigh, NC; 5) Genome Sciences Department, Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

Though it is believed that changes in developmental gene expression play a role in organismal evolution, the molecular basis for such change is poorly understood. We are studying how enhancers controlling developmental gene expression change over different evolutionary time scales, and how these changes affect regulatory output and contribute to morphological diversification. Evolutionary analyses in *Drosophila* suggest that developmental enhancers are highly plastic, with functional constraints manifesting themselves in the composition and architecture of transcription factor binding sites rather than linear sequence conservation. However, there is insufficient sequence and functional divergence among the sequenced *Drosophila* species to fully characterize the dynamics of enhancer evolution. To overcome this limitation, we have sequenced 18 developmentally important loci in 11 species of sepsids and tephritids. The families were chosen to provide optimal evolutionary comparisons with drosophilids, and individual species were chosen to facilitate identification of functional sequences by intrafamily comparison. We are identifying potential regulatory regions in these targeted loci and testing their function in transgenic *D. melanogaster* embryos. In parallel we are characterizing the endogenous gene expression patterns in select species by RNA *in situ hybridization*. By studying enhancer evolution in increasingly divergent species we are developing a better understanding of natural variation in functional enhancers and how changes in these enhancers lead to the diversity in Diptera.

343A

Characterization of cis-regulatory elements of the glial gene *repo*. Bradley W. Jones, Jamie W. Lamberton. Department of Biology, The University of Mississippi, Oxford, MS.

Previous investigations identified the *glial cells missing* (*gcm*) gene as a “master regulator” of glial cell fate in the fruit fly *Drosophila* . However, *gcm* is also expressed in and required for the development of larval macrophages and tendon cells, and lamina neurons in the adult CNS. Thus, Gcm protein activates the transcription of different sets of genes in different developmental contexts. How Gcm regulates these different outcomes is not known. Different collaborators, co-activators, and/or repressors must be involved in regulating Gcm target genes in different tissues. Our goal is to identify collaborators that act with Gcm to promote the transcriptional activation of Gcm target genes specifically in glial cells, or prevent their activation in the other tissues in which Gcm is expressed. Here we have focused on the transcriptional regulation of a well-characterized glial-specific Gcm target gene, the transcription factor *reversed polarity* (*repo*); we aim to understand how the transcription of the glial-specific Gcm target gene *repo* is regulated by Gcm and other factors. We have located three different cis-regulatory elements that drive cell-specific expression independent of Gcm binding sites: 1) A distal element that promotes expression in dorsolateral epidermis; 2) A repressor element that suppresses expression in the epidermis; and, 3) A proximal element that promotes expression in a subset of cell body glia. Currently we are characterizing these elements to define minimal sequences required for expression or repression with the goal of identifying interacting factors using genetic, biochemical and bioinformatic approaches.

344B

Transcriptional regulation of *nerfin-1* expression during *Drosophila* neurogenesis. Alexander Kuzin, Mukta Kundu, Thomas Brody, Ward F. Odenwald. Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, MD.

nerfin-1 belongs to a conserved subfamily of Zn-finger transcription factors present in all metazoans including man. Our characterization of loss- and gain-of-function mutants reveals that *nerfin-1* is required for interneuron axon guidance (1). During embryonic, larval and adult nervous system development, *nerfin-1* mRNA is dynamically expressed in both neural precursor cells and nascent neurons. For example, during embryonic CNS development, *nerfin-1* expression is detected in all early delaminating neuroblasts and also in PNS secondary precursor cells. To better understand the regulation of *nerfin-1* expression we have begun to characterize its cis-regulatory elements. An 11 kb *nerfin-1* genomic fragment that contains 6 kb of upstream sequence serves to rescue a *nerfin-1* null mutation. EvoPrinter analysis (2) identifies multiple putative enhancer regions upstream and downstream of the transcribed region. Each of these regions contains multiple conserved sequence blocks (CSBs) that are shared in all *Drosophila* species. *In vivo* transgene analysis of each of these regions reveals that the cis-regulatory enhancer elements that regulate *nerfin-1* expression are modular. For example we have identified an enhancer that drives expression in subsets of neuroblasts and in GMCs, and other enhancers that drive expression in neurons and in PNS secondary precursor cells. To understand the roll of the CSBs within the neuroblast/GMC enhancer, we have generated transgenic reporter lines that contain different combinations of CSBs. Thus far, deletion of a single conserved sequence block within the neuroblast/GMC enhancer alters its expression so that expression is no longer evident in GMCs.

1. Kuzin et al (2005). Dev. Biol. 277: 347-65, 2005
2. Odenwald et al (2005). Proc. Natl. Acad. Sci. 102: 14700-5.

345C

Study of the Transcriptional Regulation of *homothorax* Expression in *Drosophila* Eye. Wei-Wen Lan[1,2], Su-Yi Chang[1,2], Ju-Yu Wang[1], Y.Henry Sun[1,2]. 1) Institute of Molecular Biology, Taipei, TW; 2) Faculty of Life sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, TW.

homothorax (hth) plays multiple roles in *Drosophila* development. It encodes a homeodomain (HD) protein and is essential for the nuclear localization of another HD protein, Extradenticle (Exd). Whereas *exd* is ubiquitously expressed, *hth* is expressed in very specific patterns. Therefore, the expression of *hth* defines the spatio-temporal specificity of the Hth-Exd complex. To understand the regulatory network required to control *hth* expression, we analyzed its *cis*-regulatory elements by fusing various genomic fragments upstream to the report gene *gfp*. We identified a *6.8kb* fragment within the transcribed region of *hth* that drives GFP expression in the eye disc in a pattern similar to that of endogenous *hth*. This fragment was then further dissected, and the *cis*-regulatory region was narrowed down to the size of *1.5kb*. We tested the regulation by several candidate factors and our results showed that Ey, Tsh and Wg signaling positively regulate *hth* transcription and Dpp signaling negatively regulate *hth* transcription. Finally, sequential deletion of the *1.5* kb region narrowed down the major *cis*-regulatory region in the eye disc into *508 bp*.Candidate transcriptional activating and repressing regulators are being tested for their ability to regulate *hth* expression through this *cis*-element.

346A

Spatial regulation of achaete the leg microchaete primordia global activation and repression by Hairy and Delta. Ji Inn Lee, Meghana Joshi, Teresa Orenic. Biological Sciences, University of Illinois-Chicago, Chicago, IL.

During vertebrate and invertebrate development, organs and tissues must be precisely patterned and periodic proneural gene expression is an early and essential event in neuronal patterning. On the surface of the Drosophila leg, a group of small mechanosensory bristles, called microchaetae, are organized in a series of longitudinal rows around the leg circumference. This orderly arrangement of the leg bristles provide a simple model system with which to investigate the molecular mechanisms involved in the precise proneural gene expression. In the prepupal leg, the proneural gene, achaete(ac), is expressed in longitudinal stripes which comprise the leg microchaete primordia. We have found that Hairy and Delta function concertedly and non redundantly to define periodic ac expression. This process involves broad and late activation of ac expression and refinement in response to a prepattern of repression, which is established by Hairy and Delta. This findings have allowed us to formulate a general model for generation of periodic bristle patterns in the adult leg and this model is supported by the analysis of an enhancer that specifically directs ac expression in the leg microchaete proneural fields. This enhancer contains a small activation element, which directs broad expression of ac along the circumference of prepupal legs, and two repression elements, one which is Dl/N-responsive and the other which is Hairy-responsive.

347B

Genome Wide Mapping of the In Vivo DNA Binding Sites of Transcriptional Regulators of the Pregastrula Gene Network. Xiao-Yong Li[1], S. MacArthur[1], R. Bourgon[2], D. Nix[1], N. Ogawa[1], H.C. Chu[1], L. Zeng[1], M. Stapleton[1], L. Simirenko[1], V. Iyer[1], D. Pollard[1], V. Sementchenko[3], T.R. Gingeras[3], M.D. Biggin[1], M.B. Eisen[1]. 1) Berkeley Drosophila Transcription Network Project, Lawrence Berkeley National Lab, Berkeley, CA; 2) Statistics Department, UC Berkeley; 3) Affymetrix, Inc.

Early Drosophila development is driven by a transcriptional regulatory network that specifies patterning along the anterior/posterior and dorsal/ventral axes. As part of a comprehensive approach by the BDTNP to model this network, we have used ChIP-chip to map the in vivo binding sites for regulatory transcription factors throughout the genome. To date we have data for more than ten factors critical for A/P and D/V patterning. We have identified a surprisingly large number of high confidence binding regions - between a hundred to several thousands, depending on the factor. Multiple criteria imply that the bound regions may be functional Cis Regulatory Modules (CRMs). The binding regions for different factors often overlap with each other, forming clusters typical of CRMs. All previously known CRMs of these factors are bound in our studies. The thousands of additional, novel regions identified tend to be associated with key developmental genes or genes that show patterned expression in early embryos, including many miRNA genes. The DNA binding recognition sequences of these factors are enriched in the regions they bind. Comparative studies indicate that these recognition sites evolve in a manner consistent with them being under evolutionary selection as functional DNA sites. Interestingly, A/P and D/V regulatory factors generally bind the same target genes, many of which have mixed A/P and D/V expression pattern in embryos, but others of which have classically been considered to be either D/V or A/P specific genes. Further, the A/P and D/V regulators often bind the same regions within these genes, suggesting that they form CRMs that integrate D/V and A/P developmental signals. Our ChIP - chip analyses have identified many potential CRMs and provide information about the early gene regulatory network governing Drosophila embryogenesis.

348C

A combinatory mechanism sets the border of a low-level Dpp target gene, pannier, in early Drosophila embryos. Hsiao-Lan Liang, Nikolai Kirov, Christine Rushlow. Biology, New York Univ, New York, NY.

During early embryogenesis, Decapentaplegic (Dpp) forms an activity gradient with peak levels in the dorsal-most region and lower levels in the dorsal-lateral region. A number of candidate Dpp target genes in the dorsal region are differentially expressed in response to this gradient. pannier (pnr) is a low-level Dpp target which is expressed in a broad domain encompassing approximately 32-35 cells. Besides the activation from Dpp, an additional mechanism, Brinker (Brk) repression, is crucial to establish the pnr expression broader. Other higher-level Dpp targets, such as ush and Race, are Brk-independent. In order to understand how pnr responds to the threshold set up by Dpp and Brk, we have identified and analyzed an early pnr enhancer. Our results showed that the expression of pnr comprises two patterns: a dorsal patch and five stripes along the AP axis. Only the dorsal patch is responsive to Dpp. Within the dorsal patch enhancer, putative Mad and Brk binding sites have been identified by gel shift analysis. In vivo mutagenesis has shown that a particular site, which binds to both pMad and Brk, is critical for the expression of the dorsal patch, suggesting that Mad and Brk may compete with each other to set up the pnr expression border. Interestingly, this Mad site has a binding affinity comparable to, instead of higher than, those of high-level Dpp targets, such as Race and C15. This result suggests that a co-factor may exist to enhance Mad binding, which consequently competes effectively with Brk repression. *pannier, ush, pnr, Race, C15*.

349A

Regulation of odor receptor genes in trichoid sensilla of *Drosophila*. Carson J Miller, John R Carlson. MCDB, Yale University, New Haven, CT.

Olfaction—the detection, discrimination, and decoding of airborne chemicals—plays a critical role in the biology of many animals, including the identification of conspecifics and potential mates, the location of food, and the detection of predators. In flies, odors are detected by olfactory receptor neurons (ORNs) of the peripheral nervous system. Each ORN expresses a small number of *Or* (odor receptor) genes which confer upon it unique odor sensitivities. Individual ORNs project to the antennal lobe in the brain in a stereotypical pattern, and in contrast with mammals, misexpression of odor receptors does not affect this pattern. Consequently, for the CNS to receive accurate odor information, the choice of *Or* genes by ORNs must be strictly regulated. I am studying *Or* gene choice in trichoid sensilla of the antenna, some of which detect pheromones and may contribute to a fly's ability to discriminate males from females. Using the GAL4 expression system, we have defined a minimal upstream fragment of *Or88a* that drives expression similar to that of endogenous *Or88a* expression. We are working to understand which DNA motifs in the *Or88a* minimal promoter are necessary for expression and which transcription factors may be involved in regulating expression of *Or88a*.

350B

Harnessing natural sequence diversity to explore regulatory function. Brant Peterson[1], Emily Hare[1], Rudolf Meier[2], Rick Kurashima[3], Brian Wiegmann[4], Michael Eisen[1]. 1) Dept Molecular & Cell Biol, Univ California, Berkeley, Berkeley, CA; 2) Dept of Biol Sci, Nat Univ Singapore, Singapore; 3) Pacific Basin Agricultural Research Center, UDSA ARS, Honolulu, HI; 4) Dept of Entomology, North Carolina State Univ, Raleigh, NC.

Primary sequence of cis-regulatory elements is of crucial importance in mediating the action of sequence-specific transcription factors on a particular gene at a particular time, however our understanding of functional constraints acting on any given element, and therefore of the consequences of any given sequence change (e.g. through evolution) is exceeding primitive. This reflects a two-fold deficit in understanding of both how these constraints change over evolutionary time (e.g. between closely related species) and the impact that these constraints have on rates of sequence change (e.g. what constitutes a functionally "conservative" change in a given context). To address both of these questions, I am investigating the regulation of a group of key transcription factors responsible for the establishment and maintenance of the highly evolutionarily dynamic Dipteran extraembryonic tissue(s). Here I report our ongoing analyses of fosmid sequence data generated as part of a deep comparative study of regulation of several key regulators of developmental processes in 24 species of higher flies from 3 major families (*Sepsidae*, *Tephritidae* and *Drosophilidae*). Specifically, I address challenges of comparing inhomogeneous rates of primary sequence evolution even among enhancers in the same patterning system, as well as in annotating and analyzing enhancers which have no detectable primary sequence homology to their presumptive *Drosophila* orthologs. In addition, we have begun analyses of embryonic expression of these genes in their native context and the output of their regulatory regions in transgenic *Drosophila melanogaster* in order to investigate network level conservation of the function of these regulatory systems.

351C

Cis-regulatory control of slp1 expression during segmentation. L. Prazak[1], M. Fujioka[2], J. Chang[1], X. Wang[1], J. P. Gergen[1]. 1) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, N.Y; 2) Thomas Jefferson University.

The simple combinatorial rules responsible for the metameric expression of *sloppy paired* (*slp1*) make this an attractive model for investigating mechanisms of action of pair rule transcription factors. Studies with various *slp1-lacZ* reporter genes have identified two elements that drive expression at the gastrula stage of development, a proximal early stripe element (PESE) located from -3.4kb to -1.4kb and a distal early stripe element (DESE) located from -8.7 kb to -6.6kb upstream of the *slp1* promoter. The PESE element expresses the even stripes at the blastula stage with the odd stripes not being expressed until germ band extension. This reporter is repressed by ectopic expression of Runt and Ftz and the stripes expand anteriorly in both $ftz^{-/-}$ and $runt^{-/-}$ embryos. Runt and Opa however do not activate this reporter. The distal element expresses both odd and even numbered stripes and responds to both activation and repression by Runt. Several lines of evidence indicate the DESE element is relatively insensitive to repression by Eve. Interestingly, when these two elements are combined the expression pattern of the reporter responds in a manner similar if not identical to *slp1* suggesting the proximal element can mediate Eve repression. To identify Eve-responsive regions within the proximal element and determine what regions are needed for even stripe expression, we have made deletion constructs within this element alone as well as in the context of a composite reporter. To further characterize the ability of the distal element to respond to Runt dependent regulation we have mutated 5 high affinity Runt binding sites in this reporter. In order to maximize our ability to reliably detect subtle and quantitative differences in expression we have utilized ΦC31 integrase mediated site-specific integration of the different reporter genes into the same chromosomal location.

352A

A genetic selection to identify regulatory information driving the earliest zygotic transcription in *Drosophila melanogaster*. William J Rowell, Warren C Lee, Thomas W Cline. Dept Molecular & Cell Biology, Univ California, Berkeley, CA.

In *Drosophila melanogaster*, X-linked genes called X-chromosome signal elements (XSEs) are counted by the master sex-determination gene, *Sex lethal* (*Sxl*), to tell the young embryo which sex it is to be. XSEs are expressed in the pre-blastoderm embryo before the onset of widespread gene transcription. All of the XSEs have non-sex specific functions in development that predate their role in sex determination. In the course of being recruited to the sex determination pathway, these genes had to acquire regulatory information to allow for such unusually early expression. We are interested in determining the availability and molecular nature of regulatory information that can be co-opted for this purpose, and to determine the extent of pre-blastoderm gene expression.

Towards this end, we have exploited our understanding of sex determination to devise a functional selection for regulatory information that can drive the earliest transcription in the *D. melanogaster* embryo. Females mutant for the XSE *scute* (*sc*) die from a lack of *Sxl* activity. A P(sc^{+}) transgene rescues these females. Key regulatory elements were mutated in the P(sc^{+}) transgene generating P(sc^{blind}), which no longer rescues sc^{-}/sc^{-} females. When mobilized, P(sc^{blind}) can regain its ability to rescue sc^{-}/sc^{-} females by inserting near regulatory information that can drive its pre-blastoderm transcription in all somatic cells. This functional enhancer trap screen has provided at least 35 independently isolated rescuing insertions which we are in the process of verifying and further characterizing. This unbiased approach could uncover early genes and regulatory information that have been missed by previous experimental strategies.

353B

Study of the transcriptional regulation of *unpaired* expression in *Drosophila* eye development. Chuan-Ju Wang[1,2], Ya-Hsin Liu[1], Y. Henry Sun[1,2]. 1) Academia Sinica, Institute of Molecular Biology, Taipei, TW; 2) Institute of Genome Sciences, National Yang-Ming University, Taipei, TW.

unpaired (*upd*) encodes a secreted ligand for the Jak/STAT pathway. It is expressed at the central point of the posterior margin in the eye disc, the initiation site of the morphogenetic furrow (MF). *upd* regulates the cell divisions anterior to the MF in the eye disc through the Jak/STAT signaling pathway. It is not clear what factors specify the expression of *upd*. To understand the regulatory network controlling *upd* expression, a 10-kb genomic fragment containing *upd* was divided into fifteen 1-3 kb sub-fragments and cloned into GFP-expressing vectors to establish transgenic fly lines. Two of them show patterns similar to the endogenous *upd* pattern. There were several clusters of putative binding sites, including Su(H), STAT and dTCF binding sites, residing in the two sub-fragments. Testing of gain-of-function and loss-of-function effects of these pathways on the expression of *upd-lacZ* suggested Hh and Dpp pathway may cooperate with N signaling to activate upd expression at the central site of posterior margin in the eye disc. Furthermore, we found that Wg signaling may negatively regulate *upd* expression, and Upd was also involved in positive auto-regulation of its own expression.

354C

Promotor analysis of Bällchen, a kinase specifically expressed in Drosophila melanogaster embryonic neuroblasts. Toma Yakulov, Alf Herzig, Herbert Jäckle. Max-Planck-Institut für biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, Am Fassberg 11, 37077 Göttingen, Germany.

The Vaccinia Related Kinase ortholog Bällchen/NHK1 is required for the maintenance of germline stem cells (GSCs) in Drosophila (Herzig, A. et al., submitted). In the absence of Bällchen (Ball) GSCs progress normally along the program of terminal differentiation. *ball* expression is not restricted to GSCs, which suggests that ball has a permissive function in responding to self-renewal cues from the somatic niche. Initial experiments indicate that Ball is also required for maintenance of neuroblasts, the neuronal stem cells. During larval development, similar to the germline, *ball* is expressed both in neuroblasts and in differentiating neuronal cells. However, in the embryonic nervous system *ball* expression is restricted to neuroblasts.

To identify trans-acting factors required for the temporal and spatial regulation of ball expression we constructed transgenic Drosophila lines containing the *lacZ* reporter gene under the control of ball promotor fragments. Here we demonstrate that a 123 bp genomic sequence upstream of the *ball* transcription start site and 284 bp of the *ball* 3'UTR can drive lacZ expression in Drosophila embryos in a pattern similar to the native *ball* expression. Additionally, data regarding the upstream regulatory mechanism will be presented.

355A

Evolution of the Ventral Midline in Insect Embryos. Robert Zinzen[1], Jessica Cande[2], Matthew Ronshaugen[2], Dmitri Papatsenko[2], Michael Levine[2]. 1) Developmental Biology Unit, EMBL, Heidelberg, DE; 2) MCB-GGD, University of California, Berkeley, CA, USA.

The ventral midline is a source of signals that pattern the nerve cord of insect embryos. In dipterans such as the fruitfly *Drosophila melanogaster* and the mosquito *Anopheles gambiae*, the midline is narrow and spans just 1-2 cells. However, in the honeybee, *Apis mellifera*, the ventral midline is broad and encompasses 5-6 cells. *slit* and other midline patterning genes display a corresponding expansion in expression. This difference appears to be due to divergent *cis*-regulation of the *single-minded gene*, which encodes a bHLH-PAS transcription factor essential for midline differentiation. *single-minded* is regulated by a combination of Notch signaling and a Twist activator gradient in *Drosophila melanogaster*, but is activated solely by Twist in *Apis melfilera*. This change in regulation can account for the generally broader honeybee midline, and we suggest that the Twist-only mode of regulation - and the broad ventral midline - represents the ancestral form of CNS patterning in Holometabolous insects.

356B

Role of oligomeric state in determining the transcriptional regulation of Wingless signaling targets by *Drosophila* CtBP. Chandan Bhambhani[1], Ming Fang[2], Ken Cadigan[1]. 1) MCDB, University of Michigan, Ann Arbor, MI; 2) GDB, Southeast University Medical School, Nanjing, China.

CtBP is commonly known as a transcriptional co-repressor and regulates several processes early in development. Our previous report established the role of *Drosophila* CtBP (dCtBP) as a gene-specific regulator of Wingless (Wg) signaling, showing a novel positive and negative role in Wg target gene transcription. Here we present evidence to support that this differential activity may be dependent on the oligomeric state of CtBP.

Structural analysis of the Human CtBP1 (hCtBP1) suggests that it is a dimer, and this dimerization is required for its well-established role as a co-repressor. dCtBP has high sequence identity to hCtBP1, and we have shown that mutations in the dimerization interface of dCtBP, which are predicted to abolish dimerization, do not perturb the activation function of dCtBP. This monomeric form of dCtBP is as potent as the wildtype dCtBP in enhancing the activation of an Armadillo-dependent reporter gene in cultured cells. In flies, the monomeric dCtBP can enhance a small eye phenotype caused by Armadillo expression. In contrast, wildtype dCtBP suppresses this small eye phenotype suggesting that its negative role may be predominant over the positive role in regulating Wg signaling in the eye. However, when these genes are expressed in wing imaginal discs, both forms of CtBP are able to activate Wg target Distal-less but wildtype CtBP represses Wg target Sensless. We are currently testing the regulation of Sensless by monomeric CtBP. The wing phenotypes suggest that wildtype CtBP antagonizes Wg signaling leading to wing notches while expression of monomeric CtBP does not lead to a loss of Wg phenotype. We propose a model where monomeric dCtBP enhances Wg signaling while dCtBP dimers repress the pathway. To confirm this model, we are generating a mutant dCtBP that can only exist as a dimer, which would be predicted to lose activation function but retain inhibitory activity.

357C

Sources of DNA-binding specificity in the Hox protein Ultrabithorax. Sarah Bondos, Ying Liu, Kathleen Matthews. Dept Biochemistry & Cell Biol, Rice Univ, Houston, TX.

Animal development requires accurate and reliable tissue-specific gene regulation by the Hox transcription factor family, which specifies and sub-divides body axes. Crucial Hox functions in vivo appear inconsistent with the low DNA binding specificity of their highly conserved homeodomains in vitro. This dichotomy raises two key questions: (i) how does a single Hox protein select unique gene targets in different tissues, and (ii) how does DNA binding site selection vary among Hox family members? To address these issues, we have explored DNA binding in the full-length Drosophila melanogaster Hox protein Ultrabithorax (Ubx) and its variants. We find Ubx and Ubx HD bind DNA with differing affinity and specificity, and these two species exhibit different responses to their environment (oxidation, pH, osmolytes). The N-terminus of the protein mediates a subset of these effects. The hexapeptide (YPWM) motif and alternatively spliced microexons impact sequence-specific DNA binding by the homeodomain. Regulation by the hexapeptide motif is, in turn, modulated by the microexon region. These in vitro effects correlate nicely with in vivo results from other laboratories. This mechanism may allow Exd binding or alternatively splicing to determine tissue-specific DNA target selection. Finally, the structure of Ubx is altered by DNA binding in a sequence-specific manner, potentially allowing target DNA to dictate the mode (activation or repression) of DNA binding and to direct protein interactions. We hypothesize that regulation of sequence-specific DNA binding by the variable regions outside the homeodomain distinguishes Hox family members in vivo.

358A

Genomic analysis of the ecdysone response. Lucy Cherbas[1,2,3], Yi Zou[1,2,3], Philip Knollman[1,4], Tyler Iams[1], Peter Cherbas[1,2,3]. 1) Dept Biol, Indiana Univ, Bloomington, IN; 2) Drosophila Genomics Resources Center, Indiana University, Bloomington, IN; 3) Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN,; 4) Notre Dame University, South Bnend, IN.

We have assessed the extent, cell type specificity, and general characteristics of the very early transcriptional response to ecdysone by microarray analyses using 10 Drosophila cell lines of diverse tissue origin. Very early (5 hr) responses are highly cell type specific. Though several thousand genes are able to respond rapidly in one or more cell lines, a small fraction respond in any one line, the intersections of the response sets are modest, and very few of genes respond universally. The numbers of inductions and repressions are approximately equal. Individual genes may be induced in some lines but repressed in others. Some genes are primary (very early) responders in some lines but late (secondary) responders in other cell lines. These results greatly extend earlier results using one or two “model” responsive genes and they establish the framework for mechanistic studies of the ecdysone response pathways in a variety of cell types.

359B

Hormonal regulation of tanning at eclosion. Monica M. Davis[1], David A. Primrose[2], Sandra L. O'Keefe[1], Ross B. Hodgetts[1]. 1) Biological Sciences, University of Alberta, Edmonton, Alberta, CA; 2) Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, CA.

A complex neuropeptide signaling cascade that includes ecdysis-triggering hormone, eclosion hormone (EH) and crustacean cardioactive peptide (CCAP), controls ecdysis in Drosophila melanogaster. At eclosion, this signaling is further relayed by bursicon (BURS), through its receptor rickets (RK). EH and CCAP genetic ablation and burs and rk mutant adult flies fail to properly tan and harden their adult cuticle following eclosion. Tanning and hardening of the cuticle requires metabolites of dopamine. The rate limiting step in the production of dopamine is the conversion of tyrosine to Dopa by tyrosine hydroxylase (TH), which is encoded by the pale (ple) locus. Many levels of regulation of TH activity have been shown in Drosophila. The major site of activation of TH involves phosphorylation at Ser32 by protein kinase A (PKA). Interestingly, RK has been shown to activate PKA via an increase in the second messenger cAMP. We have shown that ple transcripts accumulate prior to eclosion and that TH protein accumulates following eclosion in wild type flies. While transcription is normal in all hormone pathway mutants, TH protein does not accumulate in EH and CCAP genetic ablation mutants, suggesting that these hormones are required for proper TH translation. However, TH accumulation does occur normally in burs and rk mutants, revealing that the loss of tanning in these mutants may be a result of a loss of TH activation. In fact, phosphorylation of Ser32 by PKA is lost in burs and rk mutant flies. Loss of TH phosphorylation by PKA is correlated with a loss of activity. Furthermore, injection of 8-Br-cAMP to eliminate the need for RK signaling in newly eclosed hormone pathway mutant flies, restores TH translation, phosphorylation, and activity. We thus conclude that TH is regulated at both a translational and an activational, but not a transcriptional level by this neuropeptide cascade to control tanning of the adult cuticle following eclosion.

360C

Scr provides a new look for Hox specificity. Rohit Joshi[1,3], Jonathan M. Passner[2,3], Alona Sosinsky[1], Remo Rehs[1], Barry Honig[1], Aneel K. Aggarwal[2], Richard Mann[1]. 1) Biochemistry and Mol. Biophy, Columbia University, New York, NY; 2) Department of Molecular Physiology and Biophysics Mount Sinai School of Medicine New York, NY; 3) Contributed equally to the work.

Hox genes encode a family of transcription factors that play an important role in anterior-posterior patterning in *Drosophila* and vertebrates. A long-standing question in the field of Hox biology has been, what determines the distinct *in vivo* target gene specificities for Hox proteins when they are known to have very similar *in vitro* DNA binding properties? We have employed a multidisciplinary approach, including structural biology, genetics, computational predictions, and biochemistry, to address this problem for a specific Hox protein, *Scr*, and its previously characterized target enhancer, Fkh250, from the *forkhead* gene. Our results, in particular the visualization of novel protein-DNA interactions seen in an Scr/Exd/Fkh250 crystal structure, have given us new insights into the mechanisms of target gene selection by Hox proteins. The details of our results will be presented.

361A

Functional significance of miRNA sequence differences between species. Supriya Kumar, Chung-I Wu. Department of Ecology & Evolution, University of Chicago, Chicago, IL 60637.

The number of miRNAs in any genome must be determined empirically because we do not have enough information to accurately predict miRNA genes. We are sequencing miRNAs from multiple tissues of closely-related species of *Drosophila* using 454 sequencing to determine the complexity of the miRNA transcriptome. Our interest is in identifying fast-evolving miRNAs, i.e. miRNAs whose mature sequence and/or pre-miRNA sequence has accumulated sequence changes over evolution. This would result in a new miRNA in the former case, and possibly in a different level of expression between species in the latter case. Such changes may have functional significance, with different target mRNAs being repressed in different species. Within the 78 *Drosophila* miRNAs known at the time of writing of this abstract, 5 are fast-evolving. The miR-310 cluster, comprising 4 miRNAs in *D. melanogaster*, has only 3 miRNAs in *D. pseudoobscura*. The 3 existing miR-310 cluster members in *D. pseudoobscura* have many changes compared to the sequence in *D. melanogaster* and one change even in the seed region of one of the miRNAs in the cluster. This suggests that functional differences of the cluster may exist between these two species. To determine whether the *D. pseudoobscura* cluster can completely substitute for the *D. melanogaster* cluster, we are using homologous recombination to generate a knock-out of the cluster in *D. melanogaster*. We will then attempt to rescue the knock-out phenotype by expressing either the *D. melanogaster* or the *D. pseudoobscura* cluster under GAL4-UAS control. We have generated flies expressing either cluster under GAL4-UAS control; preliminary results suggest that ectopic overexpression under Act5C-GAL4 control in a wild-type *D. melanogaster* w^{1118} background results in lethality.

362B

Molecular basis of the systemic RNAi response in a beetle *Tribolium castaneum*. Sherry C. Miller, Susan J. Brown, Yoshinori Tomoyasu. Biology, Kansas State University, Manhattan, KS.

RNA interference (RNAi) is a widely conserved mechanism of post-transcriptional gene regulation and has become a common method to knock down gene function in many model systems. However, in some model systems the method of introducing dsRNA to initiate the RNAi response poses inherent problems. In Drosophila, dsRNA needs to be introduced directly into cells to initiate RNAi. This makes RNAi in post embryonic stages difficult, since it requires transgenic hairpin constructs to produce dsRNA within cells. Conversely, RNAi can be applied easily even in post-embryonic stages of organisms that exhibit a systemic RNAi response, in which cells uptake dsRNA and transmit the RNAi effect throughout the entire body. Therefore, understanding the molecular basis of systemic RNAi might give us a clue how to overcome the current limitations of RNAi as a genetic tool in some organisms.

In the last few years, several insects have been shown to have a systemic RNAi response. Fortuitously, one of these insects is the red flour beetle, Tribolium castaneum, an emerging model system whose genome has recently been sequenced. In this project, we have characterized the systemic RNAi response in Tribolium by analyzing important features of dsRNA (size, dose, and specificity) as well as heritability, tissue vulnerability, and duration of the RNAi effect. We are also characterizing Tribolium homologs of the genes essential for dsRNA uptake in other organisms. These approaches will reveal the conserved and divergent aspects of the systemic RNAi machinery, perhaps revealing ways to improve the application of RNAi, as well as giving insight into the evolutionary history of this response.

363C

Identification of functional domains and target genes of the Hindsight zinc-finger protein. Liang Ming[1,2], Ronit Wilk[1,2], Amanda Pickup[2], Howard Lipshitz[1,2]. 1) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, CA; 2) Program in Developmental and Stem Cell Biology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, CA.

The Drosophila hindsight gene encodes a nuclear protein containing 14 C2H2 zinc fingers and plays a crucial role in regulating epithelial integrity during Drosophila development. At the cellular level, HNT regulates the actin cytoskeleton and the Jun kinase signaling pathway. Two HNT DNA binding elements, HNT site A and B, were identified by in vitro selection assays. The three most C-terminal zinc fingers bind to site A, and the adjacent two zinc fingers to site B. The nuclear localization signal (NLS) maps to the N-terminal region of the HNT protein. Endogenous HNT is expressed in late third instar salivary glands. Thus, to identify HNT's in vivo binding sequences and direct target genes, we mapped HNT protein distribution on polytene chromosomes to 57 sites. The strongest site, 60C, was further refined to a cytological region between 60C4~60C6, which covers ~40 kb and includes 5 genes. The expression levels of three genes within this region are altered when HNT is overexpressed in salivary glands. These candidate target genes are being focus of further analysis. The understanding of HNT target genes and its functional domains may shed some light into the molecular mechanisms that regulate epithelial integrity.

364A

Studies on the role of MLE in dosage compensation. Rosa Morra, Edwin Smith, John Lucchesi. Biology, Emory University, Atlanta, GA.

The MSL complex of Drosophila is responsible for the chromatin modifications that result in a doubling, on average, of most X-linked genes in males thereby achieving dosage compensation. The core complex contains two components with enzymatic activities that are required for the spreading and function of the complex: MOF, a histone acetyl transferase that specifically acetylates histone H4 at lysine 16 throughout transcriptional units and MLE, an ATP-dependent RNA helicase. To attempt to better understand the role that MLE plays in dosage compensation, we have initiated a mutational analysis.

365B

***In vitro* search of the tissue-specific trans-acting factor interacting with regulatory sequences of the testis expressed *Stellate* genes in *Drosophila melanogaster* .** Oxana M. Olenkina, Ludmila V. Olenina, Sergei A. Lavrov, Vladimir A. Gvozdev. Institute of Molecular Genetics, Moscow, RU.

In animals and plants, RNA silencing pathways regulate endogenous gene expression and protect the genome against viruses and selfish genetic elements such as retrotransposons and repetitive sequences. In the *Drosophila* germline, these elements are suppressed by rasiRNAs. In testes of wild-type males, hyperexpression of tandemly repeated X-linked *Stellate* genes is prevented by homologous, bidirectionally transcribed Y-linked *Suppressor of Stellate* repeats (*Su(Ste)*). It was shown that deletion of *Su(Ste)* leads to abnormalities of spermatogenesis and to activation of *Stellate* expression accompanied by disappearance of *Su(Ste)* rasiRNAs. Previously it was found that 134 bp of the 5' fragment of the *Stellate* gene is sufficient to confer *Su(Ste)*-dependent silencing. However nothing was known about specific transcriptional factors interacting with *Stellate* gene regulatory sequence. To identify such factors we performed electrophoretic mobility shift assays. We divided the 130 bp regulatory region of the *Stellate* gene into four subregions and analyzed the binding of each with proteins from the testis nuclear extract and 0-12h embryo nuclear extract. We detected the formation of specific protein-DNA complexes for 3 subregions of *Stellate* regulatory sequence in testis extract but not in embryo extract. With the aid of computational analysis we found three binding sites (**E-boxes**) for basic helix-loop-helix (**bHLH**) motif-containing proteins. We demonstrated that (1) the binding of putative bHLH factor(s) with E-boxes is testis-specific, (2) the mutation in the consensus sequence of the E-box disrupts the binding, (3) the testis-specific factor(s) binding three subfragments of *Stellate* promoter region in the testis extract are the same. A few biochemical properties of putative bHLH factor(s) were demonstrated such as (a) Mg^{2+}-dependence of complex formation, (b) enhancement of binding in the presence of polyamines, (c) redox-dependence of protein-DNA interaction.

366C

A dMyc-Groucho complex regulates neuronal stem cell fate and mitosis. Amir Orian[1], Jeffrey Delrow[2], Alicia Rosales Nieves[2], David Metzger[2], Mona Abed[1], Hanaa Knaneh[1], Ze'ev Paroush[3], Robert Eisenman[2], Susan Parkhurst[2]. 1) Rappaport Research Institute, Technion, Haifa, IL; 2) Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 3) The Hebrew University, Jerusalem, Israel.

Integration of patterning cues via transcriptional networks to coordinate gene expression is critical during morphogenesis and is mis-regulated in cancer. The Myc oncoprotein functions within the context of a highly conserved basic-helix-loop-helix-zipper Myc/Max/Mxd(Mad-Mnt) transcriptional network that is required for both cell growth/size and cell proliferation. However, recent genetic and genomic experiments suggest that a set of interactions outside the canonical Myc/Max/Mad network also regulate some of Myc's functions. We identified a novel complex between the *Drosophila* dMyc oncogene and the Groucho co-repressor that antagonistically regulates a subset of non-canonical direct dMyc targets. This target subset is not shared by other Myc network members, and many targets lack canonical dMyc binding sites in their regulatory regions. Most of the targets in this group can be functionally subdivided into those affecting fate or mitosis particularly during neurogenesis, suggesting that the dMyc-Groucho interaction may coordinate fate acquisition with mitotic capacity during development. Transcriptionally and biologically we find an antagonistic transcriptional relationship between dMyc and Groucho that mimics the antagonistic interactions found for EGF and Notch signaling: dMyc/EGF signaling are required to specify neuronal fate and enhance neuroblast mitosis, whereas Groucho/Notch signaling are required to maintain epithelial fate and inhibit mitosis. Importantly Groucho function is independent of the dMyc antagonist dMnt, and therefore defines a novel repression mechanism antagonizing dMyc function during neural progenitor development. Taken together our results suggest that the dMyc-Groucho complex serves as a molecular junction that phenocopies EGF and Notch inputs to regulate neural stem cell development.

367A

The Role of E23 in Regulating Tissue-Specific Hormone Responses. Elana Paladino[1], Andrew Andres[1], Dan Garza[2]. 1) Department of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) Novartis Institutes of Biomedical Research, Cambridge, Massachusetts, USA.

Throughout *Drosophila* development, the steroid hormone 20-hydroxyecdysone (20E) initiates a cascade of gene expression that signals tissues to undergo a variety of changes. For example, at metamorphosis the salivary gland commences cell death; whereas, the imaginal discs begin to proliferate into adult structures. Understanding how 20E directs these tissue specific responses is an ongoing interest of our lab. A key player in this response may be *E23*, a gene directly induced in the late larval salivary gland in response to 20E. Since *E23* encodes an ABC transporter, we hypothesize that its role in the normal development of some tissues may be to limit the gland's exposure to 20E in order to define the precise order of gene expression cascades that are regulated by changing hormone titers. To test this hypothesis we have ectoptically overexpressed *E23* in a variety of larval and imaginal tissues before different *in vivo* hormone pulses. In all cases examined the tissues fail to respond to the subsequent 20E pulse. We are currently exploring models to explain how E23 could control global hormone responses in specific cell types.

368B

***zucchini* and *squash* encode two novel components required for RNAi processes in the Drosophila germline.** Attilio Pane, Kristina Wehr, Trudi Schupbach. Dept. of Molecular Biology, Princeton University, Princeton, NJ.

RNAi is a widespread mechanism by which organisms regulate gene expression and defend their genomes against viruses and transposable elements. Here we report the identification of Drosophila *zucchini* (*zuc*) and *squash* (*squ*), two members of the "spindle" class genes which function as novel factors in germline RNAi processes. *zuc* and *squ* mutant females are sterile and produce eggs with a range of dorsal-ventral defects due to the down-regulation of Gurken during oogenesis. We show that Zuc and Squ localize to the perinuclear nuage and interact with Aubergine, a protein belonging to the PIWI class of Argonaute proteins. These observations point to a function for Zuc and Squ in RNAi processes. In support of this conclusion, we find that Oskar protein is ectopically expressed in early oocytes, where it is normally silenced by RNAi mechanisms. Furthermore, mutations in *zuc* and *squ* induce the upregulation of Het-A and Tart, two telomere-specific transposable elements, and the expression of Stellate protein in the Drosophila germline. Our results demonstrate that *zuc* and *squ* are required for RNAi mediated silencing of transposable elements and tandem repeats in the germline and have profound effects on axis formation during oogenesis.

369C

Non-coding RNAs in the Bithorax Complex. Jessica C. Piel, Matthew Ronshaugen, Michael Levine. Division of Genetics and Development, Department of Molecular Cell Biology, Center for Integrative Genomics, University of California, Berkeley, CA 94720.

The Drosophila Bithorax complex contains three genes that encode homeodomain proteins involved in directing segment identity during the early development of the embryo. While the function of these proteins are well-characterized, it is the exact timing and location of their expression that is not fully understood. Precise regulation of their activity is necessary for the proper development of the embryo. It has been suggested that this regulation may be refined by the activity of non-coding RNAs expressed within the intronic and intergenic regions of this complex. To investigate this, we have employed the use of tiling arrays to identify potential expressed sequences in the early embryo. Their expression patterns were then analyzed through the use of *in situ* hybridization. This process has identified several non-coding RNAs within the introns of the Ubx gene that not only exhibit different timing regulation than the protein-coding gene but also unique expression patterns. Further analysis of these transcripts may reveal homeotic regulatory functions.

370A

Identifying the Proteins Responsible for the Anti-Insulator Function of the Promoter Targeting Sequence in the Abdominal-B Locus. Jamie L. Planck, Qi Chen, Kaycie Hopkins, Sheryl T. Smith, Jumin Zhou. Wistar Institute, Philadelphia, PA.

Evolutionarily conserved in organisms like Drosophila and in all vertebrates from fish to humans, Hox gene clusters are responsible for patterning at nearly all stages in development. In Drosophila, one of these clusters, the Bithorax Complex (BX-C), is responsible for the segment identity of the posterior of the fly. Within BX-C, the Abdominal-B (Abd-B) locus controls the identity of the most posterior parts of the fly via approximately 60kb of 3' regulatory sequence, which includes multiple enhancers and insulators. One of the regulatory elements of Abd-B is the Promoter Targeting Sequence (PTS), which has both promoter targeting and anti-insulator function. Little is known about the molecular mechanism of the PTS. In an effort to elucidate the mechanism by which the PTS mediates its anti-insulator function, the 625bp PTS was narrowed into a 62bp sequence that maintained anti-insulator function. EMSA analysis was then performed showing specific binding activities associated with a 27bp fragment. A series of mutant competitors revealed two distinct binding sites within the fragment. Utilizing these sites, DNA affinity chromatography was performed in an effort to isolate the specific binding proteins of PTS. Preliminary results identified the DNA binding protein HMG-D; however, further testing needs to be done to confirm this result. Transgenic flies with the double mutant, where both binding sites are mutated in the 27bp PTS fragment, are in the process of being made so that the in vivo relevance of the binding sites can be determined.

371B

Genetical Genomics in Drosophila - Combined Microarray-QTL Studies Identify Several PbAc-induced Trans-expression-QTL Signaling Pathways. Douglas M Ruden[1], Grier Page[2], Parsa Rasouli[1], Daniel Shriner[2]. 1) Inst. for Environ. Health Sci., Wayne State Univ., Detroit, MI; 2) Department of Biostatistics, Univ. AL BHM, Birmingham, AL.

Whole transcriptome scans for expression quantitative trait loci (eQTL) using combined microarray-QTL technologies is also known as genetical genomics. One of the most exciting discoveries in genetic genomics is the identification of major-effect trans-eQTL that regulate the expression of hundreds or even thousands of genes in a tissue- or cell-type-specific manner. Major effect trans-eQTL have been identified in mouse but not in yeast or plants. Here, we describe the first genetical genomic study in Drosophila melanogaster using 5-10 day old whole males fed from hatching either control food or food containing the heavy-metal salt PbAc. We have identified 8 major effect trans-eQTL that each regulate the expression of hundreds of genes. Five of the major effect trans-eQTL appear only in the presence of PbAc, whereas, the other three appear only in the absence of PbAc. Traditional phenotypic QTL analyses of flies fed control, PbAc-containing food, or paraquat-containing food, has identified several QTLs which overlap with the 8 major effect trans-eQTL. Our results show for the first time that D. melanogaster has major-effect trans-eQTL, that these trans-eQTL are regulated by PbAc, and that phenotypic QTL analyses can probably be used to identify the major-effect trans-eQTGs.

372C

Like its vertebrate homolog, Wilm's Tumor Suppresor-1, klumpfuss is localized to both the nucleus and cytoplasm and may bind RNA. Jamie C. Rusconi, Barbara Zaffo, Erica Hutchins, Kelly Romano. Dept Biological Sciences, University at Albany, Albany, NY.

Drosophila klumpfuss (klu) encodes a zinc finger transcription factor of the EGR-class. Members of this class of transcription factors can act as both transcriptional activators and repressors and contain a DNA binding domain of three C2H2 zinc-fingers. klu and the vertebrate Wilm's tumor suppressor-1 (WT-1) protein are unique within this class in containing four instead of three zinc-fingers. Using our newly generated klu antibody we have found that klu is localized to the cytoplasm as well as the nucleus in every tissue examined. In addition this cytoplasmic expression is found in puncta or cytoplasmic speckles, similar to the expression of WT-1 in recent studies. We are working to characterize the cytoplasmic puncta/speckles using a series of organelle and ribosomal markers in double labeling experiments to identify the subcellular localiztion of klu. New work from a number of groups has demonstrated that WT-1 shuttles in and out of the nucleus, binds RNA and is even associated with translating polysomes. This data together with our expression analysis has led us to propose a role for klu as an RNA binding protein. To date we have shown that mRNAs precipitate with klu using a technique called RNA immunoprecipitation or RIP and are working to see if this klu is directly bound to RNA. In addition we have just generated a transgenic fly line that expresses the mouse WT-1 under the control of a heat shock promotor and will present our analysis of this line and its ability to rescue klu mutants.

373A

Domain analysis of the dBlimp-1, an ecdysone inducible and labile transcription factor in *Drosophila melanogaster*. Moustafa M. Sarhan[2], Hitoshi Ueda[1,2]. 1) Department of Biology , Okayama University, Okayama, Okayma, JP; 2) Graduate School of Natural Science and Technology, Okayama University, Okayama,Jp.

dBlimp-1 is a transcriptional regulator found as a binding factor to the promoter region of the *ftz-f1* gene which induced after decline of ecdysone and plays an important role in molting and metamorphosis in *Drosophila melanogaster*. dBlimp-1 is induced directly by ecdysone and expressed at high level periods of this hormone. Furthermore, it is supposed to have a function to activate the *ftz-f1* gene after its disappearing. dBlimp-1 determines timing of FTZ-F1 expression and pupation by working as a repressor and has several conserved motifs among different species. To know the function of these motifs, several dBlimp-1 mutants carrying deletions at the conserved motifs were expressed under control of the heat shock promoter in the mid-stage prepupae. Functional domains were analyzed by detecting expression pattern of FTZ-F1 by Western blotting and observing pupation timing. When we deleted the most conserved motif, except the DNA binding domain, dBlimp-1 still can act as a repressor and obvious delay in FTZ-F1 expression and pupation timing was observed. On the contrary, after the deletion of the proline-rich containing region such delay was not observed as in the case of wild type. Surprisingly, intact dBlimp-1 could be detected for only one or two hours after induction in the early-stage prepupae. Meanwhile, dBlimp-1 tended to be highly stable when the proline-rich containing region was completely deleted. These results suggest that the proline-rich containing region has an essential role for dBlimp-1 to act as a repressor and for the determination of pupation timing. On the other hand, the conserved domain has no role in this process but it may have another function which has not been shown yet. Additionally, the instability of dBlimp-1, which evoked by the proline-rich containing region, is important for determining the ecdysone-induced pathway.

374B

The role of RNA localization in controlling the translation of isoforms of the nuclear receptor E75. Carol Schwartz[1], Henry Krause[1,2]. 1) Donnelly CCBR, Univ Toronto, Toronto, ON, CA; 2) Dept. of Molecular and Medical Genetics.

RNAs corresponding to different isoforms of the E75 nuclear receptor are transcribed in response to pulses of ecdysone and juvenile hormone during Drosophila development. Combining whole mount RNA in situ and antibody staining techniques, we find that these RNAs are often retained in the nucleus for several hours without transport into the cytoplasm and without translation. The profile of RNA localization and translation varies from tissue to tissue. We look at the effect of ectopic expression of E75 isoforms on RNA localization and protein expression. We discuss possible mechanisms of RNA localization and postranscriptional regulation of E75 expression. We comment on the the importance of this postranscriptional regulation on developmental timing.

375C

An interaction study of the male specific lethal (MSL) complex and trans-acting dosage effects in metafemales of Drosophila melanogaster. Xiaoping Sun, James Birchler. Dept Biol, Univ Missouri, Columbia, MO.

The effect of ectopic expression of male specific lethal 2, msl2, on chromatin modification and gene expression was studied in Drosophila diploid females and metafemales (3X;2A). Results show that ectopic expression of MSL2 in transgenic msl2 females and metafemales sequesters the MOF histone acetylase to the X, which occurs concordantly with an increase of histone acetylation. Gene expression studies indicate that the X-linked genes in ectopically expressed MSL2 females and metafemales are not affected by direct targeting of the MSL complex to the X chromosomes, suggesting one function of the MSL complex is to override the effect of a high level of histone acetylation. Autosomal gene expression is generally decreased in ectopically expressed MSL2 females, which correlates with the reduced autosomal histone acetylation. Metafemales show dosage compensation of X-linked genes with some autosomal reductions in expression. They were confirmed to have no MSL complex. Interestingly, in metafemales with ectopically expressed MSL2, the autosomal expression is returned to a more normal level. There is a lower autosomal level of histone acetylation compared to the normal metafemales, suggesting a nullifying effect on the negative dosage effect of the X chromosome as previously suggested to occur in normal males. The results falsify the hypothesis that targeting the MSL complex to the X chromosomes causes a two-fold upregulation of their expression. These findings suggest an interaction of genomic balance, the MSL proteins and histone acetylation on gene expression.

376A

***Drosophila* Blimp-1 is a transient transcription repressor that controls timing of the ecdysone-induced developmental pathway.** Masayoshi Takai[2], Kazutaka Akagi[2], Moustafa Sarhan[2], Hitoshi Ueda[1,2]. 1) Department of Biology, Okayama University, Okayama, JP; 2) Graduate School of Natural Science and Technology, Okayama University, Okayama, JP.

dBlimp-1 is a binding factor to the promoter region of the *ftz-f1* gene, which is induced after ecdysone pulse, is expressed temporally restricted manner and plays an important role in embryogenesis, molting and metamorphosis in *Drosophila melanogaster*. To elucidate the functions of dBlimp-1, transgenic fly lines carrying dBlimp-1 cDNA fused to the heat shock promoter were established and effects of forced expression were analyzed in the mid-prepupal stage. Our results showed that the forced expression of dBlimp-1 leads to delay in βFTZ-F1 expression and pupation timing. On the other hand, it turned out that induced dBlimp-1 disappears rapidly. Taken together, we conclude that dBlimp-1 is programmed to disappear rapidly and this control is essential for determination of the precise timing of βFTZ-F1 expression and pupation.

377B

Identifying novel gene targets of Hunchback that regulate early-born neuronal identity in the Drosophila CNS. Khoa D. Tran, Chris Q. Doe. Institutes of Neuroscience and Molecular Biology, Howard Hughes Medical Institute, University of Oregon 1254, Eugene Oregon 97403 USA.

The zinc-finger nuclear transcription factor, Hunchback (Hb) specifies early-born neuronal identity in the central nervous system (CNS) of the developing *Drosophila* embryo. Hb is required to maintain neuroblasts in a "young" state and promotes continued production of first-born neurons. However, the mechanisms through which Hb regulates temporal identity, and its downstream targets, remain unknown. We have designed chimeric Hb proteins to act as a constitutive activator (Hb:VP16) or a constitutive repressor (Hb:EnR) to assay for Hb CNS function. The ability of the chimeric proteins to replicate Hb function in regulating known targets such as *Kr*, *pdm*, *zfh2*, and *runt* will allow us to address the mechanism by which Hb specifies early-born identity. We also plan to identify Hb direct and indirect targets in the CNS. We will use a combination of bioinformatics tools and tissue specific microarray experiments to pinpoint Hb targets. The discovery of novel Hb-regulated genes sufficient to specify early-born identity as well as genes that are required to repress later-born identities will allow a more complete understanding of how diversity is generated during neurogenesis.

378C

Ecdysone signaling and microRNAs. Jishy Varghese, Stephen Cohen. Developmental Biology, EMBL, Heidelberg, DE.

The ecdysone receptor and its ligand the steroid hormone ecdysone are responsible for developmental transitions and metamorphosis in insects. In *Drosophila* several studies strongly implicate the role of ecdysone signaling during embryonic development, larval molting, pupariation, regulation of autophagy and apoptosis and morphogenesis of the various adult organs. In adult flies it regulates oogenesis and life span. microRNAs are believed to be post-transcriptional regulators of gene expression which has direct roles in organismal development and homeostasis. I will present data which shows that ecdysone signaling acts through microRNAs to maintain optimal levels of ecdysone receptor during metamorphosis.

379A

Inhibition of RNA interference by cell death signaling. Weiwu Xie, James A. Birchler. Biological Sci Div, Univ Missouri-Columbia, Columbia, MO.

Targeted gene silencing can be achieved in Drosophila melanogaster by RNA interference through a transgene expressing dsRNA homologous to the target gene. One such construct, driven by an eye-specific promoter GMR, encodes inverted repeat sequences of the white (w) gene exon 3 and has been shown to repress w expression to a pale yellow color. Several commonly used eye mutant markers partially restore the eye color whereas the others cannot. Interestingly, the restored color in the Bar eye mutants surrounds the region where cell death occurs. By comparing the two groups of marker genes, those that restore color produce irregular cell death during eye development. Therefore, we hypothesized that the inhibition of RNAi is caused by cell death signaling. Excessive cell death can be induced by over-expression of the canonical apoptotic genes Grim, hid and rpr and other genes Strica and ttk. When combining these transgenes with the RNAi construct, we observed eye color restoration in all cases. When a cell death inhibitor gene is added and the eye shape restored, w gene silencing is also restored. The restored color pattern of the Bar eyes also disappeared when the cell death was suppresed by acetamine. We further tested other constructs used to silence the w gene. The constructs were designed to generate a 1.4 kb dsRNA covering exons (2 to 4) and introns (2 and 3) by either transcribing from both directions or by transcribing the inverted repeats to produce a long stem-loop molecule. Although differences occur, we observed inhibition of RNAi of all transgenes when combined with at least some transgenes causing cell death. Our results suggest that cell death signaling inhibits RNAi of these w transgenes.

380B

Regulation of Retinoblastoma protein stability and function by the COP9 signalosome. Martin Buckley[1], Zakir Ullah[2], Geoffrey Williams[3], David Arnosti[1], R. William Henry[1]. 1) Department of Biochemistry and Molecular Biology, and Program in Genetics, Michigan State University, East Lansing, MI 48823; 2) The National Institutes of Health NICHD, Building 6/3A-15, 9000 Rockville Pike Bethesda, MD 20890; 3) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Box G-B187 Providence, RI 02912.

Retinoblastoma (RB) proteins are canonical tumor suppressors, which play important roles in cell cycle regulation and are implicated in a third of all human tumors. The mammalian RBs, as well as their *Drosophila* counterparts Rbf1 and Rbf2 exert their control by regulating the transcription of genes involved in cell cycle, as well as, differentiation, apoptosis and growth. However, the mechanism by which RB exerts its regulation at physiological targets remains obscure. Increasing evidence suggests RB regulation is facilitated by recruitment of protein cofactors. We have taken advantage of the less complex Rbf-network in *Drosophila* to isolate such cofactors and analyze their functional significance. We have biochemically purified a number of Rbf2 associated cofactors. From embryonic extracts we identified the COP9 signalosome (CSN1-8) complex, a novel protein complex, not previously linked to Rbfs. Here we show that COP9 genetically and physically interacts with Rbf2 during embryogenesis. Diminished levels of individual COP9 subunits leads to the reduction of Rbf1 and Rbf2 via a proteasome mediated pathway. The CSN4 subunit of the COP9 signalosome co-occupies Rbf target genes promoters with Rbf1 and Rbf2, suggesting an active role for the COP9 signalosome in transcriptional regulation. Intervention by the COP9 signalosome at target gene promoters may extend Rbf protein lifespan and enable appropriate programs of retinoblastoma gene regulation.

381C

Regulation of the Groucho corepressor by phosphorylation. Einat Cinnamon, Ronny Helman, Ze'ev Paroush. Department of Biochemistry, Faculty of Medicine, The Hebrew University, Jerusalem, ISRAEL 91120.

Negative transcriptional regulation is a strategy that has been commonly selected in evolution for setting up and maintaining gene expression patterns. We are studying Groucho (Gro), a ubiquitously expressed corepressor, that potentiates the repressor function of a vast number of transcription factors, including those acting in various signaling cascades such as Notch, Dpp and Wingless. For many years the prevailing view had been that Gro-mediated repression is regulated simply by the spatial and temporal distribution of its dependent DNA-binding repressors. It has recently emerged, however, that Gro's repression capability itself is regulated. Specifically, we have shown that Gro is phosphoryated, likely by MAPK, in response to signaling mediated by the EGFR pathway, and that this modification downregulates its repressor function. To further our understanding of this mode of regulation, we are currently aiming to determine whether other RTK signaling cascades, besides the EGFR pathway, also lead to phosphorylation of Gro, for example those mediated by the Torso and FGF receptors. Similarly, we are testing the possibility that all MAPK family members, including JNK and p38, target Gro. To address these questions, we have generated an antibody that specifically recognizes the phosphorylated form of Gro, allowing us to follow its phosphorylation state during the different stages of embryonic development. We find that Gro is phosphorylated at the poles of the blastoderm embryo, in regions coinciding with terminal Torso RTK activity, and that the domain of Gro phosphorylation overlaps with that of activated MAPK. Importantly, mutant analysis confirms that Gro's phosphorylation depends on signals relayed by the Torso pathway. At present we are in the process of establishing if phosphorylation plays a role in downregulating Gro-dependent repression at the poles, by misexpressing different derivatives of Gro (i.e., an unphosphorylatable form and a pseudo-phosphorylated form) in early embryos, and assessing the molecular and morphological consequences of these manipulations.

382A

Maternally supplied hey protein is a repressor of early Sxl expression. Elena Kozhina, Hong Lu, Dun Yang, James Erickson. Dept Biol, Texas A&M Univ, College Station, TX.

To determine its sex, a fly embryo must know how many X chromosomes it carries. Assessment of X chromosome dose depends primarily on four X-linked signal elements (XSEs) that function to activate the female-specific SxlPe promoter in the early embryo. The ability to accurately measure a 1X (male) or 2X (female) dose of XSE proteins depends on several positively-acting maternally supplied co-factors for the XSEs, as well on a negative regulators that interact with SxlPe. The autosomally encoded bHLH repressor encoded by deadpan has been shown to modulate the response to XSE proteins by binding SxlPe. Consistent with Dpn's role as a repressor, mutations in dpn lead to weak ectopic activation of SxlPe in male embryos. We analyzed the role of Dpn binding sites at SxlPe using SxlPe-lacZ transgenes and found, paradoxically, that mutations in the Dpn binding sites had stronger, and earlier, effects on SxlPe expression that did elimination of dpn protein. This suggests that additional Dpn-related repressors also act to control SxlPe. One such protein is product of the hey gene (Hairy/E(spl) related with YRPW motif). Female embryos from mothers bearing hey germline clones have elevated expression from SxlPe while sibling male embryos exhibit ectopic SxlPe activity. The effect of hey is weaker than the effect of Hey/Dpn binding site mutations suggesting that additional maternal repressors of SxlPe exist.

383B

The hN13 RING finger protein is a Novel Cofactor for the HES Family of Transcription Factors Involved in Segmentation and Neurogenesis. David Metzger[1], Dorit Kenyagin[2], Taryn M. Phippen[1], Amir Orian[2], Susan M. Parkhurst[1]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) The Rappaport Faculty of Medicine and Research Institute, Techion-Israel Institute of Technology, Haifa, Israel.

RING finger proteins have been shown to be involved in a number of biological processes. Most notable are their roles as E3 ubiquitin ligases, E3 SUMO ligases, and transcriptional repressors. However, much is still not understood about this class of proteins. We have identified a new E3 ubiquitin ligase in a yeast two-hybrid screen for Hairy transcriptional cofactors that we are currently calling hN13. Hairy is a member of the Hairy/E(spl)/Dpn (HES) family of basic HLH proteins that are known to be involved in segmentation, sex determination, and neurogenesis. Binding of hN13 has been mapped to Hairy's basic domain, a conserved region among all HES family proteins, and consequently hN13 binds all members of this family. While we find that hN13 efficiently ubiquitinates Hairy *in vitro*, it is not required for Hairy degradation. We find that hN13 functions as a transcriptional repressor in reporter assays. Both its ligase activity and transcriptional repression are dependent upon a functional RING finger domain. Using a polyclonal antibody we generated against hN13, we have observed that hN13 is ubiquitously expressed throughout embryogenesis and that it is strictly kept in the cytoplasm except during specific stages during development such as segmentation and neurogenesis, when it localizes to the nucleus. A number of other cofactors have been shown to be required for Hairy-mediated repression including Groucho, dCtBP, and dSir2. Using a chromatin profiling technique known as DamID, we find that hN13 overlaps with the majority of Groucho targets and not those of dCtBP or dSir2, suggesting a common mechanism whereby hN13 and Groucho act in combination to modify chromatin structure as a means of transcriptional repression.

384C

Architectural principles shaping the formation of Hox-containing complexes. Barbara Noro, Richard Mann. Biochemistry/ Molec Biophysics, Columbia University, New York, NY.

Eukaryotic transcriptional regulation often requires the coordinated recruitment of several transcription factors to a defined DNA surface, allowing the integration of multiple regulatory signals. The Hox family of transcription factors is central to the specification of morphological traits throughout D. melanogaster development, and an extensive wealth of data suggest that Hox factors partly achieve such phenotypic specificity by coordinating the assembly of multiprotein complexes on their DNA targets. To understand the function of such complexes as a whole, the relationships between the individual components needs to be elucidated. Our analysis focuses on the identification of the protein interfaces which mediate the interactions between the abdominal Hox protein AbdominalA (AbdA) and its cofactors Homothorax/Extrandenticle (Hth/Exd) and Engrailed (En). In vitro EMSA and GST pull-down analysis identified novel regions of AbdA, outside of the highly conserved homeodomain and YPWM motif, crucial to mediate the interaction to both Hth/Exd and En. Cuticle defects and Distalless (Dll) repression upon ectopic expression of AbdA mutants in vivo confirmed the in vitro results. In addition, we identified the molecular surfaces of En necessary to complex with Hth/Exd or AbdA by both in vitro and in vivo means. These data provide insight into the architectural principles underlying the formation of Hox-containing complexes and the transcriptional regulation of Hox target genes.

385A

Identification of Groucho as a component of the Knirps repressor complex. Sandhya Payankaulam, David Arnosti. Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48823.

Knirps is a Drosophila gap gene that functions as a short range transcriptional repressor. To understand the mechanism of repression, we have sought to identify putative cofactors by purifying Knirps from embryonic extracts. While evidences so far implicate Groucho as a corepressor primarily associated with the long range Hairy/E(spl) family of proteins, our analysis showed Groucho to be a component of the Knirps complex. We further tested for the genetic interaction between *knirps* and *groucho* by examining the expression of *even-skipped*, a well characterized target of Knirps. Over 9% of the embryos showed misregulation in the *even-skipped* stripe 5 in *groucho* heterozygous mutants. Interestingly, in a $gro^{-/+}/kni^{-/+}$ transheterozygous double mutant background, over 25% of the embryos showed deregulation in the stripe 5, overlapping the region where *knirps* is expressed. The mRNA expression of *knirps* was not altered in *gro* mutants, indicating that the misregulation is not a secondary effect of altered *knirps* transcription. These results raise the possibility that Knirps may use what has been previously thought to be a long range corepressor to effect is activity. It is likely that Knirps, like Hairy or Brinker, uses multiple means to repress its target and the requirement for a specific co-factor being dictated by developmental cues which could be stage specific or tissue specific or both.

386B

Analysing the Effect of Loss of Drosophila SIN3. Aishwarya Swaminathan, Lori Pile. Biological Sciences, Wayne State University, Detroit, MI.

Transcription is a vital regulated event during the development and life of any organism. Transcription activation is generally associated with acetylation of N-terminal tails of histones H3 and H4 by enzyme complexes like histone acetyltransferases (HATs). Transcription repression is associated with the activity of histone deacetylase complexes (HDACs) that reverse the effect of HATs. The SIN3 complex is a multi-subunit HDAC that removes acetyl groups from lysine residues on the N-terminal tail of histones H3 and H4. We have determined that loss of SIN3 (the co-repressor moiety of the SIN3 complex) affects multiple stages of Drosophila development. Using the Gal4-UAS binary system we have shown that ubiquitous loss of SIN3 by RNAi in Drosophila results in lethality and that the organism fails to develop beyond the 3rd larval instar stage. Our preliminary studies indicate that induction of SIN3 RNAi by heat shock during the 1st or 2nd instar larval stage caused the flies to die during the 3rd larval instar stage. Interestingly, induction of SIN3 RNAi in 3rd instar larvae does not have any lethal effect and the flies that eclode are phenotypically normal. Furthermore, down regulation of SIN3 in the central nervous system is also lethal at the embryonic stage. We plan to extend our study to observe developmental defects that may arise in various non-essential organs of the adult fly due to the loss of SIN3 in the corresponding imaginal discs. The rationale is two-fold. First, it will help us overcome the problem of lethality arising due to complete loss of SIN3 expression. Second, it will help us elucidate the different signaling pathways that govern SIN3-regulated gene expression and thus determine the role of SIN3 in development.

387C

Molecular and genetic characterization of Atrophin proteins, a novel class of nuclear receptor corepressors. Lei Wang, Chih-Cheng Tsai. Dept Physiology/Biophysics, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854.

Nuclear receptors (NRs) constitute one of the largest families of eukaryotic transcription factors, which play important roles in a variety of biological processes including homeostasis, metabolism, development and reproduction. It has been well established that NRs work in concert with transcriptional cofactors, including corepressors and coactivators, to regulate their target genes' expression. We have recently identified Atrophin family proteins as a novel class of NR corepressors. The Atrophin protein family comprises Drosophila Grunge (Gug), vertebrate Atrophin-1 and vertebrate arginine glutamic acid dipeptide repeats encoded (RERE). Our characterization of Atrophin proteins has revealed that all these three proteins interact with several NR subfamily 2 (NR2) proteins, including Drosophila Tailless (Tll), Seven-up1 (SVP1) and their vertebrate homologues Tlx and Chicken ovalbumin upstream promoter-transcription factor (COUP-TF). We additionally found that Gug and RERE, through their shared ELM2 (EGL-27 and MTA1 homology 2) and SANT (SWI3/ADA2/N-CoR/TFIII-B) domains, interact with histone deacetylase 1 and 2 (HDAC1/2). Based on these initial findings, I will present data showing the in vivo relationships among nuclear receptors, Gug, and Rpd3 (the Drosophila HDAC1) in Drosophila. Our study of Gug and its vertebrate homologues will not only broaden our understanding of the molecular mechanisms underlying the transcriptional repressive effects of NRs, but also define a new pathway that integrates the activities of HDAC and other histone modifying factors in repressing gene transcription.

388A

Mechanism of transcriptional repression by the ETS family transcription factor YAN. Jie Zhang, Pavithra Vivekanand, Maureen Cetera, Ilaria Rebay. Ben May Institute for Cancer Research, The University of Chicago, Chicago, IL.

YAN, an ETS family transcriptional repressor, antagonizes the receptor tyrosine kinase pathway to prevent inappropriate target gene transcription. Upon signaling, phosphorylation of YAN by MAPK results in inactivation of YAN-mediated repression. *In vitro* studies have shown that self-association mediated by YAN's N-terminal SAM domain can lead to the formation of long polymers, raising the possibility that polymerization may be required for transcriptional repression. In order to investigate the *in vivo* significance of YAN polymerization, we are comparing the ability of monomeric, dimeric and polymeric YAN to repress various target genes. Preliminary results from transcription assays in cultured *Drosophila* cells and from overexpression studies in the developing eye and embryo suggest that there may be differential requirements for YAN polymerization for repression of target genes. In all assays, the YAN monomer has minimal biological activity, indicating the requirement for high order YAN-YAN association in repressing target genes. Mechanistically, we are investigating how the polymerization status of YAN affects its repression function. Specifically we are exploring the hypothesis that YAN polymerization may allow long-range association with chromatin and that the extent of spreading correlates directly with the level of transcriptional repression of a particular target gene. Preliminary chromatin immunoprecipitation (ChIP) data suggest that binding of YAN to its target genes is not restricted to the high affinity ETS binding site but that it spreads into flanking sequences. With ongoing experiments, we are attempting to further investigate the status, function and regulation of YAN polymerization *in vivo*. We will perform a ChIP-chip assay, which combines ChIP with tiled genomic microarray, to detect the genome-wide distribution of YAN and to identify YAN target genes. Determining how YAN polymerization and spreading is modulated at various target genes during development will be important for understanding the mechanism of YAN-mediated gene repression.

389B

Tranposon born microRNAs in host-pathogen interaction. Manika Pal Bhadra[2], L Mamatha[1,2], Utpal Bhadra[1]. 1) Department of Chemical Biology, Indian Institute of Chemical Technology, Hyderabad 500007, INDIA; 2) Functional Genomics & Gene Silencing Group, Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500 007 INDIA.

MicroRNAs are a family of tiny, endogenous 21-25 nt long non coding RNAs that control wide range of genes in various biological processes. Here, small RNA profile of *Drosophila* embryos and adults recovered five novel microRNAs from *Drosophila* 1360 repetitive element that initiates heterochromatin formation in *Drosophila* mini chromosome. The repression of miRNAs with concomitant upregulation of *hoppel* activity is associated with mutational effect of RNAi components, *piwi*, *Spindle E* and *Argonaute-1*. Accumulation of Pre-miRNA in *piwi* and *Ago-1* heteroallelic mutations suggests that miRNAs function in RNA silencing pathway and RNAi machinery is required for their biogenesis. They also participate as a convenient way for regulating multiple host genes and *hoppel* transposon expression. These findings demonstrate an unexpected link between transposon born microRNAs and host gene regulation. It is also postulated that despite of its role in heterochromatin, the importance of *hoppel* transposon in host parasite interaction delimits its elimination from the *Drosophila* genome by the selective pressure of evolution.

390C

The Different Functions of Smaug in Post-Transcriptional Regulation. Aaron L Goldman[1], Fiona Menzies[1], Timothy Westwood[2], Howard Lipshitz[1]. 1) Developmental & Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario, CA; 2) University of Toronto, Mississauga, Ontario, CA.

The RNA-binding protein, Smaug (SMG), has been shown to play two distinct roles during *Drosophila* embryonic development. SMG has been shown to (1) trigger maternal *Hsp83* mRNA decay; and (2) translationally repress the stem-cell fate determinant gene *nanos (nos)*. To extend our analysis of maternal RNA degradation to the genomic level, we performed a series of microarray experiments. We estimate that 55% of *Drosophila* genes encode maternal transcripts. 21% of these are destabilized in unfertilized eggs, and strikingly, 67% of the unstable RNAs are stabilized in eggs from *smaug* mutants. Thus, SMG is the major regulator of maternal transcript destabilization in the early embryo. SMG also acts as a translational repressor. Since Nos plays an evolutionarily conserved role in the maintenance of germline stem cells (GSCs) by repressing differentiation, we wish to better understand the mechanism by which SMG represses *nos* translation in somatic cells, while allowing *nos* translation in GSCs. In immunostains of *Drosophila* embryos, we show that SMG protein is found enriched in particles in the somatic cells adjacent to the GSCs. *nos* mRNA, but not *Hsp83* mRNA, is found to colocalize with these SMG particles, consistent with SMG's role in translationally repressing *nos* and destabilizing *Hsp83* mRNA. GFP-tagged Nos protein is excluded from the SMG particles and is only found in GSCs. Interestingly, in our microarray study, we found that *oskar* and *vasa* transcripts, which encode genes required for Nos translation, are destabilized in a SMG-dependent manner. These data indicate that SMG may contribute to stem cell fate determination via two independent pathways: by direct translational repression of *nos*, and by downregulation of the translational activators of Nos. Consistent with this hypothesis, we show that *oskar* and *vasa* mRNA does not colocalize with SMG protein. We also show that Vasa protein is restricted to the GSCs and is excluded from the SMG-containing particles.

391A

Live imaging of mRNA RNP particles in Drosophila ovaries. Yiyin Ho, Elizabeth Gavis. Molecular Biology, Princeton University, Princeton, NJ.

The asymmetric distribution of proteins is essential in Drosophila embryonic development for the subsequent differentiation and function of many specialized cell types. The localization of maternal mRNAs during oogenesis is required in order to establish and pattern both the anterior-posterior (A-P) and the dorsal-ventral (D-V) body axis. These mRNAs contain localization signals that cause them to be transported through complexes to their target destination. Localized mRNAs are packaged into ribonucleoprotein (RNP) particles, which are presumed to contain many copies of the mRNA.) However, it is not known whether a single RNP particle contains more than one kind of transcript.

Through fluorescent tagging of mRNAs in vivo, we are investigating whether maternal mRNAs, including *gurken*, *bicoid*, *nanos*, and *oskar*, colocalize in RNPs and are transported together. A strategy previously used in our lab for in vivo labeling of mRNAs with GFP or RFP has now been expanded to include a second tagging system. Thus, we can label two different mRNAs simultaneously, each with different fluorescent protein. The behavior of two such labeled transcripts will be analyzed by time lapse confocal microscopy.

392B

Investigating the dynamics of localized fluorescently labeled *gurken* mRNA in *Drosophila*. A.M. Jaramillo[1,2], T.T. Weil[1], E.R. Gavis[1], T. Schupbach[1,2]. 1) Howard Hughes Medical Institute; 2) Department of Molecular Biology, Princeton University, Princeton, NJ.

During *Drosophila* oogenesis, the targeted localization of *gurken* (*grk*) mRNA leads to the establishment of the axis polarity of the egg. In early stages of oogenesis, *grk* is found at the posterior of the oocyte, whereas in the later stages *grk* is positioned at the dorsal anterior corner of the oocyte. This localization provides the spatial restriction necessary for the Gurken protein to properly signal to the Epidermal Growth Factor Receptor (EGFR) in the overlying follicle cells. In order to visualize real time localization of endogenous *grk* mRNA in living oocytes, we have utilized the MS2-MCP system adapted for *Drosophila* by Gavis (Forrest et al., 2003). MCP-GFP tagged endogenous *grk* localizes to the appropriate sites within the oocyte. Fluorescence Recovery After Photobleaching (FRAP) of fluorescent *grk* mRNA particles reveal a difference in the dynamics of localized *grk* between young and older egg chambers. Current genetic and pharmacological studies are investigating the participation of the dynein motor enzyme and the cytoskeleton in the regulation of *grk* mRNA localization.

393C

Expression Patterns of Noncoding Transcripts in the Bithorax Complex. Benjamin Pease, Welcome Bender. Harvard Medical School, Dept of Biological Chemistry and Molecular Pharmacology, Boston, MA.

The bithorax complex (BX-C) is a 300-kb region of the *Drosophila melanogaster* genome that is involved in segment-specific gene expression. Loss-of-function mutations in the BX-C result in the transformation of segments to resemble more anterior segments. Curiously, the region only contains 3 protein-coding regions. However, numerous noncoding transcripts have been identified with striking segment-specificity. We used strand-specific RNA probes for *in situ* hybridizations of early embryos, targeting the 75-kb intronic regions of the *Ultrabithorax* (*Ubx*) transcription unit. We have found two ncRNAs, transcribed antiparallel to Ubx, in the bithorax (PS5) regulatory region. Mutations in this region result in the bithorax phenotype, where the anterior haltere (PS5) is transformed to anterior wing (PS4). *In situs* were also used to study the effects of gypsy mobile element insertions on a ncRNA in the *bithoraxoid* (*bxd*) region.

394A

Small interfering RNA induces transcriptional silencing in *Drosophila* independent to DNA methylation. Utpal Bhadra[1], Pushpavalli SNCVL[1], Linga Mamatha[1,2], Manika Pal Bhadra[2]. 1) Functional Genomics & Gene Silencing Group, Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500007, INDIA; 2) Department of Chemical Biology, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500007, INDIA.

The double stranded RNA (dsRNA) dependent DNA methylation is a novel device for transcriptional gene silencing (TGS) in plants, but its existence is controversial in mammals. The methylation dependent TGS, introduced by short promoter dsRNA in mammals, was later contradicted by the experimental results using similar approaches. It is reported that siRNA induced chromatin changes are independent to DNA methylation. In *Drosophila melanogaster* DNA methylation has shown barely in early embryogenesis. Here, we demonstrate that small double stranded RNAs of the eye color *white* gene regulatory sequences efficiently induce transcriptional repression by the recruiting Ez, Su(var) 3-9 proteins and enrichment of histone H3 K27 and H3 K9 methylation. The chromatin modulation occurs effectively at DNA methylation free stages. Therefore, we could accomplish small RNA induced transcriptional changes in flies occurs via histone tail methylation that might help predominantly in controlling and maintenance of development regulators and homeotic genes. It is also proposed that transcriptional gene silencing irrespective of DNA methylation is well preserved in animals and can be potentially used for application device for initiating silencing of any genes in animals at the transcriptional level.

395B

An alternatively-spliced rnp-4f mRNA isoform retaining a 5'-UTR intron is more efficiently translated and acts upstream of genes important for Drosophila CNS development. Jing Chen, Sunetra Bhatla, Malini Varadarajan, Jack C. Vaughn. Zoology, Miami University, Oxford, OH.

The 5'- and 3'-UTR and alternative splicing in pre-mRNAs play a variety of roles in regulating eukaryotic gene expression. Two major mRNA isoforms arise via alternative splicing in the 5'-UTR of the Drosophila splicing assembly factor rnp-4f, designated "long" (not spliced) and "short" (spliced). The coding potential for the two mRNA isoforms is identical, but the long isoform is unique in retaining an evolutionarily-conserved stem-loop at the 3'-splice junction of intron 0 and a highly conserved downstream tract, with unknown functional significance. Developmental Northerns show that both isoforms are present throughout embryogenesis, but their abundances relative to constitutively-expressed rp49 change dramatically. Quantification shows that both isoforms are abundant at 0-2 h, followed by rapid declines in mRNA levels at about the time of the mid-blastula transition stage. The longer isoform then increases in abundance to peak at 8-12 h, but declines by late embryo stages. In contrast, the shorter isoform is present at very low levels during mid-embryogenesis. This pattern corresponds to that described by others for RNP-4F protein levels, showing that the mRNA isoform retaining intron 0 is more efficiently translated. A DIG-labeled probe specific to the long mRNA isoform localizes preferentially within the developing CNS, and dissection studies show that this isoform is concentrated at the region which includes midline glial cells. Specific RNAi-mediated knockdown of the long isoform results in a variety of CNS developmental abnormalities, of which the most common is failure in development of commissures toward the anterior end. Some RNAi phenotypes resemble those for comm, others robo, sli, fra, ptc, smo, put, or twi. These results are interpreted to show that the long rnp-4f isoform acts upstream of many genes known to be important for CNS development, probably by interfering with pre-mRNA spliceosomal assembly.

396C

Role of Tis11 in *Drosophila melanogaster*. Robert Fedic[1], Perry J. Blackshear[2], Jasmin Kirchner[3], James M. Mason[1]. 1) Laboratory of Molecular Genetics, NIEHS/NIH, RTP, NC, 27709; 2) Laboratory of Neurobiology, NIEHS/NIH, RTP, NC, 27709; 3) Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK.

The control of gene expression is an essential mechanism that allows a cell to respond rapidly to extracellular and intracellular changes. Regulation of mRNA stability is a crucial part of this process, and actual mRNA levels in the cell are the result of mRNA turnover as well as synthesis. mRNA turnover is controlled by a complex network of *cis* and *trans* acting elements. AU-rich elements (AREs) in the 3' UTR region of certain mRNAs are one of the best studied examples. These elements bind proteins (ARE-BPs) hat affect the stability of mRNAs. TIS11 or Tristetraprolin is an ARE-BP targeting host mRNAs for rapid degradation. *Tis11* was discovered in mouse where it is a member of a family of four tandem zinc-finger proteins. *Tis11* homologues are found in vertebrates (fish, frogs, mammals) as well as in invertebrates (yeasts, nematodes, insects). While the homology is relatively high in vertebrates, in invertebrates homology is restricted to the tandem zinc finger region. Drosophila *Tis11* is a single copy gene subject of splicing resulting in four messages. All transcripts are present throughout development, but their levels fluctuate. Insertion of a P-element into the first exon leads to a wide spectrum of phenotypes, depending on the insertion site, affecting legs, halteres, and developmental time. Ubiquitous overexpression of *Tis11* using the UAS-Gal4 system is lethal. Our results suggest that Tis11 may be involved in regulation of organogenesis. We are now studying Tis11 expression in imaginal discs and a possible interaction with Dpp.

397A

Natural genetic variation in splice expression of the sex-determination pathway in *Drosophila melanogaster* . Brad R Foley, Anne Genissel, Sergey V Nuzhdin. Dept of Evolution and Ecology, UC Davis, Davis, CA.

The sex determination pathway in Drosophila is a well-characterised model genetic pathway which has been shown to possess abundant expression variation. Given its role in the regulation of sexual dimorphism, expression variation in the sex-determination pathway and its downstream targets is a likely source of variation in sexual phenotypic and behavioural traits. Using a panel of *Drosophila melanogaster* Recombinant Inbred Lines (RILs) derived from a natural population, genetic variation in transcript expression of the sex determination pathway and several downstream targets has been measured in both sexes. The regulation of sexual dimorphism in splice expression in these lines has been characterised and we have examined the consequences of upstream variation on the expression of fruitless, a gene known to play a major role in the regulation of courtship and sexual behaviour in *Drosophila melanogaster*, as well as several other candidate genes for sexual behaviour in this species.

398B

Enhancer and silencer elements associated with non-exonic recursive splice sites. A. Javier Lopez, Panagiotis Papasaikas, Michael Chen. Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Recursive splicing is associated with generation of alternative mRNAs and stepwise removal of long introns using non-exonic elements. It occurs at sequences that function sequentially as 3' and 5' splice sites. Recursive splicing has been predicted at hundreds of non-exonic sites in Drosophila and mammals. Seventeen predicted sites have been tested experimentally and found to be functional. We are working to understand the frequency, biological role and mechanisms of recursive splicing. One goal is to identify auxiliary cis-acting sequences that activate and coordinate the sequential function of non-exonic recursive splice sites. Thirty-three candidate intronic splicing enhancer motifs were indentified computationally using regular 5' and 3' splice sites, and these were found to be significantly enriched downstream of recursive splice sites. The distribution of one motif near regular and recursive splice sites suggested a function in both steps of recursive splicing. Candidate silencers are underrepresented within 100 bp of recursive splice sites but rise distally. Phylogenetic conservation and experimental results in a minigene system confirmed the role of candidate enhancer and silencer elements downstream of non-exonic site RP3 in *Ultrabithorax*, including the predicted bifunctional motif. These analyses also confirmed the role of predicted intronic silencers in suppressing the use of nearby non-conserved cryptic 5' splice sites. Intriguingly, we found that a conserved pseudo-5'splice is part of a silencer module, possibly serving as an insulator for the upstream enhancer. These results show that non-exonic recursive splice sites are complex integrated entities that have evolved to function efficiently without altering the structure of mRNA products. We are combining the prediction of cis-elements with a machine-learning model of the core sequence to improve the sensitivity of recursive splice site prediction. These results will be valuable for assessing the frequency and function of recursive splicing and for understanding of alternative splicing.

399C

Novel features in a Recursive Splice Site model generated by machine learning. A. Javier Lopez[1], Panagiotis Papasaikas[1], Aly A. Khan[2], Russell Schwartz[1,2]. 1) Biological Sciences; 2) Computer Science, Carnegie Mellon University, Pittsburgh, PA.

Recursive splicing is associated with generation of alternative mRNAs and stepwise removal of long introns using non-exonic elements. It occurs at sequences that function sequentially as 3' and 5' splice sites. An ad hoc model based on juxtaposition of nucleotide frequency matrices for standard 3' and 5' splice sites has predicted recursive splicing at hundreds of non-exonic sites in Drosophila. The distribution is strongly biased towards introns larger than 10 kb, where the frequency of predicted sites is nearly 10 times higher than random expectation. About 90% of the top 150 sites are conserved in other diptera and selected sites have been traced as far as honeybee. Seventeen sites have been tested and shown to be functional. We are working to understand the extent, biological role and mechanisms of recursive splicing. One goal is to generate more accurate models. As the number of experimentally verified sites is limited, we used a reiterative expectation maximization approach to train a model using a large amount of unlabeled sequence corresponding to the introns of Drosophila and a small subset of verified examples as initial seed. The resulting model for the core sequence differs from the original juxtaposition of standard splice sites and nearly doubles the information content. The number of strongly predicted sites was also doubled but these still exhibit conservation and the strong bias for large introns. Intriguingly, the new model reveals an interdependency between positions -7 and -8 that is not observed in regular 3' splice sites and may identify specific functional constraints. Experimental tests of six newly predicted sites and a control that violates the interdependency confirmed the activity of the predicted sites and supported the significance of the correlated sites. We are combining this new model with the identification of cis-acting auxiliary elements to improve the sensitivity of recursive splice site prediction. These results will be valuable for assessing the frequency and function of recursive splicing in Drosophila and other organisms.

400A

Dominant-negative mutation in Cdk9 reveals a role for the transcription elongation factor P-TEFb during oogenesis. Denis Basquin, Daniel Pauli. Dept of Zoology and Animal Biology, University of Geneva, Geneva, CH.

Transcriptional elongation by RNA polymerase II (RNAPII) is regulated, in part, by the positive transcription elongation factor b (P-TEFb), which promotes the transition from abortive to productive elongation by phosphorylating the carboxyl-terminal domain (CTD) of the largest subunit of RNAPII. The Drosophila melanogaster P-TEFb complex is composed of the cyclin-dependent kinase Cdk9, and a cyclin partner, CyclinT or CyclinK. To examine the role of Cdk9 in vivo, we expressed a dominant-negative form of the kinase to alter P-TEFb function at a given developmental stage or in a tissue-specific manner. We found that ubiquitous expression of the mutant Cdk9 is lethal and strongly affects larval endoreplicating tissues. Expression in the imaginal discs reduces the size of adult structures. Expression of Cdk9DN in the germline induces female sterility, and causes oogenesis defects such as egg chamber fusions and nurse cell abnormalities. We next investigated the role of CyclinK as a P-TEFb subunit. We show that tagged versions of Cdk9 and CyclinK colocalize at many sites on salivary gland polytene chromosomes. Furthermore upon heat shock treatment, both proteins are recruited to the heat shock puffs in a pattern that mimics phosphorylated RNAPII.

401B

Nanos downregulates transcription and modulates CTD phosphorylation in the soma of early Drosophila embryos. Girish Deshpande, Gretchen Calhoun, Paul Schedl. Dept Molecular Biol, Princeton Univ, Princeton, NJ.

nanos (nos) specifies posterior development in the Drosophila embryo by repressing the translation of maternal hb mRNA. In addition to this somatic function, nos is required in the germline progenitors, the pole cells, to establish transcriptional quiescence. We have previously reported that nos is required to keep the Sex-lethal establishment promoter, Sxl-Pe, off in the germline of both sexes. We show here that nos also functions to repress Sxl-Pe activity in the surrounding soma. Sxl-Pe is inappropriately activated in the soma of male embryos from nos mothers, while Sxl-Pe can be repressed in female embryos by ectopic Nos protein. nos appears to play a global role in repressing transcription in the soma as the effects of nos on promoter activity are correlated with changes in the phosphorylation status of the carboxy terminal domain (CTD) repeats of the large RNA polymerase II subunit. Moreover, the suppression of transcription in the soma by Nos protein appears to be important for normal embryonic development. Finally, we present evidence suggesting a role for TFIIH kinase, cdk-7 in this process. *nanosnosDrosophilahbnosSxl-PeSxl-PeSxl-PeSxl-Penoscdk-7*.

402C

In Vitro Translation Experiments to Identify Mechanisms of nanos Translational Regulation. Shane Andrews, Elizabeth Gavis. Dept Molecular Biol, Princeton Univ, Princeton, NJ.

Nanos is critical for patterning the posterior half of the embryo during early development. A gradient of Nanos protein is synthesized from maternally deposited nanos mRNA that is localized to the posterior. However, only a small fraction of the total pool of nanos mRNA is actually localized. Because of this, it is important that the unlocalized mRNA be translationally repressed for proper development to occur. One key component of the embryonic translational repression machinery, the protein Smaug, has been identified. However, the actual mechanism of nanos translational repression is still unclear. While Smaug appears to repress translational initiation, work from our lab indicates that translational repression occurs at a step downstream of initiation. In order to further characterize TCE-mediated repression, we are utilizing several experimental approaches. If regulation occurs downstream of initiation, the nanos TCE should be able to repress translation regardless of whether it is initiated at the cap or at an IRES. We are testing this prediction using luciferase constructs designed to monitor IRES-mediated translational initiation in the presence or absence of the nanos TCE. Recently a second protein, Glorund, has been shown to repress nanos translation during oogenesis by binding to the third stem loop of the TCE. We have developed a fly ovarian extract that faithfully recapitulates stem loop III dependent translational repression, and are using this extract to further characterize the mechanism of TCE-mediated repression during late-stage oogenesis.

403A

Molecular basis of RNA recognition by the translational repressor and hnRNP F/H homolog Glorund. Yossi Kalifa, Elizabeth R. Gavis. Dept Molecular Biology, Princeton Univ, Princeton, NJ.

Translational control of *Drosophila nanos* (*nos*) mRNA is essential for patterning of the anterior-posterior body axis. Localization of *nos* mRNA to the germ plasm at the posterior pole of the *Drosophila* embryo is required to activate *nos* translation and thereby generate abdominal segments. Inefficient posterior localization of *nos* mRNA during late stages of oogenesis leaves the majority of *nos* distributed throughout the oocyte and early embryo. Translational repression of this unlocalized *nos* mRNA is mediated by a cis-acting translational control element (TCE) in the *nos* 3' untranslated region which composed of two stem-loops designated II and III that have temporally distinct functions and different modes of recognition. Stem-loop II mediates translational repression of unlocalized *nos* RNA during early embryogenesis whereas stem-loop III mediates repression of unlocalized *nos* in late oocytes. We have purified and characterized a novel *Drosophila* hnRNP protein, Glorund (Glo) that interacts with the region of the *nos* TCE responsible for translational repression during oogenesis. Glo binds to the double-stranded UA-rich motif of TCE stem-loop III via two RRM-like motifs. The effect of mutation in TCE stem-loop III on Glo binding in vitro correlates with the ability of this element to repress translation in vivo. *glo* mutant ovaries show increased accumulation of a GFP-Nos reporter in late oocytes as well as derepression of an unlocalized, translationally silent *nos* RNA derivative in late oocytes. Based on the known solution structure of the three quasi RNA recognition motifs of the human hnRNP F we have mutated the residues that are important for RNA interaction and tested the effect of these mutations in vivo.

404B

Characterization of CG6770, a potential translational inhibitor. Sara L. Naylor, Marc Tatar. Ecology & Evolutionary Biology, Brown University, Providence, RI.

Upon refeeding after protein starvation, the detailed transcript profile of Drosophila melanogaster CG6770 is exceptionally similar to that of 4E-binding protein (4eBP). CG6770 encodes a small polypeptide of unknown function. Given its expression pattern congruity to 4eBP, we propose that CG6770 is a cofactor for d4eBP in its role as a repressor of translation. CG6770 may therefore contribute to the control of cell growth and proliferation, as well as to stress responses including starvation resistance. We are characterizing CG6770 with cellular, genetic and biochemical approaches. Through application of RNAi we show that CG6770 is required for oxidative stress resistance in S2 cells. To investigate phenotypes in whole animals we have generated a targeted knockout of CG6770. Similar to 4eBP, our homozygote mutants are viable. We shall report on traits of this mutant, the results of rescue and over-expression, and the tissue localization patterns of CG6770.

405C

The RNAi machinery represses nanos translation in the early embryo. Ben D. Pinder[1], Wibke J. Meyer[2], H. Arno J. Müller[3], Craig A. Smibert[1]. 1) Biochemistry, University of Toronto, Toronto, ON, CA; 2) Institut für Genetik, Heinrich-Heine Universität, Düsseldorf, Germany; 3) Division of Cell and Developmental Biology, University of Dundee, Dundee, UK.

In the Drosophila embryo Nanos protein is localized to the posterior where it specifies posterior cell fates. This localization is achieved through controls which ensure that only nanos mRNA localized to the posterior is translated while nanos mRNA in the bulk of the embryo is translationally repressed. Unlocalized nanos mRNA is repressed by Smaug, a sequence-specific RNA binding protein that binds cis-acting Smaug Recognition Elements in the nanos 3'untranslated region.

An affinity-based purification of potential Smaug binding proteins identified Argonaute 2, a component of the RNA interference (RNAi) machinery. We have demonstrated that Smaug and Argonaute 2 interact in the same protein complex and have also shown that Smaug associates with Argonaute 1. To investigate the role of the RNAi machinery in regulation of nanos we took advantage of the argonaute 2 allele known as drop out ($ago2^{dop1}$) which has been shown to disrupt the function of both Argonaute 1 and Argonaute 2 in embryos, resulting in a complex maternal effect lethal phenotype. We have shown that embryos derived from $ago2^{dop1}$ mutant mothers express ectopic Nanos protein, while the levels of nanos mRNA are unaffected. Taken together these results indicate that the RNAi machinery is required for translational repression of nanos. While nanos translation is repressed during both oogenesis and embryogenesis, we have shown that the role of the Argonaute proteins is limited to embryogenesis. Taken together our data suggests that a miRNA functions in the embryo to regulate nanos translation and future work will attempt to identify the miRNA and cis-elements involved. In addition we will address the functional significance of the interaction between Smaug, Argonaute 1, and Argonaute 2.

406A

Orb remodels the Cup-Bruno translational repression RNP complex to activate localized translation at the oocyte. Li Chin Wong[1], Alexandre Costa[2], Ian McLeod[3], John Yates 3rd[3], Paul Schedl[1]. 1) Dept of Molecular Biology, Princeton Univ, Princeton, NJ; 2) Stanford University, Stanford, CA; 3) The Scripps Research Institute, La Jolla, CA.

The cytoplasmic polyadenylation element binding (CPEB) protein homolog in *Drosophila*, Orb, is essential for oocyte determination and polarity establishment during oogenesis. Proper localization of Orb in the developing oocyte is essential as Orb positively regulates the translation of various mRNAs that function in polarity determination. Cup was identified by mass spectrometry, and confirmed by Western analysis, to co-immunoprecipitate with Orb. We show that this interaction is biologically significant as multiple alleles of *cup* interact genetically with *orb* to suppress a haploinsufficient DV polarity phenotype in heterozygous *orb* female. Since Cup is a translational repressor, we expected to find an increase in the level and/or activity of Orb protein in cup mutants. Accordingly, the levels of Orb protein, but not *orb* mRNA, are elevated in the developing ovary in homozygous *cup* mutants. More interestingly, we observed an increase in the hyper-phosphorylated isoform of Orb in *cup* homozygous mutants, thus indicating that Cup may suppress the *orb* phenotype by affecting both the levels and activity of Orb. An analysis of protein complexes formed *in vivo* by immunoprecipitation studies show that both isoforms of Orb bind to me31B and PABP, in addition to Cup. However, our immunoprecipitation data suggests that Orb does not bind to Bruno *in vivo*. Immunostaining of developing egg chambers reveal that although both Bruno and Orb are expressed at the posterior of the egg chamber at the early stages of development, Bruno completely ceases to co-localize with Orb abruptly at stage ten, when Orb intensifies at the dorsal-anterior of the oocyte while Bruno remains at a low level in the posterior of the oocyte. Our data suggest that when the translationally repressed Cup-Bruno RNP complex arrive at the posterior of the egg chamber of later stages, a remodeling of the RNP complex occurs where Orb replaces Bruno and activates translation of the localized Orb target mRNAs.

407B

Regulation of Wnt signaling by lipid modification. Wendy Ching, Roel Nusse. Dept Developmental Biol, Stanford Univ, Stanford, CA.

Wnt proteins compose a family of secreted signaling molecules that are highly conserved and control many developmental processes. Metabolic labeling and mass spectrometry on purified vertebrate and *Drosophila* Wnt proteins have revealed that they are modified by the covalent attachment of a fatty acid. Lipid modification of many intracellular proteins has been shown in different instances to facilitate protein interactions, membrane targeting, and structural stability. While it may seem unusual for a protein that is secreted into the extracellular space to be modified by the addition of a hydrophobic moiety, this has been observed to be the case for two secreted growth factors: Hedgehog and Spitz. Given the vastly different roles of lipid modification on Hedgehog versus Spitz, it is interesting to consider what other roles such hydrophobic modifications could play in regulating the function of secreted signaling proteins. To address this question, we are investigating the role of lipid modification in Wnt signaling in *Drosophila* by using a combination of cell culture and *in vivo* techniques. To study the properties of Wnt proteins that lack lipid modification, we are using site-directed mutagenesis against the acylated residue. We are also using genetic techniques and RNAi to eliminate the function of Porcupine, an enzyme suspected to acylate Wnt proteins.

408C

Loss-of-function in a Delta allele (Dl^{RF}) is due to protein misfolding and failure to reach the cell surface. Anton A Delwig, Matthew D Rand. University of Vermont, Burlington, VT.

Delta-Notch signaling is a fundamental pathway that controls cell fates during development. A current model for Notch activation involves “trans-endocytosis”, whereby endocytosis of the Delta ligand promotes subsequent ADAM and Presenilin proteolysis and activation of the Notch receptor on an adjacent cell. Initial evidence for this model relied on Delta mutants that reportedly fail to undergo endocytosis. One such allele, Dl^{RF}, gives a temperature-sensitive loss-of-function. Previous studies in retinal cells showed Dl^{RF} protein localizes to endocytic vesicles at the permissive temperature (18ºC) and to the cell periphery at the restrictive temperature (30ºC). We have cloned and sequenced Dl^{RF} and found two mutations in the extracellular epidermal growth factor-like (EGF) repeats, EGF3 (Gly305->Glu) and EGF4 (Cys348->Tyr). Expression in Drosophila S2 cells shows that at 30ºC the Dl^{RF} protein fails to reach the plasma membrane, instead accumulating in the perinuclear region and in large cytosolic vesicles. Co-expression of GFP-tagged Dl^{RF} and RFP-tagged wild-type Delta (Dl^{wt}) demonstrates complete segregation of the proteins at 30ºC with Dl^{RF} failing to localize to the plasma membrane. With Dl^{RF} there is an absence of the Kuzbanian-derived Delta extracellular proteolytic product in the media. We have also examined endogenous Delta localization in recently-characterized thoracic ganglion interneurons, a highly polarized cell type by which to judge membrane distribution. At18ºC, Dl^{RF} protein is enriched along the length of newly formed neurite bundles and their terminals. At 30ºC, the Dl^{RF} protein is dramatically reduced on neurites and is concentrated in large vesicles in the cell soma, again consistent with a failure in trafficking to the plasma membrane. Altogether the data indicate that the Dl^{RF} allele is a temperature-sensitive misfolding protein mutant. Thus, Dl^{RF} has limited utility for studying Delta endocytosis mechanisms but is well suited as a tool for loss-of-function studies. (supported by NIH NCRR P20RR16435-01 and NIEHS PHS R21ES013754-2 awarded to M.D.R.).

409A

Ninjurin A has two functions in regulating cell adhesion. Bernadette Glasheen, Nicholas Simms, Shuning Zhang, Caitlin Piette, Andrea Page-McCaw. Department of Biology, Rensselaer Polytechnic Institute, Troy, NY.

Our lab has previously shown in culture that the extracellular protease Mmp1 and the transmembrane protein Ninjurin A (NijA) function together in a signaling pathway. Mmp1 cleaves and releases the NijA ectodomain. The secreted NijA ectodomain functions cell non-autonomously to cause cells to release adhesion from a substrate. In recent experiments we have determined that S2 cells normally express low levels of endogenous *NijA*, and when this is knocked down cells can no longer respond to the NijA ectodomain signal. We identified the S2R+ cell line that displays a 10-fold reduction in the level of *NijA* expression compared to S2 cells and these cells do not respond to the NijA signal. After transfection of the *NijA* cDNA, S2R+ cells are able to respond to the signal and release adhesion. Thus it appears that *NijA* has two functions in the signaling pathway: it acts as the signal and is necessary to respond to the signal.

One phenotype of *Mmp1* mutants is the inability to shed their cuticle, suggesting that *Mmp1* may be involved in tracheal cell adhesion. Antibody staining demonstrates that NijA and Mmp1 colocalize in the larval trachea at the cell borders and that colocalization is developmentally regulated. We used an imprecise excision of a P element upstream of the *NijA* locus to generate a null mutant, and we are currently examining *NijA* function in vivo.

410B

Drosophila Importin-7/Moleskin alters Delta/Notch expression and activity in the developing wing. Daniel Marenda. Department of Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.

Drosophila DIM-7 (encoded by the moleskin gene, msk) is the orthologue of vertebrate Importin-7. Both Importin-7 and DIM-7 function as nuclear import cofactors, and have been implicated in the control of multiple signal transduction pathways. I performed two genetic deficiency screens using DIM-7 over-expression phenotypes in both the eye and the wing to identify deficiencies that similarly modified both phenotypes. I identified a total of 11 deficiencies, one of which removes the Delta locus, a central component of the Notch signal transduction pathway. I show that Delta loss-of-function alleles dominantly suppress DIM-7 gain-of-function in the developing wing. I further show that DIM-7 over-expression in developing wings increases both Delta protein expression and Delta transcription, but this increased Delta protein is not competent to activate Notch signaling in adjacent cells. Further, DIM-7 over-expression also increases Notch protein expression in the same cells in which Delta expression is also enhanced. These results indicate a novel function for DIM-7 in Delta/Notch regulation and signaling, and may suggest a link between DIM-7 mediated Delta/Notch signaling, and DIM-7 mediated regulation of other signaling pathways.

411C

Functional analysis in vivo reveals surprisingly robust asssembly of the Axin protein complex. Marcel Wehrli, Wynne Peterson-Nedry, Naz Erdeniz. Cell & Dev Biol/L215, Oregon Health & Sci Univ, Portland, OR.

The proper regulation of Wingless (Wg)/Wnt signaling is critical for normal development. A protein complex assembled around Axin serves to target Armadillo (Arm) for degradation in the absence of Wg, maintaining the OFF state of Wg signaling. Based on biochemical and over-expression experiments, it is thought that Axin directly binds its partners APC, Shaggy (Sgg) kinase and Arm. However, tests of this model are based on over-expression experiments and have produced conflicting data. Thus the model has not yet been tested at normal expression levels using Axin mutants that lack binding sites. Such experiments would address Axin complex assembly and activity *in vivo* and provide insights into the role of Wnt signaling in carcinogenesis, in which Axin complex components are often defective. Therefore we generated an Axin rescue construct and then designed a series of Axin mutants without binding sites for APC, Sgg, Arm, the DIX domain and large intervening domains. All constructs are expressed at comparable protein levels. We assayed for the ability of mutant Axin to maintain the OFF state of signaling (= activity of Axin) as well as the ability of the Axin complex to be inactivated by Wg signaling (= regulation). Surprisingly, all Axin mutant proteins display both activity and regulation. Our findings demonstrate that the Axin complex is very efficiently assembled *in vivo* and they are best explained by secondary interactions that occur between the binding partners APC, Sgg and Arm. Such interactions had been shown biochemically, though their functional significance remained unclear. Interestingly, and consistent with this model, constructs carrying deletions for APC and Arm binding domains, respectively, show allele-specific complementation that fully rescues the *axin* null mutant, though no other combination of our deletion mutants rescues. Therefore, extending other published data, our *in vivo* analysis revealed that Axin complex activity occurs very robustly, consistent with highly cooperative assembly through secondary and ternary interactions between the individual components.

412A

The HMG-box transcription factor, Sox-Neuro, acts with Tcf to control Wg/Wnt signaling activity. Amy Bejsovec, Whitney Jones, Anna Chao. Dept. of Biology, Duke University, Durham, NC.

Wingless(Wg)/Wnt signaling specifies cell fates in many tissues during vertebrate and invertebrate embryogenesis. To better understand how Wg signaling is regulated during development, we performed genetic screens to isolate mutations that suppress or enhance *wg* mutant phenotypes. In one screen, we found that loss of function mutations in the neural determinant, *Sox-neuro*, partially suppress *wg* mutant pattern defects. *SoxN* encodes an HMG-box containing protein related to the vertebrate Sox1, Sox2 and Sox3 proteins, which have been implicated in patterning events in the early mouse embryo. In Drosophila, *SoxN* has been previously shown to specify neural progenitors in the embryonic central nervous system. We have discovered that *SoxN* also has a role in negatively regulating Wg pathway activity in the embryonic epidermis. Loss of *SoxN* function hyperactivates the Wg pathway and overexpression of *SoxN* represses pathway activity. Unlike many other negative regulators of the Wnt pathway, *SoxN* does not act to stabilize Armadillo(Arm)/beta-catenin. Rather, epistasis analysis with *arm* and other components of the Wg pathway places the *SoxN* gene product downstream, at the level of the transcription factor Tcf in regulating target gene expression. In human cell culture assays, *SoxN* represses Tcf-responsive reporter expression, indicating that the fly gene product can interact with mammalian Wnt pathway components. In both flies and in human cells, *SoxN*-mediated repression is potentiated by adding ectopic Tcf, suggesting that SoxN protein interacts with the repressor form of Tcf to influence Wg/Wnt target gene transcription.

413B

A quantitative assessment of the Hedgehog signaling pathway. Shohreh Farzan[1], Melanie Stegman[2], Manuel Ascano[1], Stacey Ogden[1], David Robbins[1]. 1) Pharmacology & Toxicology, Dartmouth Medical School, Hanover, NH, 03755; 2) Microbiology & Immunology, Cornell Weill Medical College, New York, NY, 10021.

The Hedgehog (Hh) family of secreted proteins is responsible for many developmental processes and is implicated in the progression of various forms of human cancer, including lung carcinoma and medulloblastoma. Many components of the Hh pathway have been well-characterized and various models have been proposed as to how each interacts with one another. The Hh signal is ultimately transduced by Cubitus interruptus (Ci), a member the Gli family of zinc-finger transcription factors. Ci has been shown to act as both a transcriptional activator and repressor and is hypothesized to be involved in multiple complexes with other pathway components. These different complexes are thought to regulate Ci activity in various ways, such as stabilizing the activator form of Ci or targeting it for processing to its repressor form. The signaling complex that mediates Hh response is formed by three pathway components, two of which have been shown to associate with membranes. It is unknown how membrane association alters signaling, but we hypothesize that the membrane associated complex is physiologically relevant and its main purpose is to regulate Ci activity. In order to understand each component's contribution, we want to determine their physical stoichiometry. Here, we begin to elucidate a relevant model by establishing a method to quantify steady-state concentrations of the complex components and propose a stoichiometric model of the membrane bound signaling complex.

414C

Identification of JAK/STAT pathway target genes. Maria Sol Flaherty[1], Jiri Zavadil[2], Aloma Rodrigues[1], Laura Ekas[1], Erika Bach[1]. 1) Pharmacology, New York Univ. School of Med., New York, NY; 2) Pathology, New York Univ. School of Med., New York, NY.

The JAK/STAT pathway is highly conserved and is critical for many biological processes in both vertebrates and invertebrates including cell proliferation. Alteration of this pathway is causal in many human cancers. However, it is not well understood how this pathway regulates cell proliferation. Studies about the role of this pathway in mammalian systems are complicated by genetic redundancies due to the presence of seven STATs and four JAKs. However *Drosophila* serves as an excellent model system to study this pathway, as there is only one JAK and one STAT called Stat92E.

In *Drosophila* the JAK/STAT pathway plays a critical role in the growth of several tissues, including the eye imaginal disc, hemocytes in the larval lymph gland and stem cells in testis. Ectopic activation of this pathway leads to over-growth of these tissues, while loss-of-function mutations lead to a reduction their size. In order to better understand how this pathway regulates cell proliferation, we identified JAK/STAT target genes using a combined approach of micro-array analysis, bio-informatics and genetics. We have identified some novel potential JAK/STAT target genes which maybe involved in cell proliferation. We performed genome-wide expression profiling on eye discs from *GMR-upd* animals, in which the JAK/STAT pathway is hyper-activated and which have greatly enlarged eye discs and adult eyes. ~520 genes were differentially regulated in *GMR-upd* as compared to yw controls. To select genes for validation, we compared the genes identified in the *GMR-upd* micro-array analysis to those found in (1) a bio-informatics screen (using Target Explorer) for genes with Stat92E binding sites in non-coding regions or (2) in a sensitized genetic screen for mutations that dominantly modified the *GMR-upd* phenotype. We present the genes that have been validated by *in situ* hybridization and/or by quantitative PCR.

415A

S149 is a new Dpp target that acts as a corepressor with Brinker to promote cell death. Offer Gerlitz, Yaron Suissa, Oren Ziv, Hadar Neuman, Tama Dinur. Biochemistry, Faulty of Medicine, The Hebrew University, Jerusalem, IL.

A fundamental question in development is how growth, cell fate specification and pattern formation are spatially and temporally coordinated to control the final shape, size and cellular makeup of an organ. Part of the answer resides in the ability of a single morphogenetic molecule to provide simultaneous guiding cues for different developmental processes. The morphogen Decapentaplegic (Dpp) controls growth, survival and patterning during development of *Drosophila* appendages by differentially regulating the expression of various target genes. The binding of Dpp to its serine/threonine kinase receptor complex triggers the phosphorylation of the transcription factor Mad, which, together with associated factors, translocates to the nucleus and regulates the expression of target genes. Much of the regulation of target genes is effected through repression of a transcriptional repressor, Brinker (Brk). Loss of *brk* function leads to ectopic expression of Dpp target genes, tissue overgrowth and cell-fate transformations corresponding to elevated levels of Dpp signaling. Here we identify S149 as a new Dpp target in the developing wing that acts as a corepressor with Brk to promote cell death through induction of the JNK pathway. Interestingly, S149 function is not required for Dpp-dependent patterning in the developing wing. We further find that a previously identified corepressor of Brk, Groucho, which is implicated in patterning, does not exhibit a similar death-promoting activity. Finally, we show that overexpression of S149 and Groucho differentially affect known Dpp target genes. These results imply that Brk's choice of corepressor determines the specificity of target gene repression, thereby directing the control of Dpp-dependent patterning, growth and cell survival outputs.

416B

The role of RacGap50C in the Wingless pathway. Whitney Jones, Amy Bejsovec. Biol, Duke Univ, Durham, NC.

The Wingless(Wg)/Wnt signal transduction pathway directs a variety of cell fate decisions in developing animal embryos. Despite the identification of many Wg pathway components to date, it is still not clear how these proteins work together to generate cellular identities. We have been characterizing the role of RacGap50C, a negative regulator, in the Wg pathway. Embryos mutant for *RacGap50C* show cuticle pattern disruptions that include replacement of ventral denticles with naked cuticle, and an increase in Armadillo protein accumulation. RacGap50C has previously been shown to be required for contractile ring formation in cytokinesis, participating in a complex that mediates the interaction between the actin and microtubule cytoskeleton. However, we have shown that the role of RacGap50C in the Wg pathway is a separate function of the protein, and does not require the conserved GAP domain, which is required for cytokinesis. We have performed a yeast two hybrid screen to identify potential protein interactors with RacGap50C in the Wg pathway. One interacting protein, Pavarotti (Pav), is a member of the complex required for the formation of the contractile ring. *Pav* mutant embryos also show a phenotype consistent with a role in the Wg pathway. This suggests that the Pav-RacGap50C complex may play a role in regulating the Wg pathway. Initial characterization of other interacting proteins further indicates a potential role for RacGap50C in the cytoskeletal interactions and cellular localization of wingless pathway components. We have also analyzed the structure of RacGap50C with respect to its function, examining domains of interaction important for Wg pathway regulation, and investigating the role of these domains in the cellular localization of Wg pathway components.

417C

Negative regulation of Wingless signaling by a microRNA. Jennifer Kennell, Kenneth Cadigan. Dept of Mol, Cell & Dev Biol, Univ of Michigan, Ann Arbor, MI.

Wnts are a family of highly conserved, secreted glycoproteins that act at a short range to regulate many developmental processes. Binding of Wingless (a Wnt in *Drosophila*) to its coreceptors triggers events in the cytosol that prevent phosphorylation and subsequent degradation of Armadillo (Arm). This results in increased levels of Arm in the cytosol and translocation to the nucleus where Arm functions as a coactivator for the TCF/LEF family of transcription factors. Using a genetic screen to identify antagonists of Wingless (Wg) signaling we identified a previously uncharacterized microRNA that suppresses Wg signaling *in vivo* and *in vitro*. Misexpression of this microRNA in the developing eye suppresses the small eye phenotype caused by Wg or Arm overexpression. In addition, misexpression of this microRNA in the wing imaginal disc inhibits expression of endogenous Wg target genes including *senseless*, *distal-less* and *naked cuticle*. Using cell culture assays we found that misexpression of this microRNA inhibits Wg signaling downstream of Arm stability and nuclear localization. To test the biological relevance of this microRNA we produced mutants lacking the gene. We found that adult flies lacking this microRNA are significantly smaller and have decreased cuticle pigmentation. We are currently testing whether any aspect of this phenotype is due to increased Wg signaling. In addition, Wg signaling may positively regulate the expression of this microRNA based on its expression pattern in the developing wing. Our data suggest that this microRNA may act as a negative feedback inhibitor of Wg signaling. Experiments to test this hypothesis are in progress and will be presented.

418A

The Jak/Stat pathway acts upstream of dpp for GSCs maintenance. Lourdes López-Onieva, Ana Fernández-Miñan, Acaimo González-Reyes. Centro Andaluz de Biologia del Desarrollo. CSIC-Universidad Pablo de Olavide, Seville, Spain.

Drosophila melanogaster males and females retain populations of Germline Stem Cells (GSCs) during their adult life that are essential for fertility. Interactions between GSCs and the surrounding somatic cells create a unique micro-environment or niche that is permissive for the maintenance of stem cells in a non-differentiated state. Importantly, the Jak/Stat (Janus Kinase-signal transducer and activator of transcription) signalling pathway is required in the male germline for GSC maintenance (Kiger, White-Cooper et al. 2000) (Kiger, Jones et al. 2001). Recently, it has been described that the Jak/Stat signalling pathway plays a critical role in ovarian niche function. Similar to the testis, overexpression of one of the ligands of the Jak/Stat pathway in somatic cells in the germarium causes an hyperplastic growth of GSCs. In addition, the Escort Stem Cells, a group of somatic cells present in the ovary, require Jak/Stat signalling to maintain the wild-type population of ovarian GSCs (Decotto and Spradling 2005). In this study, we show that the activation of the Jak/Stat pathway in the supporting stromal cells of the niche induces the tumorogenic growth of GSCs. In this regard, we also demonstrate that the Jak/Stat signalling is essential in the niche Cap Cells for GSCs maintenance. These results suggest that a Jak/Stat controlled relay signalling between the soma and the germline is in place to ensure the maintenance of GSCs. In attempt to unveil the molecular mechanism responsible for this relay, we have analyzed the dpp pathway activation in Jak/Stat-induced tumorogenic ovaries. This pathway is known to have an essential role in GSCs for their maintenance. We find that the stem cells-like present in the experimental ovaries express P-mad, a reporter of dpp pathway activation. Finally we show that dpp mRNA levels are increased 3 fold in experimental ovaries compared to wild-type controls. Our results support a model in which the Jak/Stat pathway regulates dpp expression in somatic cells to allow GSC maintenance.

419B

WntD pathway-mediated regulation of NF-κB activity during development and the innate immune response. Mark A. McElwain, Dennis C. Ko, Michael D. Gordon, Michael A. Katsnelson, Roel Nusse. Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

Signaling pathways such as those regulating Nuclear Factor-kappaB (NF-κB)-like transcription factors control many aspects of embryonic development and adult homeostasis. Often, the same pathway is used repeatedly to accomplish different tasks, raising the question of how these pathways are regulated in different biological contexts. While much is known about the major pathways transduced through NF-κB family members, much is still to be learned about how other signaling mechanisms control NF-κB activity. Recently, it was demonstrated that Drosophila WntD regulates embryonic dorsal/ventral axis specification and the adult innate immune response by signaling through the Drosophila NF-κB homolog Dorsal. Identifying the WntD receptor and other downstream components is essential for understanding how the signal is transduced, and how axis specification and the immune response are regulated. Here, we describe the analysis of three candidate pathway components identified in a suppressor screen for modifiers of a WntD overexpression phenotype - Frizzled 4, Toll-6, and CG16708, a putative ceramide kinase.

420C

A chemical genetic screen to identify new Par-1 targets. Piyi Papadaki, Anne Ephrussi. Developmental Biology Unit, EMBL, Heidelberg, DE.

Par-1 kinases play instrumental roles in cell polarization in a diverse set of cellular types from all organisms in which they have been studied to date, from yeast to humans. However, the molecular mechanisms and biochemical pathways by which they exert their effects remain elusive. We are using Drosophila oogenesis, in which Par-1 functions are well studied, as a model system to identify and characterize Par-1 targets. Specifically, we are applying a “chemical genetic” approach to screen for Par-1 targets in an in vivo context. The screen is based on the production of transgenic flies that carry an “analog sensitive” (AS) Par-1 mutant, which can make use of bulky ATP analogs too large to fit in the ATP-binding pocket of all the other kinases in the cell. The exclusive ability of AS-Par-1 to utilize the ATP analog thus provides a means of specifically labeling direct Par-1 targets. We will present evidence that AS-Par-1 retains normal enzymatic activity and substrate specificity, as well as technical challenges and preliminary results of the screen.

421A

Deciphering the role of signalling cascades in epidermis differentiation. Francois Payre[1], Jennifer Zanet[1], Isabelle Delon[1,2], Philippe Valenti[1], Alistair Mac Gregor[3,4], David Stern[3], Serge Plaza[1]. 1) Centre de Biologie du Developpement, Toulouse, FRANCE; 2) The Gurdon Institute, Cambridge, UK; 3) Department of Ecology and Evolutionary Biology, Princeton NJ; 4) Institute of Genetics, Cologne, Germany.

A general feature of signalling pathways is their capacities to elicit different developmental outputs in adjacent cells. How activated signalling cascades can trigger distinct differentiation programs remains however poorly understood. We addressed this question through the analysis of the differentiation of the embryonic epidermis. In the ventral region, Wingless determines naked cuticle and the EGF-r pathway promotes the formation of denticles (or trichomes). In the dorsal region, naked cuticle is specified by intermediate levels of Hedgehog activity, while Wingless determines the formation of thin trichomes (type4). A major output of signalling pathways is their effect on the expression of Shavenbaby (Svb), a transcription factor that switch epidermal cells towards trichome production. To gain insight into signalling mechanisms, we undertook the identification of cis-regulatory elements governing the transcriptional response of *svb* to these pathways. Through the systematic analysis of genomic regions, we found that *svb* epidermal expression is controlled by 3 separate elements, scattered over 50 kb. We show here that each element, contributing to complementary aspects of *svb* expression in both dorsal and ventral regions, behaves as an independent regulatory module with respect of signalling activities. The proximal element that directs *svb* expression in ventral denticles and in type 1&3 dorsal cells is repressed by Wingless and activated by EFG-R in both dorsal and ventral regions, thus revealing overlooked roles for EGF-r and Wg in dorsal differentiation. Our data show that a given pathway can have opposite effect on the expression of a same target gene, through its action on separate cis-regulatory elements. Our identification of such elements in the *svb* promoter now allows the respective molecular mechanisms to be unravelled.

422B

***Drosophila* SnoN modulates growth and patterning by antagonizing TGF-β signalling.** Marie-Christine Ramel[1], Caroline S Emery[1], Rebecca Foulger[2], Deborah C I Goberdhan[1], Marcel van den Heuvel[3], Clive Wilson[1]. 1) Dept Physiology,Anatomy & Gen, Univ Oxford, Oxford, UK; 2) Research School of Biosciences, Univ Kent, Canterbury, UK; 3) MRC Functional Genetics Unit, Univ Oxford, Oxford, UK.

Signalling by TGF-β ligands through the Smad family of transcription factors is critical for developmental patterning and growth. Disruption of this pathway has been observed in various cancers. In vertebrates, members of the oncogenic Ski/Sno protein family act as negative regulators of TGF-β signalling, interfering with the Smad machinery to inhibit the transcriptional output of this pathway, potentially explaining the growth regulatory effects and the normal physiological functions of these molecules. However, Ski/Sno proteins can also affect other signalling pathways involving Hedgehog and Wnt homologues. We have analyzed the function of the *Drosophila* Ski/Sno orthologue, SnoN. It inhibits growth when overexpressed, consistent with it playing a tumour suppressor role in flies. SnoN can act in multiple tissues to selectively and cell-autonomously antagonise signalling by TGF-β ligands from both the BMP and Activin sub-families. By contrast, analysis of a *snoN* mutant suggests that normally SnoN has a limited function in development, inhibiting TGF-β-induced wing vein formation during pupation. Further, SnoN expression appears to be modulated by TGF-β signalling. Genetic evidence also indicates that SnoN may normally modulate levels of insulin receptor signalling, providing one explanation for its growth regulatory properties. We propose that *Drosophila* SnoN normally functions to finely adjust TGF-β signalling levels but that it can behave as an extremely potent inhibitor of TGF-β signalling when highly expressed, highlighting the significance of its deregulation in cancer cells.

423C

The roles of D-cblL and D-cblS in eye development. Hannah Robertson, Jill Ackland, Rebecca Staehr, Adrian Monk, Gary Hime. Department of Anatomy & Cell Biology, University of Melbourne, Melbourne, AU.

The Cbl family of proteins are negative regulators of cell surface receptors that function as E3 ubiquitin protein ligases and link receptors to the endocytic pathway. Mammalian c-Cbl and CblB have been implicated in a range of human diseases and conditions including diabetes, autoimmune disease, obesity, and long term memory. Biochemically, the Cbl family are very well characterised, however very little is known about the cellular consequences of their negative regulation. We have used the *Drosophila* eye to investigate how the two D-cbl proteins, D-cblL and D-cblS impact on development. We find that all phenotypes associated with absence of D-cbl or overexpression of the two isoforms can be attributed to effects on the EGFR pathway. The EGFR is known to regulate at least seven independent events during eye development: morphogenetic furrow 'birth' and 'reincarnation'; apical nuclear migration; G1 arrest of precluster cells; G2 to M transition in the SMW; recruitment of all cell types except R8; cell survival; and ommatidial rotation. The role of the EGFR in R8 spacing remains controversial. We have found a role for D-cbl in each of these events. Our data provide novel insights into the function and regulation of the EGFR during eye development and identifies multiple new cellular function of the Cbl proteins.

424A

Fluorescent imaging of the Hedgehog transduction complex. Matthieu P Sanial, Anne D Plessis. Institut Jacques Monod, CNRS-Paris 7-Paris 6, Paris, FR.

The secreted products of the Hedgehog (HH) family play key inductive roles in patterning many tissues throughout the development of both vertebrates and non-vertebrates. They also control cell proliferation in many developmental contexts and in mammals increased HH signalling has been involved in various cancers. A number of proteins have been implicated in the signal transduction including the HH receptor Patched (PTC), the GPCR related signal transducer Smoothened (SMO) and a transduction complex (called HSC) composed of the kinesin related protein Costal 2 (COS2), the Ser/Thr protein kinase Fused (FU), the pioneer protein Suppressor of Fused (SUFU) and the transcription factor Ci/GLI. To precise the composition and sub cellular localization of the HSC, we performed a systematic analysis by confocal microscopy of the HSC proteins. This work was done using tagged fluorescent proteins introduced alone or in combination in Cl8 cells, in absence or in the presence of HH. Furthermore, to better understand how the SMO and the HSC control each other localization, we looked at the effect of mutations that specifically affect their ability to interact, their biochemical activity, or their subcellular localisation. We extended our analysis to dMLF (Drosophila Myeloid Leukemia Factor), a protein that we identified as interacting with Su(fu).

425B

Molecular genetic analysis of Drosophila COP9 Signalosome subunit 8. Daniel Segal[1], Pazit Oren-Giladi[1], Daniel A. Chamovitz[2]. 1) Molecular Microbiol & Biotech; 2) Plant Sciences, Te Aviv Univ, Tel Aviv, IL.

The COP9 signalosome (CSN) is a multi-protein complex of the ubiquitin-proteasome pathway. It is composed of 8 subunits, which have high homology to the 8 subunits that form the lid of the 26S proteasome. The CSN was first identified in Arabidopsis where it is required for the repression of photomorphogenesis. CSN or CSN-related complexes have been implicated in varied processes in diverse eukaryotes. The CSN directly interacts with SCF-type E3 ubiquitin ligases, and removes the post-translational modification Nedd8 from the cullin component of the SCF. In addition to its interaction with E3s, the CSN also effects proteolysis by its association with protein kinases, deubiquitylating enzymes, and proteasome substrates. We use Drosophila for studying the roles of the CSN in animals in the context of the whole organism. We have previously shown that fly mutants in subunits CSN4 and CSN5 have defects in various developmental processes ranging from oogenesis, and embryogenesis to axon guidance and immune response. Here we report our initial characterization of the Drosophila CSN8 subunit. Two *csn8* deletion mutants were generated by imprecise excision of a nearby P element. We showed, using anti-CSN8 antibodies, that the *csn8* mutants are null. Gel filtration analysis of wild type Drosophila indicated that the CSN8 subunit is found exclusively as part of the entire CSN complex, and not as a monomer, and that proper assembly of the CSN complex is CSN8-dependent. The *csn8*^{null} mutants are larval lethal, with some reaching prepupation. The mutant larvae have defects in molting from the 2nd to the 3rd instar, evident by double mouth hooks, similar to *csn4*^{null} mutants. They also harbor melanotic capsules in their hemolymph, as do *csn5*^{null} mutants. These two phenotypes suggest that the CSN complex is required for molting and hematopoiesis. Mosaic analysis indicated that CSN8 is required in the imaginal discs for development of various adult structures. The abnormal assembly of the complex in the mutants affects CSN function since de-neddylation of cullin1 in them was found to be defective.

426C

Hindsight Mediates the Role of Notch in Suppressing Hedgehog Signaling and Cell Proliferation. Jianjun Sun, Wu-Min Deng. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

Proper development of multicellular organisms requires coordination of developmental signaling pathways temporally and spatially. Two major signaling pathways, Notch and Hedgehog (Hh), are essential in the regulation of cell proliferation and cell differentiation in *Drosophila*, but so far the interaction between them has not been clearly illustrated. The follicular epithelial cells provide an excellent model system for the study of the temporal regulation and interaction of these two signaling pathways. Hh signaling is active in the mitotic follicle cells during early oogenesis (stages 1-6), but downregulated at stage 6/7, co-incident with the mitotic cycle/endocycle (M/E) transition induced by Notch signaling. Here we show that the downregulation of Hh signaling is dependent on Notch activation, as Ci-155, the active transcription factor of Hh signaling, was upregulated in follicle cells where Notch signaling was disrupted during mid-oogenesis. We found that zinc-finger transcription factor Hindsight (Hnt) was a downstream target of Notch signaling during the M/E switch and mediated the role of Notch in suppressing Hh signaling through transcriptional regulation of *ci*. Another zinc-finger protein, Tramtrack (Ttk), acted as a co-repressor to suppress *ci* transcription in these cells. In addition, we found that Hnt was required and sufficient for the switch from the mitotic cycle to the endocycle in follicle cells by loss- and gain-of-function analyses. Our findings demonstrate that Hnt mediates most, if not all, of the functions of Notch in follicle cells and bridges the Notch and Hh pathways in follicle cell development through transcriptional regulation.

427A

Genetic mosaic analysis reveals effects of *APC2 APC1* double mutations during *Drosophila* wing development. Sandra Zimmerman, Carolyn Mallozzi, Vilma Medrano, Lesley Holot, Lauren Thorpe, Brooke McCartney. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Mutations in the colon cancer tumor suppressor, Adenomatous polyposis coli (APC), contribute to cancer initiation through APC's role as a negative regulator of Wnt/Wingless (Wg) signaling. In addition, APC is reported to regulate actin and microtubules by mediating interactions between microtubules and other cellular structures, regulating microtubule stability, and promoting cell migration. The precise mechanisms by which APC proteins act in these diverse cellular functions are not well understood. The study of *APC* mutant phenotypes in *Drosophila* is complicated by the presence of two *APC* genes, *APC1* and *APC2*, which have redundant function. Therefore, to determine the cellular consequences of loss of *APC* function, both *APC1* and *APC2* must be mutant. We and others reported that *APC2 APC1* double mutant clones in the adult wing blade exhibit cell fate transformations consistent with ectopic Wg signaling. We found that *APC2*^{g10} *APC1*^{Q8} (double null) clones in wing imaginal discs exhibit abnormal outpocketing and segregation from the surrounding epithelium. Clones with the segregation phenotype overproliferate and are largely restricted to areas of the disc not expressing Wg protein. Further, different alleles of *APC2*, in combination with a null allele of *APC1*, exhibit a range in severity of the phenotype in wing discs and in adult wings. To determine whether the segregation phenotype is due to activation of Wg signaling, we have shown that blocking the Wg pathway in *APC2*^{g10} *APC1*^{Q8} clones by expressing dominant negative TCF partially suppresses the segregation phenotype. Conversely, we have induced clones exhibiting the segregation phenotype by expressing stabilized Armadillo or mutant Axin to constitutively activate Wg target genes independent of APC function. To understand the basis for the segregation defect in *APC2*^{g10} *APC1*^{Q8} clones, we are assessing polarity, adhesion, and cytoskeletal organization in the mutant tissue throughout wing development.

428B

Examining the activation of Slipper, a JNKKK. Rebecca Gonda, Beth Stronach. Dept Biological Sci, Univ Pittsburgh, Pittsburgh, PA.

The Jun N-terminal Kinase (JNK) pathway is involved in a number of processes including cell morphogenesis, immune response, apoptosis, and cell proliferation. Thus, it is important to understand how this signal transduction pathway can elicit specific responses when activated by different signals. In Drosophila, there are several JNKKKs through which the pathway can act. One of these, Slipper (Slpr), has been shown to regulate tissue closure and cell morphogenesis. We are interested in the mechanism of Slpr activation during morphogenesis. It is likely that in order for Slpr to phosphorylate its substrate, Slpr itself must be phosphorylated. Preliminary results suggest that in vitro translated Slpr labeled with S^{35} is phosphorylated because we see a shift to a lower molecular weight upon treatment with λ-phosphatase. A kinase-dead construct behaves identically when labeled, indicating that phosphorylation is not due to autophosphorylation, but rather due to a kinase in the lysate used to label the protein. Using complimentary methodology, I will investigate whether proposed upstream JNK pathway activators, Rac, PVR, and Msn phosphorylate Slpr using kinase assays. I am also testing the effects of a putative negative regulator, the protein phosphatase PP2C, on the phosphorylation state of Slpr in vitro. Bacterially-expressed GST-PP2C has been purified and used in pull-down assays with labeled Slpr. Preliminary data indicate that PP2C and Slpr physically interact. Conditions for the phosphatase assay are currently being optimized. Overall, we hope that these experiments elucidate the mechanisms by which Slpr is modified to elicit a specific response within the JNK pathway.

429C

The function of SOCS genes in Drosophila development and signaling pathways. Qian Guo, Douglas Harrison. Dept Biol, Univ Kentucky, Lexington, KY.

The JAK/STAT pathway is an evolutionarily conserved signaling mechanism in many species. The duration and intensity of JAK signaling must be tightly regulated to prevent excessive transcriptional response and to reset the pathway to receive additional signals. Suppressors of cytokine signaling (SOCS) are the largest class of JAK/STAT regulators in mammals. Eight SOCS genes have been found in mammals, CIS and SOCS1-7. CIS, and SOCS1-3, the canonical SOCS, have been well studied in vertebrates and are transcriptionally activated by and down-regulate JAK signaling. SOCS4-7, the non-canonical SOCS, are less studied whose relationship with JAK/STAT pathway has not been well established. The *Drosophila* genome encodes three SOCS homologues, SOCS16D, SOCS36E, and SOCS44A. All of them contain a SOCS box at the carboxyl terminus, preceded by a SH2 domain, and are homologues of the non-canonical SOCS family. Expression of SOCS36E is controlled by the JAK pathway and misexpression causes phenotypes similar to that from reduction of JAK in both ovary and wing, which may make it functionally more similar to the canonical SOCS. Expression of SOCS44A is not controlled by the JAK pathway and misexpression causes JAK mutant phenotypes in wing but not in ovary. To test whether the function of *Drosophila* SOCS is to down-regulate the JAK pathway, we have made loss-of-function mutations of the three SOCS genes using P elements inserted in or near the coding region of these genes. Excision mutants of SOCS16D and SOCS44A were generated and have no visible phenotypes. Incomplete excision mutants of SOCS36E were generated which have ectopic wing vein. The mutants of SOCS36E and SOCS44A significantly enhance melanotic tumor formation in $hop^{Tum\text{-}l}$, a gain-of-function mutation of JAK that causes constitutive activation of JAK and results in overproliferation of lamellocytes. The border cell number, recruitment of which requires high JAK activity, is slightly increased in both mutants of SOCS36E and SOCS44A. These data indicate SOCS36E and SOCS44A are negative regulators of JAK/STAT pathway.

430A

Regulation of Dishevelled in Wnt/β-Catenin and Fz/planar cell polarity signaling. Andreas Jenny[1], Thomas Klein[1], Michael Boutros[2], Marek Mlodzik[1]. 1) Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY 10029, USA; 2) Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, D-69120, Germany.

The Wnt-Frizzled(Fz)/β-Catenin and Fz/planar cell polarity (PCP) pathways are related signaling pathways crucial for the induction and regulation of many developmental processes in vertebrates as well as in invertebrates. In addition to the Fz receptor, both pathways rely on the adaptor protein Dishevelled (Dsh) to transduce a signal to downstream effectors. A crucial question is how the specificity of the signal is achieved. Dsh phosphorylation correlates with PCP signaling, as well as canonical Wnt/β-Catenin signaling and is thought to be critical for the regulation of pathway activity. To gain a better understanding of the regulation and specificity of Dsh for PCP versus Wnt/β-Catenin signaling, we performed a systematic RNAi screen in order to identify kinases and phosphatases that affect the Dsh phosphorylation status in a Western blot based mobility shift assay. We will present insight about the candidates and their effect on Fz-PCP and canonical Wnt signaling in vitro and in vivo. In particular, our data suggest that CKIε is positively required for Fz-PCP signaling as well as for Wnt/β?Catenin signaling. The identification and characterization of Dsh kinases and phosphatases is a significant step forward in understanding the regulation of PCP signaling and in particular will shed light on the specificity of Dsh for Wnt/β-Catenin versus PCP signaling.

431B

FERM domain specifies apical localization of protein tyrosine phosphatase Pez. Troy M. Larson, Kavita V.S. Vadali, Kevin A. Edwards. Biological Sciences, Illinois State University, Normal, IL.

FERM-PTPs are characterized by an N-terminal FERM (Band 4.1, ezrin, radixin, moesin) domain, a central linker containing likely protein-protein interaction sites, and a C-terminal protein tyrosine phosphatase (PTP) domain. The FERM-PTP Pez was expressed using the GAL4 system in transgenic *Drosophila* and found to localize to the apices of epithelial cells. To define the Pez localization signal, we subdivided the protein and tagged each major domain with GFP. Constructs were visualized in S2 cells as well as in flies. The GFP-tagged FERM domain and central linker localizes at the membrane of S2 cells, whereas the GFP-PTP domain localizes diffusely throughout the cytoplasm. In flies, the FERM domain localizes very strongly to the apical domains of polarized cells, but it also appears on basal and lateral membranes and in cell surface projections; in contrast, full length Pez is almost exclusively apical and rarely seen in cell surface projections. We conclude the FERM domain is sufficient to mediate apical localization, but the rest of the protein contributes to apical specificity. The identity of the apical binding partner(s) is now being pursued. One distinctive feature of the apical docking site is that is it very abundant; even high-level expression of Pez does not saturate it. This suggested that Pez may interact with the apical cytoskeleton. However, likely candidates Armadillo, actin, spectrin, and moesin all failed to colocalize with overexpressed Pez. We are now testing for Pez colocalization with transmembrane proteins. These studies shed light on structure/function relationships in one of the least studied groups of tyrosine phosphatases.

432C

Phenotypic effect of the over-expression of Ras/MAPK components in the developing Drosophila wing. Neena Majumdar, Daniel R. Marenda. Department of Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.

All multi-cellular animals develop from a single cell. This is a central concept in developmental biology, underpinning our understanding of such basic cellular functions as cell differentiation, cell division, or cell growth (among others). Thus, it is the burden of this single cell to faithfully divide, and coordinately control a large number of diverse cellular processes in order to properly form a viable, fully functional organism. A striking fact of animal development is that a comparatively small number of developmental patterning signals control the vast majority of necessary and diverse cellular processes required for proper development to take place. Thus, the reiterative use of the same signaling pathways are used to elicit multiple different cellular functions. Therefore, proper regulation of these signaling pathways is critical to ensure the correct cellular function is carried out, as mis-regulation often leads to human diseases such as cancer, neurodegeneration, and developmental abnormalities. Our long term goal for this study is a deeper understanding of the regulation of the Ras/MAPK signal transduction pathway, alterations in which are associated with approximately 25% of human tumors as well as neurodegeneration. We have analyzed the effect of over-expression of various components of the Ras/MAPK pathway (activating vs. inhibitory ligands, Egfr receptor, phosphorylated MAPK) on cell growth, division, and differentiation in the developing wing, and report here the phenotypic consequence of each. We find that each effect is surprisingly specific. We further correlate the expression of Egfr protein levels, phosphorylated MAPK levels, and MAPK subcellular localization (nuclear vs. cytoplasmic MAPK) with each genotype we tested.

433A

Downregulation of Slpr-dependent signaling by Alph phosphatase. Beth Stronach[1], Caroline Baril[2], Marc Therrien[2]. 1) Dept Biological Sci, Univ Pittsburgh, Pittsburgh, PA; 2) IRIC, Laboratory of Intracellular Signaling, University of Montreal, Montreal, Quebec.

Precise intensity and duration of intracellular signaling is required to generate appropriate cellular responses. For example, sustained RAS-MAPK signaling accompanies a switch from neuronal cell proliferation to differentiation in response to NGF ligand. Previous genetic studies have shown that Jun Kinase (JNK) signaling is tightly regulated to control proper tissue morphogenesis, induction of apoptosis, and immune response. Using loss of function mutants and transgene overexpression of *slpr*, encoding a JNKKK, it is possible to modulate JNK signaling negatively and positively resulting in distinct and opposite morphogenetic phenotypes. These phenotypes reflect aberrantly low or high levels of JNK pathway activity respectively, and thus serve as the basis to identify additional modifiers of Slpr-dependent JNK activation. Indeed, we identified *alph*, a serine/threonine phosphatase related to mammalian PP2C, as a suppressor of the $slpr^{BS06}$ mutant with threshold levels of JNK signaling, reasoning that reduced *alph* activity potentiates the maternal function of Slpr in the *slpr* zygotic mutant. Additional assays confirm genetic interactions between *alph* and JNK pathway components. For example, *alph* mutants suppress *hep* (JNKK) mutations in which JNK signaling is impaired. In addition, *alph* mutants enhance a GMR-Rac induced rough eye phenotype due to JNK pathway hyperactivation. *alph* also enhances morphogenetic defects associated with *slpr* overexpression. These data demonstrate that Slpr-dependent JNK signaling is moderated by Alph phosphatase to control the shape of developing tissues.

434B

***Drosophila* protein tyrosine phosphatase dPTP61F, an ortholog of human PTP1B and T cell PTP, modulates insulin signaling through DOCK engagement.** Chia-Lun Wu[1], Han Lee[1,2], Tzu-Ching Meng[1,2]. 1) Institute of Biological Chemistry, Acdemia Sinica, Taipei, TW; 2) Institute of Biochemical Science, National Taiwan University, Taipei, TW.

Mammalian insulin regulates cell growth and metabolism through its specific insulin receptor (InR). It is now clear that the insulin signaling is evolutionarily conserved. In fruit flies *Drosophila melanogaster*, several kinases controlling this signaling cascade have been characterized as the orthologs of their mammalian counterparts. Despite the appreciated role of kinases, it is not known how phosphatases participate in the regulation of *Drosophila* insulin receptor (dIR)-mediated signaling. In the current study, we have investigated the function of a nontransmembrane protein phosphatase dPTP61F, the ortholog of human PTP1B and T Cell-PTP, both of which have been identified as negative regulators for human InR. The ablation of endogenous dPTP61F by RNA interference leads to the enhanced tyrosine phosphorylation of dInR in S2 cells stimulated with insulin, suggesting that dPTP61F may regulate insulin signaling through direct dephosphorylation of dInR. We further pursued that the mechanistic details on how dPTP61F gains an access to dInR for tyrosine dephosphorylation *in vivo*. We were particularly interested in the role of an adaptor DOCK, which is a dInR- and dPTP61F-associated protein. When ectopically expressed in S2 cells, DOCK promotes dPTP61F-mediated tyrosine dephosphorylation of dInR. The C-terminal proline-rich motifs of dPTP61F are essential for binding to DOCK, and the presence of this region is important for dephosphorylation of dInR. Interestingly, we found that NCK, the mammalian ortholog of DOCK, which forms a stable complex with PTP1B, plays a key role for effective tyrosine dephosphorylation of human InR mediated by PTP1B. Our findings not only identify a *Drosophila* PTP that controls the activity of dInR, but also provide novel insights into a regulatory mechanism through which an adaptor protein acts coordinately with a kinase and a phosphatase in fine tuning the insulin signaling.

435C

Tyrosine kinase signaling regulates the retinal determination protein Eyes Absent. Wenjun Xiong, Noura Dabbouseh, Ilaria Rebay. Cancer Biology, The University of Chicago, Chicago, IL.

Eyes Absent (EYA) is a member of the retinal determination (RD) network, a collection of highly conserved transcription factors that control the specification and development of the Drosophila eye and other tissues. The RD proteins direct specific programs of gene expression to regulate important cell behaviors such as proliferation, differentiation and survival. In agreement with their essential roles in normal development, misregulation of function or loss of RD gene expression has been implicated in cancer and other inheritable diseases. EYA, a central component of the RD network, serves as a point of cross-talk between this network and other signaling pathways. Its expression and functions are regulated by multiple signaling inputs, including wingless, dpp and EGFR signaling. Besides its role as a transcription factor, EYA was recently shown to be the prototype of a novel protein tyrosine phosphatase family, though its biological substrates are largely unknown. Both the transactivation and phosphatase activity of EYA are required for normal eye development. Previous work in the lab has shown that EYA, which itself is tyrosine phosphorylated, has autocatalytic function *in vitro* and in cultured cells. Toward the goal of understanding how EYA's two functions as a transcription factor and tyrosine phosphatase may be coordinated, we are investigating the role of tyrosine phosphorylation in regulating EYA function. Currently, we are using a combination of *in vitro*, genetic and cultured cell assays to identify the upstream tyrosine kinase signaling networks that lead to EYA's phosphorylation, to pinpoint which tyrosine residues of EYA are the potential substrates, and to understand how such regulation influences phosphatase and/or transcriptional functions. By solving these questions, we hope to answer how the phosphatase activity functions in development and how the two activities of EYA interconnect.

436A

Drosophila Sac1 role in eye development. Amir A. Yavari, Gerald Call, Raghavendra Nagaraj, Edward Owusu-Ansah, Utpal Banerjee. MCDB, Univ California, Los Angeles, Los Angeles, CA.

Yeast Sac1 gene codes for a phosphatidylinositol phosphate (PIP) phosphatase and is involved in the regulation of actin cytoskeleton, secretion, and ATP transport. Drosophila Sac1 gene serves as a negative regulator of the JNK cascade and disruption of this cascade results in dorsal closure defects in embryos. Our aim was to investigate the role of Sac1 during eye development and its putative interaction with the JNK pathway. Since over-activation of JNK has been demonstrated to result in apoptosis in third instar imaginal discs, we propose that eye-specific disruption of Sac1 during eye development overactivates JNK and results in apoptosis. We generated clones of Sac1 gene by using FLP/FRT system in the eye and observed a significant increase in the number of TUNEL positive cells. Disruption of a JNK component, Misshapen, results in a partial rescue of the cell death phenotype. Utilizing Dad-lacz, we were able to monitor Dpp expression in the Sac1 clones of third instar eye discs. Dad, Daughter Against Dpp, has been shown to be activated by Dpp, which acts downstream of JNK. Overactivation of JNK in our Sac1 clones resulted in an increase in expression of lacz in the Dadlacz-Sac1 clones compared to wildtype. Further study of Sac1 will be useful in identifying the role of PIPs in JNK signal transduction during eye development.

437B

Genetic analysis of the 31E genomic region of Drosophila melanogaster: Identification of Replication factor. Amr Amin[1], Yuebing Li[2]. 1) Biol Dept, UAE Univ, Al-Ain, UAE; 2) Neurology Department, Cincinnati University, Ohio, USA.

In an effort to isolate an enhancer of the orthodenticle gene (otd) and because of the abundance of genetic information and the availability of molecular probes, the Drosophila 31E genomic region is investigated. Drosophila replication factor C (DRFC) transcript is isolated as one of the possible candidates for 31Ef. The longest DRFC cDNA clone obtained is 1158 bp, which includes an open reading frame of 332 amino acids. No polyadenylation signal or poly A stretch is found at the 3' end of the DRFC cDNA. DRFC gene product shows a 62% identity to the human replication factor.

438C

Chifoumi is a novel negative regulator of JAK/STAT signalling. Nadège Pelte[1], Patrick Mueller[2], Martin Zeidler[3], Michael Boutros[1]. 1) Research Group Signaling and Functional Genomics, German Cancer Research Center , Heidelberg, DE; 2) Department of Molecular Developmental Biology, Max Planck Institute, Goettingen, DE; 3) Department of Biomedical Science,?The University of Sheffield, Sheffield, UK.

The JAK/STAT pathway is an evolutionary conserved signalling pathway that plays role in many physiological processes, including stem cell maintenance, oogenesis, border cell migration, embryonic segmentation, sex determination, gut, eye, and tracheal development, hematopoesis and immune response. Forward and genetic screens performed so far have led to the identification of several pathway components, including positive and negative regulators. Recently, genome-wide RNAi screens in cultured cells allowed the discovery of putative new JAK/STAT pathway components, which wait for further characterization and validation. Here we describe Chifoumi, a newly identified negative regulator of JAK/STAT signalling pathway. The current in vivo and in vitro data of its involvement in immune response and development will be presented.

439A

Signaling functions of Kurtz in embryonic patterning. Marla Tipping, Alexey Veraksa. Univeristy of Massachusetts Boston, Dorchester, MA.

Mammalian beta-arrestins have been characterized as endocytic adaptors mediating internalization and desensitization of G protein coupled receptors (GPCRs). Recent evidence suggests that beta-arrestins play a broader role in cellular regulation, functioning in several non-GPCR signaling pathways. The *Drosophila* genome encodes one non-visual beta-arrestin, Kurtz (Krz). We have previously identified Krz as a regulatory component of the Notch pathway, functioning in a ternary complex with Notch and Deltex. *krz* mutant germline clones were generated to determine its role in *Drosophila* development. The embryos derived from the mutant germline clones show severe defects, which apparently precede *krz* involvement in Notch signaling. We are investigating the molecular mechanisms of krz involvement in regulation of embryonic patterning.

440B

Upd/Jak/STAT signaling represses *wg* transcription to allow initiation of morphogenetic furrow in *Drosophila* eye development. Yu-Chen Tsai[1], Jih-Guang Yao[2], Po-Hao Chen[1], Y. Henry Sun[2]. 1) Department of Life Science, Tunghai University, Taichung, TW; 2) Institute of Mol. Biol., Academia Sinica, Taipei, TW.

The *unpaired (upd)* gene is encoded a ligand for the Jak/STAT signaling pathway in *Drosophila*. Upd/Jak/STAT signaling is involved in the multiple developmental processes including cell proliferation, cell differentiation, cell migration. During early eye development, Upd/Jak/STAT pathway acts as downstream of Notch signaling to regulate global growth in the second instar eye discs. Upd is expressed at middle of posterior margin where is differentiation initiation site. We are interested whether Upd/Jak/STAT signaling also can regulate the onset of retinal neuron differentiation. Photoreceptor differentiation begins from the central point of the posterior margin of eye disc and gradually progresses toward the anterior direction during early third instar. The site of MF initiation is at middle of the posterior margin of the eye disc. The movement of MF can be divided in to two phases: initiation and progression. MF progression requires the interplay of the positive factors: Decapentaplegic (Dpp), Hedgehog (Hh) and Notch (N) signaling pathways. Whereas multiple signaling pathways promote MF initiation, only one signaling pathway, the Wingless (Wg), is known to play a negative role. *wg* is expressed broadly in the early second instar eye disc, and becomes restricted to the lateral margins beginning from late second instar. Reduction of Wg signaling can cause MF initiation from the lateral margins (predominantly from the dorsal side). Ectopic activation of Wg signaling blocked both MF initiation and progression. In this study, we demonstrate that the Upd/Jak/STAT signaling is necessary and sufficient for MF initiation, at both the birth and reincarnation stages. The primary function of this signaling appears to be the suppression of *wg* expression.

441C

Functional characterization of Neuralized isoforms and the NHR domain, a novel domain that mediates Delta binding and Notch signaling. Cosimo Commisso[1,2], Gabrielle L. Boulianne[1,2]. 1) Developmental & Stem Cell Biology, Hospital for Sick Children, Toronto, ON, CANADA; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, CANADA.

Notch signaling, which is crucial to metazoan development, requires endocytosis of Notch ligands, such as Delta and Serrate. Neuralized is a plasma membrane-associated ubiquitin ligase that is required for neural development and Delta internalization. Neuralized is comprised of three domains that include a C-terminal RING domain and two Neuralized Homology Repeat (NHR) domains. All three domains are conserved between organisms, suggesting that these regions of Neuralized are functionally important. The Neuralized RING domain has been shown to be required for Delta ubiquitination and we have recently shown that the Neuralized NHR1 domain functions in Delta binding. Specifically, we found that *neuralized*^{1}, a well-characterized neurogenic allele, exhibits a mutation in a conserved residue of the NHR1 domain that results in mislocalization of Neuralized and defects in Delta binding and internalization. Additionally, we showed that the NHR1 domain of Neuralized is both necessary and sufficient to bind Delta. *neuralized* transcripts give rise to at least two protein isoforms, Neuralized^{PA} and Neuralized^{PC}. The less characterized isoform, Neuralized^{PC}, is essentially an N-terminal truncation of Neuralized^{PA} and exhibits cytoplasmic localization in S2 cells, in contrast to the plasma membrane localization of the well charcterized Neuralized^{PA} isoform. Interestingly, Delta expression is sufficient for Neuralized^{PC} membrane recruitment in S2 cells. Consistent with this, Neuralized^{PC} is recruited to the plasma membrane *in vivo* suggesting that in a developmental context the Neuralized protein isoforms may have redundant function. To address this, we have generated and are analyzing isoform-specific mutations and we will show preliminary data suggesting that the phenotypes of the isoform mutants differ in an unexpected way.

442A

The function of StIP in the JAK/STAT pathway. Linzhu Han, Douglas Harrison. Biol Dept, Univerity of Kentucky, Lexington, KY.

The JAK/STAT pathway is a conserved developmental pathway that is important in cell proliferation, differentiation, cell migration and apoptosis. The mechanism of activation of this pathway is identical in flies and vertebrates. Ligand-dependent activation of the JAKs results in direct phosphorylation of STATs. The phosphorylated STATs enter into the nucleus and regulate transcription of target genes. The fly JAK pathway controls many developmental processes.

In mammals, StIP is believed to be an adaptor that facilitates JAK/STAT pathway activation. It can associate with JAKs and unphosphorylated STATs, and may serve as a scaffold protein to regulate activation of STAT3. In *Drosophila*, there is one StIP homolog, CG11887. Our lab has identified three mutations in *Drosophila* StIP. Stip^{c05390} is a piggyback insertion in exon 2, and was generated in the Exelixis insertional mutagenesis screen. Two other alleles are deletions of StIP generated by the excision of a nearby P element. All of them are homozygous lethal.

Three genetic assays are being used to determine whether StIP mutations affect JAK activity. One is interaction with Hop$^{Tum\text{-}l}$, which causes hematopoietic neoplasia. Hop$^{Tum\text{-}l}$ upregulates JAK signaling and causes tumor formation. If StIP positively affects the JAK/STAT pathway, these StIP mutants should suppress tumor formation. A second assay is interaction with the ectopic wing vein phenotype caused by hypomorphic JAK activity. If StIP positively regulates JAK signaling, then StIP mutants should enhance the vein phenotype of Hop hypomorphic mutants. A third assay is follicle cell specification in oogenesis. High JAK activity is required for specification of the most terminal follicle fate. Reduced JAK activity reduces the number of these follicle cells, thus the effect of heterozygous and homozygous loss of StIP is being investigated. Lastly, physical interaction of StIP with JAK is being examined to compare with mammalian StIP. Results of these studies will be presented.

443B

***docked* - a gene interacting with *dumpy* during wing development.** Suresh K. Kandasamy, Kiley Maguire, Justin Thackeray. Biology Department, Clark University, Worcester, MA.

dumpy (*dp*) encodes a massive ECM component known to be involved in epidermal-cuticle attachment. *dp* is genetically complex, with several distinct allele classes differentially affecting development of various tissues. In an effort to better understand the role of Dp and the ECM in organogenesis, we have initiated a search for genes that interact with *dp* during wing development.

The oblique class of *dp* alleles (dp^{o}) produces a unique truncation or indentation at the distal tip of the wing, reducing the size of the wing-blade without disturbing development of veins or the margin. During a search for an unrelated gene, the Bender laboratory (University of Georgia) isolated four alleles of a gene they named *docked* (*doc*), because of the truncated wing phenotype produced when doc^{1} is heterozygous with a deficiency for the region; three additional *doc* alleles are lethal over the same deficiency. Noting the similarity between the *doc* and *dp* wing phenotype, we obtained the *doc* alleles and found a strong interaction between *doc* and *dp* mutations. We have determined that the doc^{1} mutation is dominant, and increases cell density in the wing. A transcription unit carrying a premature stop codon on the doc^{16} chromosome is a likely *doc* candidate and we are attempting to confirm this by germline rescue. Further genetic and molecular characterization of *doc* will be presented.

444C

Modulation of Notch signal transduction by endocytotic regulators Numb and the Nedd4 family of ubiquitin ligases. Koji Kawahashi[1,2], Tadashi Sakata[3], Shigeo Hayashi[1,2]. 1) Laboratory for Morphogenetic Signaling, RIKEN, CDB; 2) Graduate School of Science and Techonology, Kobe Univ; 3) Temasek Life Sciences Laboratory, Singapore.

Notch signaling is highly conserved in metazoans and is involved in diverse biological events such as development, maintenance of stem cells, and cancer. Notch receptor is activated by binding of DSL ligands on the surface of neighboring cells, followed by two proteolytic cleavages, releasing the Notch interacellular domain which functions as a transcription factor of Notch target genes. Recent studies have demonstrated that endocytotic regulation of Notch receptor and DSL ligands is essential for cell fate determination by Notch signaling. Endocytosis is a key step for determining whether internalized molecules in intracellular vesicle are destined for recycling or degradation. However how the various components of endocytosis regulate activity of Notch receptor is not well understood. To investigate the critical aspect of the mechanism by which endocytotic pathway regulates Notch receptor, we focus on two classes of endocytotic regulators of Notch, Nedd4 family and Numb in *Drosophila*. Nedd4 family is composed of Nedd4, Suppressor of deltex (Su(dx)), and Smurf, which are HECT domain-containing E3 ubiquitin ligases. We previously reported that Nedd4 binds and ubiquitylates to Notch and recruits it into endocytotic vesicles leading to down-regulation of Notch signaling. Numb is partitioned to one of the two daughter cells undergoing asymmetric cell division and is thought to down-regulate Notch via endcytotic pathway. To address the question of how Numb and Nedd4 family interact with each others to regulate Notch trafficking, we analyzed embryonic phenotypes generated by combinations of Numb and Nedd4 family mutant alleles, cellular localization of Notch receptor affected by Numb and Nedd4 family in S2 cells, and physical interaction of Notch, Numb, and Nedd4 family by using biochemical approaches. In this meeting, we are going to report and discuss the recent progress.

445A

The Functional interaction of JAK/STAT pathway ligands. Shanshan Pei, Doug Harrison. Biology, University of Kentucky, Lexington, KY.

The first characterized *Drosophila* JAK/STAT pathway ligand, Unpaired (Upd), is a secreted protein associated with the ECM. Two other recently defined JAK/STAT ligands, Upd2 and Upd3, share high protein sequence similarities in certain regions with Upd. ISH results have shown that these three genes have overlapping expression patterns during *Drosophila* early developmental stages and oogenesis. We propose that Upd family ligands physically interact, forming homo- and heterotypic complexes to activate JAK signalling.

We have successfully employed Bimolecular Fluorescence Complementation (BiFC) in *Drosophila* S2 cell culture to study the interaction between Upd and Upd3. Two proteins are fused with the N and C-termini of a fluorescent protein respectively and then co-expressed. Interaction of the proteins of interest will bring together the fluorescent protein truncations, enabling fluorescence complementation. Initial experiments have shown that Upd and Upd3 are able to form both homo- and heterotypic complexes. Moreover, we are quantifying the relative interaction strength of homo- and heterotypic complexes via competitive BiFC. In such technique, BiFC signals derived from two alternative interactions sharing one common interaction partner within a single cell will be compared to provide us a comparison between the binding strength of two ligand complexes (eg. Upd-Upd and Upd3-Upd). Ultimately, we will move the UAS-based BiFC system into flies to detect ligand interactions in vivo.

The relative ability of each ligand complex to activate JAK/STAT will also be investigated. A luciferase-based reporter assay system is being used for this purpose. Cells producing different ligand complexes are co-cultured with reporter cells possessing *firefly luciferase* gene downstream of 10XSTAT binding site. Results of these assays will be presented.

446B

Identification of regulators and interactors in the Delta-Notch signaling pathway using a transposon-based genetic screen. Nevine A. Shalaby, Marisa C. Osswalt, Annette L. Parks, Eric J. Morreale, Marc A.T. Muskavitch. Biology, Boston College, Chestnut Hill, MA.

The Delta-Notch signaling pathway functions in a wide variety of cell fate decisions during embryonic and post-embryonic development in Drosophila. Increasing evidence from many labs now implicates subcellular trafficking as an important regulator involved in Delta-Notch activation, signaling and down-regulation. In order to identify new regulators and interactors of Delta-Notch signaling, we have pursued a transposon-based genetic modifier screen in collaboration with the Artavanis-Tsakonas laboratory at Harvard Medical School. The screen makes use of a sensitized genetic background in which wild-type Delta is overexpressed in the Drosophila eye using the *GMR-Gal4* driver, resulting in a flat, glassy eye phenotype. The screen utilized a set of transposon insertion lines generated by Exelixis, and housed in the Artavanis-Tsakonas laboratory. We screened 10,500 individual fly lines, each bearing a transposon inserted at a different genomic location. Approximately 180 transposon lines passed initial positive and negative tests and have been assessed in cell biological and genetic secondary tests to validate and prioritize them for further study. The genes disrupted by these transposons include genes predicted to be involved in protein trafficking and modification, gene regulation, lipid and protein metabolism, and cell-cell signaling.

447C

Molecular and genetic characterization of *upd*, *upd3* and *os*. Liqun Wang, Douglas Harrison. Dept Biology, Univ Kentucky, Lexington, KY.

The JAK/STAT pathway is a well conserved signaling pathway from vertebrates to *Drosophila*. It responds to many ligands including cytokines and growth factors in vertebrates. In *Drosophila*, the secreted glycoprotein, Unpaired has been shown to activate the JAK/STAT pathway. Unpaired has an essential role in regulating many developmental processes such as embryonic patterning, sex determination, follicular cell patterning, and more. The *upd* gene is located on the X chromosome, in polytene band 17A. Two predicted genes, *upd2* and *upd3*, within a 70kb region of *upd*, were found to have sequence similarity with *upd* and Upd2 can activate JAK signaling. Classical mutations, described as *outstretched* (*os*), have been defined as alleles of *upd*. Two upd alleles, upd^{YM55} and upd^{YC43}, cause embryonic lethality. Three *os* alleles, os^{o}, os^{s}, os^{1}, result in outstretched wings, small eyes, or both. Allelism of *upd* and *os* is based on the failure of the zygotic lethal *upd* alleles to complement the outstretched wing and small eye phenotypes of the *os* alleles. However, additional mutations in the *upd* region were identified that genetically separate the *os* and *upd* loci, suggesting that the molecular basis of the complementation is complicated. Molecular mapping of *os* mutations is under way. Since the *upd-like* genes are close to *upd* on the X chromosome and show sequence similarity with *upd*, it was possible that *os* phenotypes may result from mutations in *upd-like* genes. To test this, a mobilization screen was done to generate mutants of *upd3* with a P element inserted in the last intron of *upd3*. The *upd3* mutants show *os* phenotypes, small eyes and/or outstretched wings. Molecular characterization of some *upd3* mutants shows deletion of part or all of the last exon of *upd3*. Genetic characterization of those *upd3* mutants with *upd* and *os* shows that the *upd3* mutants complement the *upd* mutants but fail to complement the *os* mutants. This suggests that the *os* may be caused by mutations in a common regulatory region of *upd* and *upd3*. Results of the influence of *os* on *upd* and *upd3* expression will be presented.

448A

The alleles of *strawberry notch* and *discs large* have an altered role in lipid signaling. Catherine Coyle-Thompson, Mary Lee Sparling. Dept Biol, California State Univ, Northridge, CA.

We have identified a role for the *strawberry notch* and *discs large* gene products in lipid signaling. Several of the mutant alleles of *sno* and *dlg* result in an alteration of the products in the lipid signaling pathway. The production of these altered products by *sno* and *dlg* alleles appear to be temperature sensitive and occur at several developmental periods.

449B

A multiplex in situ approach to define the precise contribution of the maternal BMP pathway in dorsal-ventral patterning of the early Drosophila embryo. Katia Carneiro, Helena Araujo. Histology and Embriology, UFRJ, Rio de Janeiro, Brazil.

During different moments throughout animal development the restriction of potential cell fates is initially perceived as differential gene expression territories (GET), which may result from distinc thresholds for activation by a specific morphogen. Thus, the shape of the morphogenetic field has drastic consequences on the fate cells will acquire later in development. The establishment of proper GET along the dorsal-ventral (DV) axis of the *Drosophila* embryo relies on controlling nuclear translocation of the Rel-like transcription factor, Dorsal (Dl). After fertilization, the maternal pathways Toll (Tl) and Dpp/BMP4 converge on the cytoplasmatic IkB homologue protein Cactus to regulate its degradation. These events give rise to a nuclear gradient of morphogenetic Dl protein in the embryo that in turn activates genes that are sensitive to different thresholds for Dl. We have recently demonstrated that a maternal Sog/Dpp pathway is able to pattern the *Drosophila* (DV) axis of the embryo by directly influencing the slope of the Dl nuclear gradient (Carneiro, Fontenele, *et al*, Dev. Biology, 2006). Although our conclusions about this issue are based on genetic evidence and careful examination of GET, they do not reflect the precise relationship between the two pathways along the entire embryonic DV axis. In order to understand the precise contribution of the Tl versus Dpp pathway on the shape of the Dl gradient along the entire DV axis, we have used a multiplex *in situ* approach. This technique allows us to analyze the pattern of expression of several genes in parallel, allowing us to ask questions regarding effects on overlap between adjacent territories. We have started using a specific genetic background in the mothers which ensures that signals from the Tl pathway are homogeneous throughout the embryo. This way, by varying the Dpp pathway, for instance, by use of a *dpp* duplication in the mothers, or reducing the Dpp antagonist *sog* in the mothers, we expect to define the contribution of the Dpp pathway alone, to gene expression target of Dl regulation.

450C

FORMATION OF A PROXIMO-DISTAL AXIS IN THE ABSENCE OF DPP SIGNALING. Carlos Estella, Richard Mann. Columbia Univ, New York, NY.

In the leg imaginal disc two major signaling pathways, Dpp and Wg, are essential for specifying and patterning both the dorso-ventral (DV) and proximo-distal (PD) axes. In DV patterning, Dpp and Wg appear to function independently: dpp regulates a set of target genes (such as omb) to pattern the dorsal half of the leg while wg regulates a different set of target genes (such as H15) to pattern the ventral half of the leg. In contrast, these two secreted molecules synergize to pattern the PD axis: the combination of both signals is required for the expression of PD target genes, such as Distalless (Dll) and dachsund (dac). The current model suggests that different levels of a combined Wg+Dpp gradient are essential to set up the different domains of gene expression along the PD axis of the leg. High levels of Wg+Dpp in the center of the leg disc activate Dll and repress dac. Moderate levels of these signals are insufficient to activate Dll but permissive for dac activation. In this study, we have found that the Dpp pathway transcriptional repressor encoded by the brinker (brk) gene is required for PD axis formation, but is dispensable for DV axis specification. Thus, in the absence of brk activity, the DV readouts dpp, wg, H15, and omb are expressed normally. In contrast, brk is required to repress Dll and dac during the first two stages of larval development. Moreover, we have found that Dpp signaling is not required for Dll or dac activation in the absence of brk. Thus, these results demonstrate that the primary role for Dpp signaling in PD axis formation is to relieve repression by brk. However, we further show that Dll and dac have different sensitivities to both Brk repression and Wg activation. Thus, our results suggest that the graded activities of both Brk and Wg establish the Dll and dac expression domains and, by extension, pattern the P/D axis of the leg.

451A

Weckle is required for the transcriptional activities of Dorsal in Drosophila. Dechen Fu, Mike Levine. Department of Molecular and cell Biology, U. C. Berkeley, Berkeley, CA.

Weckle, a Zinc finger domain protein, is identified to be essential for dorsal-ventral pattern formation in early embryos of Drosophila by F1 Genetic Screen. Mutations of weckle result in dorsalized embryos, which is similar to the dorsal loss function mutation. Recently, Weckle is found to be associated with Toll pathway, which initiates the gradient of Dorsal along dorsal-ventral axis, and thus establishes the embryonic dorsal-ventral polarity. However, the role of Weckle protein in regulating the transcriptional activity of Dorsal protein has not been characterized. Here, we found that Weckle protein, which present in both cytoplasma and nucleus in early embryos, can also work as a transcriptional co-factor by binding specific DNA sequences via its Zinc-finger domain in vitro. Mutations of those Weckle binding sites in the enhancers of some downstream genes of dorsal, including brinker (brk) and Short gastrulation (sog), result in either loss or reduced dorsal-dependent expression of these genes in early embryos. Interestingly, our Chromatin-immunoprecipitation (CHIP) results show that weckle site mutation in brk enhancer leads to disassociation of both Weckle and Dorsal protein from this enhancer in early embryos, although Dorsal protein can still bind to weckle site-mutant brk enhancer in vitro. Thus, in addition to affect the nuclear translocation of Dorsal protein via Toll pathway, Weckle protein may also regulate the transcriptional activity of Dorsal by affecting its DNA binding ability in early developmental processes of Drosophila.

452B

Formation of a BMP gradient within the neuroectoderm. Lisa Gunaydin[1,2], Claudia Mizutani[1], Ethan Bier[1]. 1) Div. of Biological Sciences, Univ. of California San Diego, La Jolla, CA; 2) Department of Biology, Swarthmore College, Swarthmore, PA 19081.

Protein gradients provide embryonic cells with information about their anterior-posterior and dorsal-ventral positions. In *Drosophila melanogaster*, the morphogen Dpp plays a well-characterized role in patterning the dorsal ectoderm. There is indirect evidence that Dpp can also signal in a graded fashion to adjacent neural cells; however, this effect is difficult to isolate from the potent organizing activity of the ventral morphogen Dorsal in the neuroectoderm. In this study, we investigated the potential role of Dpp in patterning the neuroectoderm. Levels of Dpp and of its antagonist Sog were modified in embryos carrying *lacZ* reporter constructs under the zen promoter, a Dpp-target gene normally activated in the dorsal ectoderm. In constructs bearing zen promoter mutations (*zen**), *lacZ* expression extends ventrally and does not undergo refinement to the dorsal midline, serving as a proxy for Dpp activity in the neuroectoderm. Neither ubiquitous nor local Dpp overexpression in an anterior-posterior stripe appreciably altered the pattern of *zen*-lacZ* expression. However, a local increase in Sog appeared to decrease *lacZ* expression in the neuroectoderm. This result suggests that Dpp may signal in neuroectoderm cells to activate *zen*-lacZ* expression and corroborates recent evidence that Dpp can pattern the neuroectoderm by dose-dependent repression of neural genes. Dpp signaling may thus play a broader role in dorsal-ventral patterning than previously thought, determining cell fates in the neuroectoderm as well as in the epidermis.

453C

***Drosophila* rasiRNA mutations disrupt axis specification through activation of a DNA damage checkpoint.** Carla Klattenhoff[1], Diana Bratu[1], Nadine McGinnis-Schultz[1], Birgit Koppetsch[1], Heather Cook[2], William Theurkauf[1]. 1) Program in Molecular Medicine and program in Cell Dynamics, University of Massachusets Medical School, Worcester, MA 01605; 2) Department of Biological Sciences, Wagner College, Staten Island, NY 10301.

Small repeat associated siRNAs (rasiRNAs) mediate silencing of retrotransposons and the *stellate* locus. Mutations in the *Drosophila* genes *armitage* and *aubergine* block rasiRNA production and disrupt embryonic axis specification, triggering defects in microtubule polarization and asymmetric localization of mRNA and protein determinants in the developing oocyte. Mutations in the ATR/Chk2 DNA damage signal transduction pathway dramatically suppress these axis specification defects, but do not restore retrotransposon or *stellate* silencing. Furthermore, rasiRNA pathway mutations lead to germline-specific accumulation of γ?H2Av foci characteristic of DNA double strand breaks, and formation of γ?H2Av foci is not suppressed by Chk2 mutations. We therefore propose that the rasiRNA pathway maintains germline genome integrity, and that rasiRNA pathway mutations disrupt axis specification through activation of an ATR/Chk2-dependent DNA damage response.

454A

Characterization of the pleiotropic gene *Poly* in *Drosophila melanogaster* oogenesis. Stephen Klusza, Wu-Min Deng. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

The development of the *Drosophila melanogaster* egg chamber is contingent upon correct spatiotemporal localization of mRNAs and proteins at specific stages of oogenesis. An ongoing FLP-FRT mosaic screen isolated a new allele of *poly* (designated *poly* ^{2}), in which germline clones disrupts both Staufen and Kin:β-Gal localization from the posterior of the oocyte at stage 9, as well as possessing altered nurse cell chromatin morphology after stage 5 of oogenesis. In addition to the oogenic phenotypes, *poly* ^{2} homozygous mutants display temperature-dependent subviability in concurrence with dynamic loss of the wing margin. Melanotic tumors are also frequently seen in *poly* ^{2} homozygous 3rd-instar larvae. The multiple phenotypes found in *poly* ^{2} homozygous mutants and clones support a significant role for *poly* in a variety of systems. To further characterize the potential roles of *poly* in oogenesis, overexpression of *poly* is currently being utilized in an effort to rescue the pleiotropic phenotypes of *poly*, and to complement genetic interaction experiments in the search for interacting genes.

455B

Roles of *single-minded* in the left-right asymmetric development and tissue specifications of the *Drosophila* embryonic gut. Reo Maeda[1], Shunya Hozumi[1], Kiichiro Taniguchi[1], Takeshi Sasamura[1], Ryutaro Murakami[2], Kenji Matsuno[1]. 1) Dept. Biol. Sci/Tech., Tokyo Univ Science., Chiba, JAPAN; 2) Dept. Phy. Biol. Inf., Yamaguchi Univ., Yamaguchi, JAPAN.

Many bilateral animals show left-right (LR) asymmetry in their internal organs. In vertebrates, mutants that have LR defects have been identified, and the mechanisms of the LR axis formation are well understood. In contrast, it is suggested that different mechanisms are employed for the LR axis formation in invertebrates. To elucidate the mechanism of LR development in invertebrates, we studied genetic mechanisms responsible for LR asymmetric development of *Drosophila*.

The midline structure of vertebrate embryos has important roles in LR asymmetric development both as the signaling center for LR asymmetry and as a barrier to inappropriate LR signaling across the midline. However, in invertebrates, the functions of the midline in LR asymmetric development are unknown. To elucidate these roles, we studied the involvement of *single-minded* (*sim*) in the LR asymmetry of the *Drosophila* embryonic gut, which develops in stereotypic, asymmetric manner. *sim* encodes a bHLH-PAS transcription factor that is required for the development of the midline structure. Here we report that *sim* was expressed in the midline of the foregut and hindgut primordial. The handedness of the embryonic gut was affected in *sim* mutant embryos and in embryos overexpressing *sim*. However, midline-derived events, which involve Slit/Robo and EGFr signaling and direct the development of the tissues adjacent to the midline, did not affect the laterality of this organ, suggesting a crucial role for the midline itself in LR asymmetry. In the *sim* mutants, the midline structures of the embryonic gut were deformed. The misexpression of *sim* in the hindgut primordium induced ectopic midline cells. We also found that different portions of the embryonic gut require *sim* functions at different times for normal LR asymmetry. Our results suggest that the midline structures are involved in the LR asymmetric development of the *Drosophila* embryonic gut.

456C

Expression of the Drosophila melanogaster GADD45 homologue (CG11086) affects egg asymmetric development which is mediated by the p38/JNK pathway. Gabriella Peretz[1,2], Uri Abdu[1,2]. 1) Life Sciences, Ben-Gurion University, Be'er Sheva, IL; 2) The National Institute for Biotechnology in the Negev, Be'er-Sheva, IL.

The mammalian GADD45 (growth arrest and DNA-damage inducible) gene family is composed of three highly homologous small, acidic, nuclear proteins: GADD45α, GADD45β, and GADD45γ. GADD45 proteins are involved in important processes such as regulation of DNA repair, cell cycle control and apoptosis. Annotation of the *Drosophila melanogaster* genome revealed that it contains a single GADD45-like protein (CG11086; D-GADD45). Since up-regulation of GADD45 proteins has been characterized as an important cellular response to genotoxic and non-genotoxic agents, we aimed to characterize the effects of D-GADD45 overexpression on *D. melanogaster* development. We found that, as its mammalian homologues, D-GADD45 is a nuclear protein; however, D-GADD45 expression is not elevated following exposure to genotoxic and non-genotoxic agents in Schneider cells. Overexpression of D-GADD45 in various tissues led to different phenotypic responses. In somatic cells overexpression caused apoptosis, while overexpression in the germline affected the dorsal-ventral polarity of the eggshell and disrupted the localization of anterior-posterior polarity determinants. Since mammalian GADD45 proteins are activators of the JNK/p38 MAPK signaling pathways, we tested for a genetic interaction in *D. melanogaster*. We found that egg shell defects and morphogen mislocalization caused by D-GADD45 overexpression are dominantly suppressed by mutations in the p38/JNK pathways suggesting that D-GADD45 activates the p38/JNK pathway in the Drosophila germline.

457A

Distribution of the potential morphogen Unpaired during oogenesis. Travis Sexton, Doug Harrison. Dept Biol, Univ Kentucky, Lexington, KY.

Janus kinase (JAK) activity controls differentiation of the follicular epithelium during Drosophila oogenesis by establishing a gradient of JAK activity with highest levels at the anterior and posterior poles. Unpaired (Upd), a ligand for the pathway, is secreted from the polar cells potentially establishing the JAK activity gradient. Although JAK activity has been shown to form a gradient, extracellular distribution of Upd is not known. We propose that Upd acts directly to establish the JAK activity gradient, establishing the fates of the follicular epithelium, thus acting as a morphogen. This research aims to investigate the extracellular distribution of Upd and what factors may contribute to Upd's distribution. Furthermore, upd3, a gene with some sequence similarity to upd, is also expressed in the polar cells. We also aim to determine what role, if any, Upd3 plays in follicular development. To visualize Upd protein we have used an Upd antibody as well as an Upd-GFP construct. Conventional and extracellular staining protocols has revealed Upd protein on the basal and apical sides of the follicular epithelium. While a gradient of Upd within the follicular epithelium is not obvious, the detected distribution is consistent with that of the underlying JAK activity gradient. The distribution of Upd could occur by passive diffusion or by a more active mechanism. It has been reported that some morphogens are dependent on HSPGs for distribution. HSPGs can be divided into 3 classes; glypicans, syndecans, and perlecans. Mosaic analysis using cell fate markers and JAK reporters has shown us that the glypican dally does play a role in follicular patterning but the other glypican dallylike does not. The influence of each HSPG on JAK activation and Upd distribution will be presented. Another possible influence to the JAK gradient and follicular patterning is the expression of a predicted ligand of the pathway, Upd3. upd3 ovaries have no obvious morphological phenotype, therefore, specification of each follicular fate will be quantitatively assayed using cell fate specific markers.

458B

Opposing interactions of homeodomain gene homothorax and Notch pathway genes Lobe and Serrate are required for ventral eye development. Amit Singh[1], Kwang Wook-Choi[1,2,3]. 1) Dept Molecular & Cell Biol, Baylor Col Medicine, Houston, TX; 2) Dept of Ophthalmology, Baylor Col Medicine, Houston, TX; 3) Developmental Biology Programme, Baylor Col Medicine, Houston, TX.

Axial Patterning plays a crucial role during organogenesis. In the developing eye field of *Drosophila*, generation of Dorsal (D) versus Ventral (V) compartments (DV patterning) is the first lineage restriction event. Interestingly, *Drosophila* eye begins with a ventral ground state on which the dorsal eye fate is established. During early eye development loss of ventral eye results in complete loss of eye field and is also responsible for developmental defects in higher organism. The members of Notch Signaling pathway, Lobe (L) and Serrate (Ser), play an important role in ventral eye growth and development. One of the interesting questions is what defines the boundary of the ventral half of the developing *Drosophila* eye. In a genetic screen for modifiers of the L mutant phenotype, we identified *homothorax (hth)* as a strong modifier. *hth* is known to be a negative regulator of eye development. Loss-of-function of *hth* results in ectopic eye enlargements only in the ventral margin of the eye, which is opposite to ventral-eye-loss phenotype seen in *L* or *Ser* mutant background. Hth, a homeodomain protein, forms a complex with the Hox gene Extradenticle (Exd). We have studied the developmental interactions of L (and Ser) with *hth* and *exd* and found that loss-of-function clones of *L* or *Ser* result in ectopic induction of Hth and the Hox gene Extradenticle Exd, suggesting that L is required for inhibition of the *hth/exd* function in the ventral domain. Hth forms a complex with Exd, which is required for their translocation to the nucleus. We will present the sub-cellular localization of L and Hth-Exd complex in order to discern their function. Our studies suggest that L and Ser are essential for antagonizing the function of negative regulators of eye development such as Hth. .

459C

The rearrangement of circular visceral musculature cells controlled by JNK signaling is involved in the left-right asymmetric looping of the anterior-midgut in *Drosophila*. Kiichiro Taniguchi[1], Shunya Hozumi[1], Reo Maeda[1], Shuichi Shirakabe[1], Hiroo Fujiwara[1], Takeshi Sasamura[1], Aigaki Toshiro[2], Kenji Matsuno[1]. 1) Dept Biol Sci/Tech, Tokyo Univ Science, Chiba, JP; 2) Tokyo Met.Univ., Dept.Biol.Sci., Hachioji, Tokyo, JP.

In Drosophila, the embryonic alimentary canal mainly consists of the foregut, midgut and hindgut, and each of which shows stereotypic left-right (LR) asymmetry. We performed a genetic screen to identify the mutations affecting LR asymmetry in the embryonic gut and identified *puckered* (*puc*). Homozygous embryos for *puc* showed randomization of the anterior-midgut (AMG) handedness.

puc encodes a *Drosophila* JNK phosphatase, which mediated negative feedback regulation on the signal cascade of JNK encoded by *basket*. We found that Puc was expressed in the AMG or its primordial and their surrounding tissues, but LR asymmetric expression of Puc was not detected in any stages examined. *puc* was required in the circular visceral musculature (CVMU) cells at stage 11-14 for the proper LR patterning of the AMG. The LR defects in the AMG in *puc* was caused by the hyper activation of JNK signaling in the CVMV cells. In wild type embryos, the rearrangement of CVMV cells surrounding the AMG was organized LR asymmetrically before the AMG looping. In contrast, *puc* mutant failed to arrange these cells LR asymmetrically. In addition we demonstrated that not only the CVMU but also the endosomal epithelium was required for the LR asymmetry in the AMG. Our results suggest that the down regulation of JNK signaling by Puc in the CVMV is requited for the LR asymmetric rearrangement of these cells, and contribution of both CVMU and endosomal epithelium contribute to LR looping of the AMG.

460A

Lgl and its phosphorylation by aPKC regulate Par-1 localization and oocyte polarity. Ai-Guo Tian, Wu-Min Deng. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

Drosophila oocyte anterior-posterior (AP) polarity, which is the basis for formation of the two major body axes, requires posterior localization of the serine/threonine kinase Par-1 and re-orientation of the microtubules during mid-oogenesis. Here we show that tumor suppressor Lgl is required for localization of oocyte polarity markers and the correct arrangement of the microtubule cytoskeleton. In our studies, Lgl phosphorylation by atypical protein kinase C (aPKC) in the germline cells also played a critical role in oocyte polarity formation; overexpression of a non-phosphorylatable form of Lgl (Lgl-3A), or apkc loss of function mutations in the germ line, resulted in severe oocyte polarity defects. Overexpression of the wild-type Lgl, on the other hand, caused a mild oocyte polarity defect, a phenotype that was enhanced by an apkc heterozygous mutation and alleviated by overexpression of aPKC. In addition, we found that Lgl was enriched at the posterior oocyte cortex which was regulated by aPKC, co-localizing with Par-1. Lgl and Par-1 formed a stable protein complex in the germline cells. Co-overexpression of Lgl-3A and Par-1 caused Par-1 to be mislocalized along the oocyte cortex. Our results suggest that aPKC phosphorylation of Lgl is involved in setting up different oocyte cortex domains, which is essential for AP axis specification in the oocyte.

461B

Differential expression of Echinoid drives epithelial morphogenesis in *Drosophila*. Caroline Laplante, Laura A. Nilson. Dept Biol, McGill Univ, Montreal, PQ, Canada.

Interaction between distinct cell types is thought to drive epithelial morphogenesis by locally altering the cytoskeleton. Our study of Echinoid (Ed), a putative cell adhesion molecule, has shown that differential expression of Ed between neighboring groups of cells is sufficient to trigger the assembly of a contractile actomyosin cable at their interface. In the ovary, we identified an endogenous Ed expression border between the two follicle cell types that form the epithelial tubes that secrete the dorsal appendages of the eggshell and showed that elimination of this border causes defective appendage tube formation. Additionally, we found an endogenous Ed expression border between the two cell types involved in embryonic dorsal closure, where the two lateral sheets of embryonic epidermis converge dorsally, covering the amnioserosa and enclosing the internal organs of the embryo. Ed is expressed in the epidermis but absent from the amnioserosa creating an endogenous Ed expression border that corresponds to the location of a well-characterized actomyosin cable. Embryos mutant for *ed* lack the Ed expression border and fail to assemble the actomyosin cable resulting in aberrant dorsal closure. Similarly, ectopic expression of Ed in the amnioserosa thus eliminating the Ed expression border also impedes the assembly of the actomyosin cable. These results suggest that differential Ed expression between two tissues triggers the assembly of the actomyosin cable. Additionally, we found that the expression of Ed during dorsal closure is independent of the JNK pathway suggesting that both the JNK pathway and the establishment of an Ed expression border are required for proper dorsal closure. This research proposes a mechanism by which the differential expression of a cell adhesion molecule locally modulates the cytoskeleton required for morphogenetic processes.

462C

Role of Nm23/Awd in border cell migration. Gouthami Nallamothu, Tien Hsu, Vincent Dammai. Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC.

The tumor suppressor gene Nm23 reduces metastatic potential in invasive cancer cell lines in vivo. However, the mechanism of Nm23 function is not known. We use the Drosophila genetic system to study the in vivo function of Nm23. Border cell migration in particular is an excellent system for studying regulation of cell migration. Border cells are a group of specialized follicular epithelial cells that become invasive during oogenesis. During border cell migration in wild type Drosophila ovary the expression of Nm23 homologue, Awd, is reduced. Over expression of Awd/Nm23 delayed migration of border cells indicating that Nm23 is a negative regulator of cell migration. Constitutive activation of growth factor receptor Pvr, the homologue of human PDGFR/VEGFR and known to guide posterior migration of border cells, also delayed migration due to a loss of chemotactic directionality. The migration defects were rescued when Pvr and Awd were co-expressed, concurrent with a reduction of the exogenous Pvr level, indicating that Awd is a negative regulator of Pvr expression. Down regulation of the receptors involves Dynamin dependent endocytosis. Ovaries expressing dominant negative Dynamin showed delayed border cells migration and elevated Pvr levels and more importantly, this phenotype can be rescued by co-expression of Awd. This indicates that Awd promotes growth factor receptor endocytosis and turnover mediated by Dynamin. We propose that Nm23/Awd is a negative regulator of surface accumulation of chemotactic receptor. Down regulation of Nm23/Awd is therefore prerequisite for receptor accumulation during cell migration. This provides a plausible mechanism for the anti-metastasis function of Nm23.

463A

CHARACTERIZATION OF A DORSAL CLOSURE GENE, *piragua (prg)* IN *Drosophila melanogaster*. Nestor Nazario-Yepiz, Juan Rafael Riesgo-Escovar. Dept Development Biol, UNAM, Inst de Neurobiologia, Queretaro, Querétao, MX.

We are studying dorsal closure, a process that occurs during embryonic development of *Drosophila melanogaster*. Dorsal closure involves changes of shape of epithelial cells. We are particularly interested in communication between cells, a critical step for dorsal closure to occur. A MAPK-like signal transduction pathway called the Jun N-terminal kinase (JNK) pathway orchestrates this process. Failure of dorsal closure leads to a dorsal open phenotype. In the lab we generated and isolated mutations with a dorsal open phenotype that disrupt the *piragua (prg)* gene. We are characterizing the *prg* mutant phenotypes. *prg* is a highly conserved gene present from yeasts to humans and codes for a zinc finger transcription factor. Preliminary evidence suggests that *prg* expression is regulated by multiple factors. *in situ* hybridization and immunostaining experiments show expression of transcript and protein from early embryonic stages. Before dorsal closure, Prg is in the cytoplasm of lateral epithelial cells and migrates to the nucleus during dorsal closure. Genetic interaction experiments have shown that *prg* interacts with *Djun* and *Dfos* during dorsal closure. In addition, *prg* acts in a parallel pathway to JNK in this process.

464B

Regulation of planar cell polarity and proximodistal patterning by the DHHC transmembrane protein Approximated. Hitoshi Matakatsu, Seth Blair. Zoology, University of Wisconsin, Madison, WI.

The protocadherins Fat (Ft) and Dachsous (Ds) are required for several processes in the development of *Drosophila*, including planar cell polarity (PCP) and the proximodistal patterning of appendages such as wing and legs. Recent work from our own and other laboratories indicates that some or all of these effects are mediated by a signaling pathway that is modulated by binding between Ft and Ds. However, it is still unclear what molecules are involved in Ft-Ds signaling pathway. We have therefore been analyzing mutations that show similar phenotypes. *approximated* (*app*) mutants have a proximodistal patterning defect, and we have obtained new *app* mutants by EMS screening that show PCP defects in wing and abdomen. We found that *app* encodes a multipass transmembrane protein containing a DHHC zinc finger motif. DHHC proteins have been recently found to add palmitate fatty acids to cytoplasmic proteins, thereby regulating their association with cell membranes. Most DHHC proteins are localized to, and thought to act in, the ER and Golgi. However, we found that App is concentrated at the apical cell cortex, overlapping the region where Ds and Ft are concentrated. We will present experiments analyzing the relationship between App and Ft-Ds signaling.

465C

The function of *inturned*, *fuzzy* and *fritz* in controlling planar polarity. Jie Yan, Chunming Zhu, Haeryun Lee, Paul Adler. Dept Biol, Univ Virginia, Charlottesville, VA.

The *frizzled* signaling pathway plays a key role in establishing and regulating planar cell polarity (PCP) in Drosophila. Many proteins are involved in this pathway. Fuzzy, Inturned and Fritz are PCP effectors, which are downstream of PCP core proteins (such as Frizzled, Disheveled, and Van Gogh) in the *frizzled* signaling pathway. Mutations in *inturned*, *fuzzy* and *fritz* result in very similar phenotypes that more than one hair form from each wing cell with abnormal polarity. Genetic studies suggest these genes function together. Recently, we observed that the accumulation of Inturned is altered when over-expresses *fuzzy* or *fritz*. By molecular approaches, we found that these 3 proteins can be co-immunoprecipitated from transgenic flies and our yeast-two-hybrid experiments confirmed that there are physical interactions between Inturned and Fritz and Inturned and Fuzzy. The yeast-two-hybrid assay was also applied to map the domains, which were important for protein-protein interactions.

466A

Prepatterning the lateral thorax: specific roles of the *iroquois* genes. Aissam IKMI, Dario COEN. Gènes, Développement, Neurogenèse, UMR 8080, CNRS et Université Paris Sud, 91405 Orsay, FR.

The Drosophila thorax exhibits 11 pairs of large sensory organs (macrochaetae, MC) identified by their unique position. This pattern is remarkably precise, providing an excellent model system to study the genetic basis of pattern formation. In imaginal wing discs, the *achaete-scute* proneural genes are expressed in highly resolved patterns that prefigure the positions of each MC. It is thought that the activities of "prepatterning" genes provide positional cues controlling this expression pattern. The *iroquois* complex (*iro-C*) comprises three homeobox genes, *araucan* (*ara*), *caupolican* (*caup*) and *mirror* (*mirr*) all expressed in the lateral thorax. Previous studies have shown that the *iro-C* is essential for the expression of proneural genes and for the development of sensory bristles in the lateral thorax. However, the precise roles of each *iro-C* gene during this process have not been clarified.

We have addressed this question with a combination of loss-of-function approaches (mutations and RNAi) and functional replacement. For this purpose, we have generated *GAL4* insertions in each of the *iro-C* genes.

We show that *iro-C* genes products are intrinsically different in respect to their contribution to the development of sensory bristles: *caup*, but not *ara*, appears required for the specification of all the MC in the lateral thorax; *mirr* is only required for the formation of a subset of MC; although not required, *ara*, when overexpressed in the same domain, is sufficient to rescue the loss of function of *caup*. In addition, we show that *caup*, but not *ara*, is required for *stripe* expression, and therefore to specify flight muscle attachment sites, in the lateral thorax.

Thus *caupolican* appears to be a common prepattern gene for the specification of the entire structure in the lateral thorax, similarly to *pannier* in the medial thorax.

467B

Novel Modifiers of the Hedgehog Signaling Pathway. David J Casso[1], Stacey K Ogden[2], David Iwaki[1], Songmei Liu[1], David J Robbins[2], Thomas B Kornberg[1]. 1) Biochemistry and Biophysics, UCSF, San Francisco, CA; 2) 1Dartmouth Medical School, Department of Pharmacology and Toxicology, Hanover, NH.

We conducted a genetic modifier screen to identify new components of the Hedgehog (hh) morphogen signal transduction pathway. The Smoothened (smo) gene is required for Hedgehog signaling, and we found that expressing an RNAi against smo causes a weak Fused phenotype in the adult wing. A screen of the Bloomington deficiency kit identified 18 loci on chromosomes 2 and 3 which enhance or suppress this phenotype. The hh, engrailed, and patched loci scored in the screen. Here, we will describe this screen in detail, and present three of the genes we have identified. We will show that these genes modify Hh signaling using both in vivo and in vitro assays. We have identified one of the smo RNAi enhancers as the mts gene, which encodes the catalytic subunit of Protein Phosphatase 2A. We also identified 2 novel smo suppressor genes which, like mts, function in the cells which receive the Hh signal. We will present data showing that these new smo suppressor genes function to limit the potency of Hh signaling in the developing wing.

468C

Epidermal Growth Factor Receptor (Egfr) regulated cell adhesion in *Drosophila melanogaster* eye-antennal and wing imaginal discs. Eduardo J. Gonzalez, Layne Dylla, Jennifer Curtiss. Biology Department, New Mexico State University, Las Cruces, NM.

Many developmental events, such as tissue formation, compartmentalization and even disease related processes like cancer metastasis depend upon differences in cell affinities, mediated in part by cell adhesion molecules. In several recent papers, the *Drosophila melanogaster* receptor tyrosine kinase Egfr (Epidermal Growth Factor Receptor) pathway, has been shown to affect different processes that involve cell affinity in a variety of tissues. However, there are some questions that remain unsolved. Are the previously studied mechanisms for Egfr-mediated cell affinity universal for every system requiring cell adhesion? Do changes in cell affinity mediated by EGFR regulate non-morphological events like compartmentalization or cell sorting in epithelia? What is the mechanism by which EGFR regulates E-Cadherin cell mediated adhesion? To address some of these questions, the FLP/FRT method was used to generate loss- and gain-of-function clones affecting EGFR signaling in the *Drosophila* eye imaginal disc and the wing disc. Clone roundness (determined by the ratio of circumference and area) was used to determine the relative interactions between mutant and surrounding wild-type tissues. Both loss- and gain-of-function of multiple components of the pathway showed round clones. These results suggest that not only is the EGFR pathway necessary to regulate cell affinity but also that its effects are concentration dependent. The fact that multiple members of the pathway produce the same effect shows that EGFR acts through the canonical pathway. We will compare the location of twin spots versus clones to assess any sorting out of cells in the imaginal discs as an indicator of a role in compartmentalization. Also, we will test if the transcription or translation of DE-Cadherin is being affected. Finally, confocal microscopy will be used to address the possibility of subcellular mislocalization of DE-cadherin.

469A

The gene regulatory network involved in DV boundary formation in the Drosophila wing. Héctor Herranz[1], Oriol Canela[2], Fransesc Sagués[3], Ramón Reigada[3], Javier Buceta[2], Marco Milán[1]. 1) ICREA and Institute for Research in Biomedicine (IRB), Parc Científic de Barcelona, Josep Samitier, 1-5, 08028 Barcelona, Spain; 2) Centre especial de Recerca en Química Teòrica (CeRQT), Parc Científic de Barcelona, Josep Samitier, 1-5, 08028 Barcelona, Spain; 3) Departament de Química-Física, Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.

Gene regulatory networks in developing organisms have been conserved during evolution. The Drosophila wing and the vertebrate hindbrain share the gene network involved in the establishment of the boundary between dorsal (D) and ventral (V) compartments in the wing and adjacent rhombomeres in the hindbrain. By means of a Systems Biology approach that combines a powerful mathematical modeling and in silico as well as in vivo experiments in the Drosophila wing primordium, we model herein this regulatory gene network. We show how short-range cell interactions, mediated by the receptor Notch and its ligands, together with long-range cell interactions, mediated by the Wingless signaling molecule, shape the boundary and produce the gene expression pattern that is observed in vivo. We present in vivo and in silico evidence that a novel property, conferred by the activity of Notch in boundary cells and mediated by its target gene Cut, is required for the formation of a stable DV boundary: refractoriness to the Wingless signaling molecule. Such property modulates the regulatory interactions between Notch and Wingless, promotes mutually exclusive domains of their activities, and confers stability to the DV boundary. Concepts like the spatiotemporal refinement of Notch activity and the polarized signaling between Notch receptor and its ligands can be also addressed and explained herein in terms of the refractoriness. A robustness analysis of the regulatory network by means of in silico experiments complements our results and ensures the biological plausibility of the proposed developmental mechanism.

470B

Ubx controls Dpp mobility and haltere development through regulation of the glypican dally. Michael Crickmore[1], Richard Mann[2]. 1) Deptartment of Biological Sciences, Columbia University, New York, NY; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

Animal bodies are composed of structures that vary in size and shape within and between species. Selector genes generate these differences by altering the expression of effector genes whose identities are largely unknown. Our previous work showed that the Hox gene Ubx decreases the production and mobility of the morphogen Dpp to limit the growth of the haltere in comparison to the wing, a serially homologous structure, in which Ubx is not expressed. We showed that Ubx limits the spread of Dpp from its source by increasing the levels of the Dpp receptor, tkv. These findings led us to the general hypothesis that selector genes create differences in size and morphology between tissues by altering the expression components of morphogen signaling pathways, which control growth and patterning during development. In our present study we show that Ubx controls both the proportioning and size of the haltere through regulation of the glypican dally. Specifically, Ubx, in combination with engrailed, which is expressed only in posterior (P) cells, represses dally expression in the P compartment of the haltere. Low levels of dally expression in the posterior haltere contribute to a reduced P compartment size and an overall smaller appendage size. We also show that one molecular consequence of dally repression in the posterior haltere is to reduce Dpp diffusion into and through the P compartment. Our results suggest that Dpp mobility is biased towards cells with higher levels of Dally and that selector genes exploit this phenomenon to modify organ development by regulating glypican expression.

471C

A novel role for *bowl* in the patterning of the *Drosophila* antenna. Catarina Bras-Pereira[1,3], Fernando Casares[1,2]. 1) Centro Andaluz de Biologia del Desarrollo (CABD)- Universidad Pablo de Olavide - CSIC, Seville, Spain; 2) Instituto de Biologia Molecular e Celular (IBMC) - Universidade do Porto, Porto, Portugal; 3) Programa Doutoral de Biologia Experimental e Biomedicina, Universidade de Coimbra, Coimbra, Portugal.

In Drosophila, antennae and legs are serially homologous ventral appendages. This means that despite their very different structure and function, they develop from a common developmental ground state. This ground state includes the activation by hedgehog (hh) of two opposing wedges of the signaling molecules wingless (wg) and decapentaplegic (dpp) in their respective imaginal discs, which in turn, establish the proximo-distal (PD) axis of the appendage. It is the segment-specific selector gene expression that, acting upon this ground state, defines the appendage-specific morphologies. In legs, the odd-skipped family genes are required for segmentation. Transcription of odd-skipped (odd), drumstick (drm) and sister of odd and bowl (sob) is activated by Notch in concentric rings, and this leads to the activation of a fourth member of the family, brother of odd with entrails limited (bowl), that shows a widespread expression pattern, at the prospective leg joints.

Here we report that, in the antenna, bowl has a dramatically different role: bowl is expressed in the ventral antennal disc, to prevent inappropriate expression of wg. The removal of bowl function leads to activation of wg in the dpp-expressing domain. This new intersection of wg and dpp results in a new PD axis that promotes non-autonomous antennal duplications. Therefore, bowl is to be placed downstream of hh and homothorax (hth - the antennal selector) to ensure the correct establishment of the PD axis specifically in the antenna. In addition, the loss of bowl function results in ectopic eye differentiation in a specific domain where both the antennal gene cut, and the eye selector, ey, are co-expressed. Therefore, bowl would be also required to make this ambiguous domain take upon and antennal identity.

472A

Regulation and function of hairy in the Drosophila notum. Denis Bulanin, Teresa Orenic. Dept Biological Sci, Univ Illinois, Chicago, Chicago, IL.

Patterning of mechanosensory organs in the Drosophila leg requires spatially defined expression of the proneural genes achaete (ac) and scute (sc). We have previously shown that the hairy gene is a key regulator of ac expression in the leg and establishes periodic ac expression in the microchaete primordia. is also required for propoer patterning of notal. Here, we examine the function and regulation of hairy in the notum, where it is also required for proper mechanosensory organ patterning. We show that, hairy is expressed in anterior compartment stripe within the scutellar primordium. We find that hairy expression in the lateral notum is regulated in repsonse to the Hedgehog, Wingless and EGFR and Decapentaplegic (Dpp) signals. Our previous studies in the leg have shown that expression along the leg's A/P boundary is regulated similarly. However, we find a key difference in response to Dpp signaling in the leg vs. notum.

473B

Characterizing the role of *distal antenna related* in regulating the expression of *atonal* during eye development. Micheal Burnett, Erin Archuleta, Jennifer Curtiss. Dept Biol, New Mexico State Univ, Las Cruces, NM.

The morphogenetic furrow (MF) marks the beginning of differentiation in the *Drosophila* eye. Cells behind the furrow differentiate under the control of signaling pathways such as the Notch pathway. Within the MF, expression of the transcription factor Atonal (Ato) marks the beginning of differentiation. Ato is initially expressed in all cells immediately ahead of the furrow is gradually restricted to a subset, and then to a single cell which differentiates into the R8 photoreceptor. R8 is the first photoreceptor to develop and is required to recruit the remaining seven photoreceptors. The precise mechanism of *ato* regulation is therefore of great interest. Distal antenna related (Danr) is a Pipsqueak family transcription factor capable of inducing ectopic eyes. Gain- and loss-of-function mutations in *danr* show a disruption in ommatidial patterning and a loss of photoreceptors. Antibody stainings show that Danr is expressed in the developing eye in a pattern similar to Ato, particularly within and immediately posterior to the MF. To determine whether this similarity has any biological relevance we generated loss-of-function clones of *danr*. Within these clones Ato expression was reduced, indicating that *danr* plays a role in regulating Ato. It has been shown that restriction of Ato to the R8 photoreceptor is mediated by the Notch pathway. A genome-wide protein interaction map (Giot et al) has demonstrated a physical interaction between Danr and C-terminal Binding Protein (CtBP), a transcription factor in the Notch pathway. We have used GST-pulldowns to verify this physical interaction. We also show that a loss-of-function *CtBP* mutation is capable of enhancing an eye-specific gain-of-function *danr* mutant phenotype. We are examining Ato expression in clones lacking *CtBP* alone or both *CtBP* and *danr* to determine if the role of Danr in regulating Ato is mediated through CtBP.

474C

Defining Transcriptional Domains in the Undifferentiated Retina. Lucy C Firth, Nicholas E Baker. Molecular Genetics, Albert Einstein Coll. of Med, Bronx, NY.

Uncommitted cells in the undifferentiated retina express different combinations of retinal determining transcription factors that based on their expression patterns define distinct transcriptional domains within this region: Hth; Hth, Tsh & Ey; and Tsh, Ey, Dac & Eya. Although Dpp signaling has been shown to play a role in repressing Hth, how these different transcriptional domains are exactly established is unclear. We propose that different combinations of multiple developmental signals emanating from the differentiated retina regulate are required to define these transcriptional domains. Although the retinal determination gene network may itself be a self sustaining transcriptional network, for alterations in the transcriptional profile of a cell, spatial and temporal input from different combinations of the extracellular developmental signals, Hh, BMP and Egf receptor are required.

475A

Characterization of the role of Homer in retinal apoptosis. Erica J. Hutchins, Jamie C. Rusconi. Department of Biological Sciences, University at Albany, Albany, NY.

Apoptosis is necessary for the proper development of all organisms. It functions to refine the morphology of developing limbs and tissues, to balance cell proliferation rates, and to eliminate unnecessary or abnormal cells or structures. In *Drosophila*, we study the regulation of apoptosis during retinal development, where abnormalities in this process result in improper patterning due to atypical numbers of interommatidial cells. Our lab has previously shown that *klumpfuss* (*klu*), a zinc-finger transcription factor, is a regulator of apoptosis in the pupal retina. Mutants with a loss of function of *klu* demonstrate an increase in the number of interommatidial cells, while mutants over-expressing *klu* show a loss of interommatidial cells, indicating that *klu* is both necessary and sufficient for apoptosis. Microarray data suggests that *homer*, which encodes a G-protein coupled receptor-signaling molecule, is upregulated in response to *klu* overexpression during retinal apoptosis. We have shown that Homer is expressed in the cells that will “choose” life or death in the retina, and is required for apoptosis during retinal development. Retina from *homer* loss-of-function mutants display a decrease in apoptosis, leading to extra cells in the retina. Additionally, we show that *homer* genetically interacts with *klu*, as loss of only one copy of *homer* is able to rescue the *hs-klu* phenotype. Further, the loss of only one copy of *homer* is able to rescue the *hid*-induced eye phenotype; *hid* is a proapoptotic gene that binds dIAPs to promote their degradation, implicating a role for *homer* within the apoptotic pathway.

476B

Induction of Eye Development in Drosophila. Justin Kumar, Claire Salzer. Dept Biol, Indiana Univ, Bloomington, IN.

Two defining characteristics of members of the retinal determination (RD) cascade in Drosophila are the absence of retinal tissue in loss-of-function mutants and the induction of eye formation in forced expression assays. The latter feature provides a spectacular opportunity to compare the molecular dynamics of tissue specification during normal development with instances in which tissue fates are redirected. The RD cascade is comprised of the Pax6 homologs eyeless (ey) and twin of eyeless (toy), the Pax6(5a) genes eyegone (eyg) and twin of eyegone (toe), the Six family members sine oculis (so) and optix, a relative of the Ski/Sno proto-oncogene dachshund (dac), the PTP eyes absent (eya), the novel zinc finger protein teashirt (tsh) and the TALE class homeobox homothorax (hth). The ability to induce ectopic eyes is neither unequal nor unlimited among RD genes. As a step towards understanding the rules of eye specification we have used the UAS/GAL4 system to individually express the RD genes in 220 developmental patterns. We then assayed the ability of each gene to induce ectopic eyes and the ability of each tissue to respond to these instructions. Through this approach we demonstrate that the cascade is more complicated than we currently envision and that additional factors may regulate eye formation. In an effort to increase the range of cell types that can be converted into retinal tissue we have made a series constructs in which combinations of eye specification genes are simultaneously expressed during development. Excitingly, in a few instances simultaneous expression of multiple genes was sufficient to induce eye development in locations that were resistant to the effects of each individual gene. However, in other instances the co-expression of multiple genes does not behave as current models predict. For example, it is thought that the SO and EYA proteins from an obligate biochemical complex and that this step is crucial for inducing eye development. Interestingly, the co-expression of SO and EYA was insufficient in most instances to redirect non-retinal tissues into an eye fate. We will present our findings on the expression of individual and combinations of members of the RD gene network.

477C

miRNA Regulation of the Eye Specification Cascade. Justin Kumar, Arthur Luhur. Dept Biol, Indiana Univ, Bloomington, IN.

The genes of the retinal determination (RD) cascade function within an interwoven and complicated regulatory network. These genes include the Pax6 homologs eyeless (ey) and twin of eyeless (toy), the Pax6(5a) genes eyegone (eyg) and twin of eyegone (toe), the Six family members sine oculis (so) and optix, a relative of the Ski/Sno proto-oncogene dachshund (dac), the PTP eyes absent (eya), the novel zinc finger protein teashirt (tsh) and the TALE class homeobox homothorax (hth). Together this cascade functions to direct a group of cells towards adopting an eye fate. A significant amount of effort by many laboratories has been put towards understanding how these genes are regulated at the transcriptional level with a lesser amount directed towards understanding how signaling pathways alter the activities of selected network proteins. We are interested in determining if and how the miRNA regulatory system functions to fine-tune the activity of the RD pathway. An initial search of several databases has identified a number of putative miRNA genes that are predicted to target the 3‘ UTR regions of several RD genes. As a first step towards understanding the role that miRNAs may play in eye specification we have initiated four lines of investigation. First, we are currently determining if which, if any, of these predicted miRNA genes are expressed in the developing eye. We are particularly interested in those that are expressed coincident with the RD genes ahead of the advancing morphogenetic furrow. Second, the 3’ UTR sensor approach that has been developed by the Cohen group is being used to experimentally confirm that each miRNA gene actually regulates its RD target. Third, each miRNA gene has been isolated and the UAS/GAL4 system is being used to forcibly express each one within the developing fly eye. Fourth, selected ey-GAL4/UAS-miRNA combinations will be used as starting materials for genetic screens aimed at uncovering new miRNA pathway elements. We will present our findings on the role that miRNA genes play in regulating the retinal determination pathway in Drosophila.

478A

Molecular Dissection of Pax6 Proteins During Drosophila Eye Development. Justin Kumar, Bonnie Weanser. Dept Biol, Indiana Univ, Bloomington, IN.

The Pax6 genes eyeless (ey) and twin of eyeless (toy) sit atop the known retinal determination (RD) network in all seeing animals including insects and vertebrates. Members of the Pax6 family bind to DNA through the PAIRED (PD) and HOMEOBOX (HD) domains. One of the most striking characteristics of these proteins is their ability to redirect the fates of non-retinal tissues towards that of an eye. Although both genes are Pax6 homologs there are discernable differences in their abilities to induce eye formation outside of the normal retinal epithelium. Expression of ey is able to convert a broader range of tissues than toy. Additionally, ey is able to induce the formation of significantly larger eye fields than toy. In order to understand the biological basis that underlies these differences we have generated and expressed a series of deletion and chimeric proteins in the dpp-GAL4 expression pattern. Our deletions include a set of constructs in which individual domains have been removed from the EY and TOY proteins. Our chimeric molecule set includes proteins in which individual domains of EY have been substituted with the equivalent domains of TOY. We have assayed the ability of each deletion and chimeric protein to induce eyes and have identified a number of crucial domains that confer functional differences between the two Pax6 homologs. Our results indicate that the B domain, which resides between the two DNA binding motifs, and the C-terminal regions of the Pax6 genes play important roles in the function of these genes. For example, expression of a chimeric protein in which the B domain of ey has been replaced with that of toy can convert the fly genitals into compound eyes, a feat that is not observed when either wild type gene is expressed. We will present these and other findings on the functional differences that underlie the differential activities of eyeless and twin of eyeless.

479B

Six Class Transcription Factors in Drosophila Eye Development. Justin Kumar, Brandon Weasner. Dept Biol, Indiana Univ, Bloomington, IN.

In Drosophila three members of the Six class of homeobox transcription factors exist and regulate development: sine oculis (so), optix and DSix4. Each family member is characterized by the presence of a SIX domain (SD) for protein-protein interactions and a homeobox (HD) DNA binding domain. The first two genes play pivotal roles in eye development as loss-of-function mutants lead to retinal defects while forced expression in non-retinal tissue is sufficient to induce ectopic eye formation. We have previously conducted a structure/function analysis of these proteins in an attempt to elucidate the biological basis for observed differences in the roles that so and optix play in eye specification. In these studies we demonstrated that the activity of the HDs are partially conserved while the SD and C-terminal regions of the proteins confer functional specificity upon members of the Six family. One particularly interesting result focuses on the C-terminal tails of these proteins. While the C-terminal tail of SO appears to be dispensable for its function the C-terminal domain of OPTIX appears to be functionally required. We have further dissected this segment of OPTIX by creating and expressing constructs harboring deletions of the C-terminal tail. Our functional dissection has identified short stretches that are required for OPTIX function and these regions are conserved in the mammalian homologs, SIX3 and SIX6. We have also extended our functional analysis to the vertebrate family members (SIX1-SIX6) by attempting to rescue so loss-of-function mutants and inducing eye formation. Our results suggest that only SIX1 and SIX2 appear capable of restoring eye development in so mutants. These results are particularly interesting in light of the fact that SIX1 and SIX2 do not play a significant role in vertebrate eye development, while SIX3 and SIX6 are the predominant players. We will present our findings on the functional studies that we have done on the fly and mammalian Six family members.

480C

Evolution of the Eye Specification Cascade. Justin Kumar, Rhea Datta. Dept Biol, Indiana Univ, Bloomington, IN.

The retinal determination (RD) cascade has been best studied in the fruit fly, Drosophila melanogaster. Over a decade and a half of experimentation has identified a number of key players that play pivotal roles in the specification of the eye. The RD cascade is comprised of the Pax6 homologs eyeless (ey) and twin of eyeless (toy), the Pax6(5a) genes eyegone (eyg) and twin of eyegone (toe), the Six family members sine oculis (so) and optix, a relative of the Ski/Sno proto-oncogene dachshund (dac), the PTP eyes absent (eya), the novel zinc finger protein teashirt (tsh) and the TALE class homeobox homothorax (hth). Numerous laboratories have identified homologs in these genes from organisms as diverse as ribbonworms to humans. Additionally, functional homologies have been worked out with select members of the cascade. We are interested in extending these results and our aim is to examine the evolution of the eye specification cascade as a whole. As a lever into this question we have searched several databases and have identified homologs of the RD genes in 11 additional Drosophila species, 2 species of mosquitoes, the honeybee and the flour beetle. We have used this information to address several issues. First, the RD cascade contains three pairs of genes that arose through duplications. We are interested in identifying the points in evolutionary history at which these duplications occurred. Second, each of RD genes has distinct binding targets and activities although several belong to gene families (ie SO and OPTIX are SIX class homologs). We are interested in identifying the amino acid residues (in key protein-protein interaction and DNA binding domains) that are under positive and/or negative selective pressures. Third, the RD cascade (as it is conceived today) consists of at least ten nuclear factors that undergo a complicated set of interwoven regulatory interactions. We are interested in determining the points in evolutionary history at which each gene was recruited into the RD network. We will present our findings on the evolutionary history of the retinal determination cascade.

481A

Regulation of dachshund by the Hox Genes During Drosophila Development. Justin Kumar, Jason Anderson. Dept Biol, Indiana Univ, Bloomington, IN.

The retinal determination gene dachshund (dac) is expressed in a dynamic pattern throughout development including the embryonic head, optic lobes, brain and central nervous system. It is also expressed in a number of postembryonic imaginal discs including the eye, antenna, wings, legs halteres and genitals. We had previously conducted a genetic screen for genes that regulate dac within the embryonic visual primordium and identified a number of factors including the homeobox (HD) transcription factor zerknullt (zen) as a regulator of dac transcription. zen is located within the Antennapedia complex and shares a high level of sequence conservation with that of the Hox genes within the HD. Others have shown that selected members of the Hox genes are also able to regulate dac transcription. In order to examine the possible regulatory relationship between the Hox genes and dac, we expressed each of the eight Hox genes within several imaginal discs via the dpp-GAL4 and assayed the effect on dac expression. In several instances we observed alterations in the distribution of DAC protein. One of the more interesting observations is that the expression of Deformed (Dfd) within the wing imaginal disc is sufficient to induce dac expression. Interestingly, both Dfd and the eye specification gene eyeless (ey) induce dac transcription in the same location within the developing wing when forcibly expressed along the A/P axis. The major difference is that expression of ey results in the formation of ectopic eyes in this location while Dfd can induce dac expression but not eye development. In order to determine if this regulation is solely due to the Dfd HD we made a series of chimeric proteins in which individual sections of Dfd has been replaced with the corresponding parts of zen. We have observed similar regulatory interactions between zen and other Hox genes such as proboscipedia (pb) and Ultrabithorax (Ubx). We will present our findings on our molecular dissection of the mechanism by which selected Hox genes regulate dachshund transcription.

482B

Identification of Targets of the SIX Family of Transcription Factors. Justin Kumar, Abigail Henderson. Dept Biol, Indiana Univ, Bloomington, IN.

Members of the SIX family of transcription factors are characterized by the presence of a Six domain (SD) for protein-protein interactions and a homeobox (HD) for DNA binding. In Drosophila there are three such genes, sine oculis (so), optix and DSix4, of which the first two play pivotal roles in eye specification while DSix4 is involved in the development of mesodermal derivatives. Through the course of evolutionary history each gene has been duplicated thus vertebrates have six homologs: SIX1-SIX6. The consensus binding sites for a few members of the Six family have been identified and it appears that multiple SIX family members can bind to the same consensus sequence and can regulate a single target gene. As a step towards unraveling the complexity of SIX-factor regulation we have initiated a series of genetic screens that are aimed at identifying targets of SO, OPTIX and DSix4. We are using the UAS-GAL4 system to express each gene ahead and behind the morphogenetic furrow using ey-GAL4 and GMR-GAL4 respectively. Each combination results in a unique disruption of the adult compound eye. These six fly lines are being used as starting materials for our genetics screens; each line is being crossed to the Bloomington Drosophila Deficiency Kit. This approach has allowed us to identify regions of the genome that are harboring putative targets of the SIX transcription factors. We are in the process of refining our genetic maps through the use of single gene disruption mutations. Additionally, we are using DNA microarrays to interrogate all transcripts in eye discs of flies that are forcibly expressing either SO, OPTIX or DSix4. We are interested in identifying targets that are commonly regulated by all three genes as well as those that are differentially regulated in the eye. We will present our DNA microarray and genetic results and place them in context with our efforts to understand how SIX proteins regulate development, particularly in the eye.

483C

A mosaic screen to identify X-linked genes required for the normal pattern of photoreceptor differentiation. Kevin Legent, Josefa Steinhauer, Jessica Treisman. Skirball Institute, NYU Medical Center, New York, NY.

Drosophila eye development involves signal transduction cascades very well conserved through evolution and often misregulated in human cancers. The eye primordium is specified by the transcription factors Twin of eyeless, Eyeless, Eyes absent, Sine oculis and Dachshund. Photoreceptor differentiation initiates at the posterior margin of the eye disc in the third larval instar and progresses anteriorly across the disc. Initiation and progression of this wave require signaling by Hedgehog (Hh) and Decapentaplegic. Hh specifies the R8 photoreceptor within each cluster, and Epidermal growth factor receptor (EGFR) signaling recruits the remaining seven photoreceptors. The Notch pathway is required to prevent excessive R8 differentiation, and Wingless specifies the regions of the eye disc that give rise to head tissue. Nevertheless, eye development is not yet fully understood and future studies may illuminate the connection between specification genes and signaling pathways. Our aim is to identify novel genes required for this process, including new components of these signaling pathways. We have previously reported a mosaic genetic screen using FLP recombinase driven by the *eyeless* enhancer to generate clones of cells homozygous for random EMS-induced mutations only in the developing eye, allowing the discovery of genes based only on their function in the eye. We select mutations that prevent homozygous cells from differentiating as retinal tissue, but allow the cells to survive long enough to disrupt eye patterning. Screens of the autosomes have already identified novel genes that include components of the Hh and EGFR pathways. We have now modified this method to screen the X chromosome and have recovered mutations with a variety of phenotypes. We plan to identify the genes affected, characterize their molecular functions and examine their interactions with known signaling pathways. The unbiased nature of this method provides an opportunity for unexpected findings, allowing a fuller understanding of the genetic basis of specification and differentiation of a specialized organ.

484A

The transcriptional cofactor Chip sets the boundary of the eye field. Jean-Yves Roignant, Kevin Legent, Florence janody, Jessica Treisman. Dept Developmental Genetics, Skirball Inst, New York, NY.

Differentiation of the *Drosophila* eye imaginal disc begins in the third larval instar with the formation of the morphogenetic furrow, which progresses from posterior to anterior as a wave of differentiation across the disc epithelium. Lateral and anterior regions of the eye disc instead develop into head cuticle. Photoreceptor differentiation in these domains is prevented by the transcription factors Pannier dorsally and Homothorax ventrally, and by the signaling molecule Wingless. In a mosaic genetic screen for genes that affect photoreceptor differentiation we isolated one allele of the *Chip* gene. *Chip* mutant clones of cells in the anterior ventral region of the eye disc form ectopic fields of differentiating photoreceptors, which develop into ectopic eyes in the ventral head cuticle. These ectopic eyes are entirely composed of mutant tissue, suggesting that the effect of the mutation is cell-autonomous. Mutant clones in the compound eye develop normally, suggesting that the normal function of *Chip* in adult head development is to delimit the eye field and set the boundary between eye and head tissue. Chip is a transcriptional cofactor for LIM-homeodomain (LIM-HD) and other transcription factors; its LIM protein interaction domain is required to rescue the ectopic eye phenotype. Misexpression of the LIM-HD proteins Arrowhead, Lim1 and Islet inhibits photoreceptor differentiation in a *Chip*-dependent manner, suggesting that one or more of these proteins may act with Chip to prevent photoreceptor differentiation in regions of the eye disc destined to become head tissue. We are analyzing expression patterns and mutant phenotypes to identify the partners of Chip in this process.

485B

Differential Delta expression underlies diversity of sensory organ patterns among the Drosophila legs. Stuti Shroff, Teresa Orenic. Dept Biological Sciences, Univ Illinois Chicago, Chicago, IL.

In vertebrate and invertebrate species, regional identity along the anterior/posterior (A/P) axis is specified by the Homeotic (Hox) genes. Although Hox gene function in A/P patterning has been extensively studied, the downstream mechanisms through which Hox genes specify morphology are not fully understood. The legs of the Drosophila adult are an excellent model system to investigate this process because, although they are homologous, each of the three pairs of legs exhibits unique morphological features. We are investigating the roles of two Hox genes, Sex combs reduced (Scr) and Ultrabithorax (Ubx), in generating leg-specific sensory organ patterns in the Drosophila adult. Our focus is on a group of small bristles, which are organized into a series of transverse rows (T-row bristles) found on the legs of the first thoracic (T1) and third thoracic (T3) segments, but not on the legs of the second thoracic (T2) segment. Sensory organ pattern in the legs and other adult structures is established through position-specific expression of the proneural gene achaete (ac), which functions to specify a neural cell fate. ac expression in legs is regulated by the hairy (h) and Delta (Dl) genes, each of which is expressed in a precise spatially-defined pattern. We find that, leg-specific differences in ac expression underlie the unique pattern of T-row bristles on each leg. In addition, it appears that the unique patterns of ac expression among the three legs result from differential expression of the Notch-ligand, Dl. Furthermore, we observe that the expression of the Hox genes, Scr and Ubx is up-regulated in the T-row primordia of the T1 and T3 legs respectively. This up-regulated expression is required to establish differential expression of Dl in T1 and T3 legs. These observations suggest that Scr and Ubx generate unique leg morphologies, in part by modulating Dl expression which, in turn, leads to differential patterns of N activation among the three pairs of legs.

486C

The role of RBF and RNO in Drosophila eye development. Latishya J. Steele, Jinhua Xu, Wei Du. University of Chicago, Chicago, IL.

The retinoblastoma protein (RB) was the first tumor suppressor identified, and has been well-characterized as a key regulator of the cell cycle. Interestingly, consequences of RB loss are distinct in different cell types, suggesting that other signaling pathways or cell-intrinsic factors might modulate the effect of RB removal. However, little is known about the specific genes or pathways that function in this regard. To address this, we have conducted a mosaic genetic screen to identify and isolate mutations that perturb differentiation programs concomitant with loss of RBF (fly RB) in the developing *Drosophila* eye. We have isolated an allele of the *rhinoceros* (*rno*) gene, which encodes an antagonist of differentiation believed to function through modulation of epidermal factor growth receptor (EGFR) signaling [Voas and Rebay, Genetics 165: 1993 (2003)]. Removal of *rno* leads to over-recruitment of photoreceptor and non-photoreceptor cell types (cone and pigment cells), as well as enhancement or suppression of phenotypes within genetic backgrounds that lead to increased or decreased EGFR signaling respectively. Analysis of mitotic clones doubly mutant for both *rbf* and *rno* reveals further defects in eye development: a striking delay in the onset of photoreceptor differentiation, abnormal development of non-photoreceptor cells during larval and pupal eye development, and aberrant morphology of the adult eye. These findings suggest that RBF and RNO might have partially redundant roles throughout the differentiation of these cell types. Here, we present phenotypic characterizations of the *rbf/rno* double mutant phenotype, as well as preliminary evidence of a potential mechanism through which this interaction occurs.

487A

Dip3: a regulator of Drosophila eye/antenna development that transforms eyes to antennae. Cheng-wei Wang[1], Hao A. Duong[2], Y. Henry Sun[1], Albert J. Courey[2]. 1) Dept Academia Sinica, Inst Molecular Biology, Taipei, TW; 2) Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569 USA.

The Drosophila eye and antenna arise from a single epithelium termed the eye-antennal imaginal disc. However, the mechanisms responsible for subdividing this epithelium into distinct eye and antennal fields are poorly understood; and it has not been previously possible to find experimental conditions that convert the eye primordium to an antennal fate. We show here that over-expression of the gene encoding the MADF/BESS family transcription factor Dip3 in the eye-antennal disc produces both antenna duplications and eye-to-antenna transformations - two phenotypes that apparently result from different developmental processes. Co-expressed CycE or Eyg could fantastically rescue loss of eye phenotype caused by ey>dip3 whereas p35 could not. It indicates over-expressed Dip3 caused a negative regulation on cell proliferation, not activation of apoptosis. Using clonal induction analysis we demonstrated the early antennal marker Cut could be activated, while the early eye markers Ey and Eya were inhibited in Dip3 over-expressed clones of the 2nd larval eye discs. Although the Dip3 mutant flies display no obvious eye defect, our data provided the evidence of how ectopic expressed Dip3 induced eye to antenna transformation.

488B

Phylogenetic inference based on patterning mechanisms in flies. Urs Schmidt-Ott[1,2], Steffen Lemke[1], Matteen Rafiqi[1], Michael Stauber[2], Sean Ferguson[1], Philip Shaw[2], Alexander Prell[2], Oliver Schön[3], Helmut Blöcker[3]. 1) Organismal Biol & Anatomy, Univ Chicago, Chicago, IL; 2) Max-Planck-Institute for Biophys. Chemistry, Göttingen, Germany; 3) Helmholtz Centre for Infection Research, Dept. Genome Analysis, Helmholtz Centre for Infection Research, Braunschweig, Germany.

Most Evo-Devo work is concerned with projecting developmental data on the phylogenetic tree thereby revealing developmental constraints or cases of parallel evolution. However, Evo-Devo data provide also characters for inferring the phylogeny. We show that the early radiation of cyclorrhaphan ('higher') flies including Schizophora, its sister taxon Syrphoidea, and lower Cyclorrhapha, which has been inferred from nuclear gene sequences and the morphology of male genitalia, is challenged by developmental genetic data. We have assessed the radiation of Cyclorrhapha using as characters Bicoid-dependent patterning of the anterior bodyplan and specifics of the extraembryonic gene network. First, we demonstrate that Bicoid-dependent patterning is constraint in cyclorrhaphan evolution. We show this at the level of cis-regulatory DNA in a lower cyclorrhaphan fly (*Megaselia*) using Bicoid-dependent *hunchback* regulation as a model, and by identifying *bicoid* orthologues in diverse lower Cyclorrhapha. Second, we provide evidence that bicoid is absent in Syrphoidea. We base this conclusion on PCR screening for a bicoid orthologue in *Episyrphus* (Syrphoidea), sequence analysis (63 kb) of the *Episyrphus* Hox3 locus (*bicoid* is a class 3 Hox gene), and the comparative functional analysis of *hunchback* cis-regulatory DNAs. Finally, we provide evidence that *Episyrphus*, rather than having lost *bicoid* as suggested by the current phylogeny, belongs to a branch that split from other cyclorrhaphans before bicoid evolved. This conclusion is supported by the extraembryonic fate of the anterior-most blastoderm in *Episyrphus*, and by the expression of *Episyrphus hunchback* in the extraembryonic blastoderm, which *Episyrphus* shares with non-cyclorrhaphan insects, unlike cyclorrhaphan species with *bicoid*.

489C

Conserved and divergent wing vein patterning mechanisms in the red flour beetle Tribolium castaneum. Yoshinori Tomoyasu, Robin E. Denell. Div Biol, Kansas State Univ, Manhattan, KS.

The two pairs of wings that are characteristic of ancestral winged insects have often undergone evolutionary modification. In dipteran insects such as Drosophila, the hindwings have been extensively modified and form dwarf structures called halteres, while the forewings retain a more ancestral wing morphology. The differences between the membranous forewings and the halteres in Drosophila depend on the Hox gene Ubx. Ubx represses genes that are important for wing development, promoting haltere identity. The forewings develop without Hox input, and are therefore considered a Hox-free state. In beetles such as Tribolium, however, it is the forewings that are modified (to form body covers; elytra), and instead the hindwings retain a morphologically more ancestral identity. We have previously reported that, despite their extensively modified morphology, elytra are the Hox-free state, and Ubx represses the modification in Tribolium hindwings. To understand how beetle elytra have evolved without any Hox input, we have been analyzing the development of elytron-specific features in Tribolium. Elytral veins form a unique parallel pattern, which differs significantly from the typical insect wing vein pattern, suggesting that vein patterning has been evolutionary modified in elytra. Comparison of the expression of genes important for providing vein positional information (such as en, hh, and dpp) suggests that the positional information in hindwing and elytra is very similar. We noticed, however, that some of the genes that normally respond to this positional information to form each vein (e.g. iro, knot) are not expressed in elytra. Instead, elytra seem to have acquired a novel vein pattering mechanism, in which new vein primordia intercalate between the already formed vein primordia to form a parallel vein pattern. RNAi analysis has revealed that Notch signaling might be involved in this process. We will discuss these results, as well as the differences between the wing gene networks of beetles and flies that might have contributed to the morphological differences between these species.

490A

Developmental changes of intracellular distribution of Dd4 protein and its homologue Tth in *Drosophila melanogaster*. Dina Kulikova[1,2], Denis Igumnov[2], Olga Simonova[1,2], Vladimir Buchman[3], Leonid Korochkin[1,2], Ilja Mertsalov[2]. 1) Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Vavilov St. 26, 119334, Moscow, Russia; 2) Institute of Gene Biology, Russian Academy of Sciences, Vavilov St. 34/5, 119334, Moscow, Russia; 3) School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK.

The *Drosophila* Dd4 and Tth proteins comprise a distinct group of presumptive modulators of transcription, homologous to d4 family proteins of vertebrates. The Dd4 protein has a characteristic structural d4 domain in C-terminus of the molecule, containing two tandemly arranged PHD fingers. The Tth protein has no d4 domain in the structure. However N-termini of both Dd4 and Tth have a significant similarity to unique N-terminal 2/3 domain of d4 family proteins. Developmental changes of intracellular distribution of mammalian d4 family proteins have been recently observed in cerebellar neurons of mouse brain. Here we present an examination of intracellular location of Dd4 and Tth proteins during *Drosophila* development. Using polyclonal antibodies raised against Dd4 and Tth we have shown that distribution of the proteins in nuclei and cytoplasm is different on certain stages of *Drosophila* development. To analyze physiological function of *tth* and *dd4* we also produced transgenic fly lines that can express corresponding short hairpin RNAs under control of GAL4 driver. The results of RNAi silencing experiments will be presented.

491B

Experimental and computational methods to determine the shape of the Spitz and Argos gradients. Gregory T. Reeves[1,2], Stanislav Y. Shvartsman[1,2]. 1) Dept Chemical Engineering, Princeton Univ, Princeton, NJ; 2) Lewis-Sigler Institute of Integrative Genomics, Princeton Univ, Princeton, NJ.

Despite the prevalence of morphogen gradients in developmental pattern formation, direct *in vivo* measurements of morphogen concentration profiles have been few and far between. In particular, the protein gradients of the EGFR pathway components Spitz and Argos have not been characterized. Here we demonstrate a method of using indirect experimental measurements, in conjunction with mechanistic modeling, to predict the concentration profiles of such diffusible proteins. We use the EGFR-mediated patterning of the ventral ectoderm as a model tissue, and show how a mathematical model can use the behavior of marker genes in various genetic backgrounds to quantitatively predict values of biophysical parameters.

In embryonic St. 9, the ventral ectoderm (VE) is exposed to a morphogen-like gradient in the EGFR ligand Spitz. Its interactions with the receptor, as well as with a diffusible inhibitor (Argos), have been well-characterized biochemically [1]. In our previous analysis of a mechanistic model of EGFR-mediated patterning in the VE, we were unable to obtain estimates of the relative length scales of Spitz and Argos using the current literature data as inputs [2]. However, further analysis of the model has revealed a set of quantitative experimental measurements which will allow us to probe the values of these parameters [3]. Preliminary results of these experiments suggest that the Spitz gradient may be sharper than Argos gradient, as was suggested by previous genetic experiments [4-6]. Further tests are in progress to verify these initial predictions.

[1] Klein, et al. (2004). Nature, 430: 1040-1044. **[2]** Reeves, et al. (2005). Dev. Biol., 284: 523-535. **[3]** Reeves and Shvartsman, in preparation. **[4]** Golembo et al. (1996). Development, 122: 223-230. **[5]** Freeman et al. (1992). Cell, 69: 963-975. **[6]** Freeman, M. (1994). Mech. Dev., 48: 25-33.

492C

O-linked glycan expression during Drosophila development. E Tian, Kelly Ten Hagen. Developmental Glycobiology, NIDCR/NIH, Bethesda, MD.

Mucin-type O-linked glycosylation is an evolutionarily conserved protein modification that is essential for viability in Drosophila melanogaster. However, the exact role of O-glycans and the identity of the crucial apoproteins modified with ?-linked O-GalNAc remain unknown. In an effort to elucidate the O-linked glycans expressed during Drosophila development, we have employed fluorescent confocal microscopy using a battery of lectins and an antibody specific for the GalNAc?-Ser/Thr structure (Tn antigen). Confocal microscopy provides high resolution images of the diversity of glycans expressed in many developing organ systems. In particular, O-glycans are highly expressed on a number of ectodermally derived tissues such as the salivary glands, developing gut and the tracheal system, suggesting a role for O-glycans in cell polarity and tube formation common to these organs. Additionally, O-glycans are found in the developing nervous system and within subregions of developing tissues known to be active in cell signaling events. These studies provide us with temporal and spatial information regarding O-glycan expression as well as a set of reagents for isolating glycoproteins from specific developmental stages and organ systems. This information will aid us in identifying the in vivo substrates of the polypeptide GalNAc transferases, in a continuing effort to define the biological role of O-linked glycoproteins during development.

493A

Spatial control of BMP pathway by EGFR signaling in Drosophila oogenesis. Nir Yakoby[1], Jessica Lembong[1], Christopher A. Bristow[1], Trudi Schupbach[2], Stanislav Y. Shvartsman[1]. 1) Dept of Genomics and Chemichal Engineering,Princeton University, Princeton, NJ; 2) HHMI, Dept of Molecular Biology, Princeton University, Princeton, NJ.

The BMP and EGFR pathways interact in a large number of developmental contexts, but the mechanisms of signal integration are only beginning to be explored. Signal integration can occur at the level of common transcriptional targets, and at the level of signal transduction. We show that during Drosophila egg development, the EGFR pathway can potentiate BMP signaling at the level of signal reception, by regulating the expression of multiple BMP receptors. In oogenesis, the EGFR and BMP pathways pattern the follicular epithelium by the emanating oocyte-derived Gurken ligand and Dpp secreted from the stretch cells. The first stage of BMP signaling is uniform along the DV axis has only the AP polarity, and mediated by Tkv and Put receptors uniformly expressed in the follicular epithelium. We discovered that later in oogenesis BMP signaling acquires a clear DV polarity. At this stage of signaling the BMP receptors Tkv, Put and Wit are expressed in dorsal-anterior patterns, which overlap the expression of known dorsal-anterior genes, such as Br and Rho. We demonstrate that these patterns are directly controlled by the EGFR pathway, and show that they are translated into the DV pattern of the BMP signaling at the level of pMad nuclear localization. These observations lead to a model where EGFR signaling provides the spatial control of BMP pathway by regulating the expression of its receptors. This model predicts the existence of targets that exhibit coordinate responses to variations in the levels of signaling through both pathways. We demonstrate that this is indeed the case for Br, a gene that marks the roof of the future dorsal appendages. We provide further support for this model by presenting the analysis of our genome-wide transcriptional profiling of EGFR and BMP signaling in the follicular epithelium. We argue that this type of pathway transactivation provides a novel strategy for the spatiotemporal coordination of signals in development.

494B

Characterization of klumpfuss expression in the cytoplasm during retinal development. Barbara J. Zaffo, Barbara Zaffo, Jamie Rusconi. Biological Sciences, Univrsity at Albany, Albany, NY.

klumpfuss (klu), the Drosophila Wilm's tumor suppressor-1 homologue, is both necessary and sufficient for apoptosis in the developing retina. We have previously shown that klu mRNA is the first and to date only molecule identified that is differentially expressed prior to apoptosis in the interommatidial cells, i.e., the cells that choose to live or die in the developing retina. We have generated an antibody to klu protein to further our analysis of klu in this process. Previous work with klu had shown that klu protein localized to the nucleus which seems reasonable given its role as a transcription factor. Using our newly generated antibody we have found that klu expression is not restricted to the nucleus. Instead, klu is clearly localized to the cytoplasm in some cells, the nucleus in others and in both the nucleus and cytoplasm in others. Interestingly, this klu in the cytoplasm is restricted to discreet cytoplasmic puncta or speckles. We have found that klu is expressed in all interommatidial cells as well as the primary pigment cells during pupal development. In these cells klu is localized to both nuclei and cytoplasm. While this has confounded the simple question of, "Does expression of klu kill a cell?", it has raised the more interesting question of, "What is the role of the cytoplasmic vs. nuclear localiztion of klu play in the regulation of apoptosis?" We will present our analysis of klu protein expression during the time course of apoptosis. Through double labeling experiments with klu and markers for apoptosis we will identify the subcellular localization of klu in cells that will survive vs. cells that will die.

495C

Sequential organization of leg segment patterning, growth and morphogenesis. Lina Greenberg[1,2], Victor Hatini[1,2]. 1) Anatomy and Cellular Biology, Tufts University, Boston, MA; 2) Program in Cell, Molecular, & Developmental Biology.

It has long been appreciated that organizers are used to control growth, morphogenesis, and cellular differentiation across cellular fields. However, the molecular mechanisms that link organizer function to these fundamental cellular behaviors remain poorly understood. In the Drosophila leg, DSL signaling (DSL ligands- Delta, Serrate, and Lag-2) controls the growth of leg segments and the morphogenesis of leg joints by regulating the expression of several downstream target genes. Understanding the mechanism of function of these genes would help to answer many of the remaining questions regarding the control of organ growth and shape in this system. Through various genetic and molecular manipulations we have broken down the process of leg segment development into several steps. In the first step, leg segments are subdivided into smaller territories of gene expression by several distinct mechanisms. DSL signaling activates the expression of leg segment-specific genes Bowl, dAP-2, and Nubbin. Bowl helps resolve the pattern by promoting dAP-2 expression and repressing Nubbin expression in Bowl expressing cells. Mutual repression between dAP-2 and Nubbin further refines the pattern and maintains the subdivision of the field into non-overlapping and adjacent territories. In the second step, the smaller territories promote epithelial cell proliferation and groove formation using both autonomous and non-autonomous mechanisms. These activities may underlie leg segment growth and joint morphogenesis. Based on our findings it is clear that the contribution of DSL signaling to proper growth and morphogenesis is quite indirect. DSL signaling initiates the formation of distinct territories. The territories then control growth and morphogenesis via cell-autonomous and cell non-autonomous mechanisms. Our studies provide a conceptual framework for the identification of these growth promoting and morphogenetic signals.

496A

The role of Bicoid in the sharp border of anterior Hunchback: a deterministic and stochastic analysis. Francisco Lopes[1,2,3], Carlos Vanario-Alonso[1,2,3], Alexander Spirov[1,2], Paulo Bisch[3], Fernando Vieira[4], David Holloway[5]. 1) Dept Applied Mathematics, Stony Brook Univ., Stony Brook, NY; 2) Developmental Genetics, Stony Brook Univ., USA; 3) Inst. de Biofisica, Univ. Fed. do Rio de Janeiro, Brazil; 4) Dept de Quimica, Univ. de Brasilia, Brazil; 5) Mathematics, British Columbia Institute of Technology, Burnaby; Chemistry, Univ. of Brit. Col., Vancouver; Biology, Univ. of Victoria, B.C. Canada.

There are at least three different aspects to how the gap gene *hunchback* (*hb*) reads the maternal gradient of Bicoid (Bcd) protein. First, *positioning* locates the anterior Hb expression domain at ~50% egg length in wild-type embryos, and can be shifted by mutations which vary *bcd* dosage. Second, *sharpening* turns the initially shallow border of the early Hb domain into a sharp step-function. Third, *precision* maintains precise location of the domain border, despite natural environmental variability (e.g. Bcd variability, temperature, etc). Fitting expression patterns (for wild-type and mutant embryos, as well as for artificial *hb* promoter constructs), we have developed a predictive bistable regulatory model for *hb* activation. Experiments and computations indicate that the three aspects of *hb* activation are relatively independent. Positioning depends mainly on Bcd concentration, while sharpening requires both Bcd and Hb. Bcd serves to raise Hb to a threshold activation level, allowing the *hb* self-regulation mechanism to generate bistability, with a sharp on-off expression pattern. Other work in our group indicates that Hb precision may require other (gap) factors in the segmentation network. Within the Bcd/Hb system, though, we can address the issue of robustness to intrinsic molecular fluctuations, expected to be significant for gradient amplification at low molecule number. Stochastic simulations of our model fit well with observed stochastic activation of expression patterns, and indicate that multiple Bcd binding sites and a fairly fast timescale to DNA binding/unbinding are required for the observed smooth steady state expression patterns.

497B

CHARACTERIZATION OF *aaquetzalli (aqz)*, A GENE REQUIRED DURING EMBRYOGENESIS IN *Drosophila melanogaster*..

Miguel Mendoza-Ortiz, Juan R. Riesgo-Escovar. Dept Developmental Biol, Inst Neurobiologia, UNAM, Queretaro, Querétaro, MX.

Segmentation, induction and development of the nervous system, dorsal closure, and head involution are morphogenetic processes that occur during embryogenesis. These processes have an important role in the organization and shape changes of embryonic tissues, and they require coordinate cytoskeletal reorganizations. A network of interacting signaling molecules coordinates these reorganizations. Previous studies have shown that *aqz* is required for embryonic dorsal closure and head involution using germline clones of hypomorfic alleles. In addition, it has been reported that *aqz* loss of function mutant embryos using RNAi show nervous system developmental defects. We have characterized a new allelic series of *aqz* mutations all of which are embryonic lethal with dorsal closure, segmentation, and head involution defects. We determined that these new alleles show phenotypic variability, and mapped the molecular lesions for some alleles. We also investigated *aqz* expression: It occurs during embryonic development and adult stages, and is dynamic. *aqz* codes for a hypothetical protein containing a proline rich sequence domain (PRS). This PRS domain is typical for protein-protein interactions, and is characteristic of proteins with a nuclear function. The location of defects in the mutant alleles correlates well with the expression pattern of the *aqz* transcript and protein. Gene expression analysis in adults shows that both the apparent transcript and protein sizes are higher than the annotation of *aqz* in fly base.

498C

Within-embryo noise of primary and secondary morphogenetic gradients in early *Drosophila* segmentation. Alexander Spirov[1], Theodore Alexandrov[2], Nina Golyandina[2], David Holloway[3], Francisco Lopes[1]. 1) Developmental Genetics, State Univ of New York, Stony Brook, NY; 2) Statistical Simulation, St.Petersburg State University, Russia; 3) Mathematics, British Columbia Institute of Technology, Burnaby, Canada.

Development in multicellular eukaryotes reveals noise characteristics inherently different than what is seen in prokaryotes, particularly concerning noise and variability in morphogenetic gradients. To quantify the level of noise and range of variability in the primary and secondary morphogenetic gradients in the *Drosophila* blastoderm, we have developed a suite of computational tools to decompose noisy data into signal (trend) and noise. Our techniques include Singular Spectrum Analysis, wavelet decomposition, and Fourier transformation. We decompose both one-dimensional profiles and two-dimensional data (confocal images of blastoderms stained for protein or mRNA). We find that signal-noise decomposition reveals at least four hierarchical components in the raw data. First, at the highest level, there is a low-frequency smooth signal (the expression pattern). The second component is a middle-frequency variability caused by nuclear positions in the blastoderm. The last two components are high frequency noise: one component is caused by short-range structure in nuclei and cytoplasm, particularly chromatin granules in nuclei and compartmentalization of cytoplasm; the second component is from the noise of photon detection in the photomultiplier tube. The hierarchical nature and spatially-dependent nature of noise for these confocal images makes analysis of expression variability in multicellular embryogenesis inherently more complex than studying gene expression noise in prokaryotes.

499A

The structure and function of follicle cell ring canals. Stephanie Airoldi, Lynn Cooley. Dept Genetics, Yale University, New Haven, CT.

Ring canals in somatic cells are similar to germline ring canals in that they result from arrested cleavage furrows, and allow for direct contact of cytoplasm between bridged cells. EMs have revealed the presence of ring canals in follicle cells and leg discs. These somatic ring canals contain actin, but very little is known about their structure or function. Previous reports have indicated that Anillin, Pavarotti KLP, Nasrat, and Pole hole all localize to follicle cell ring canals. A protein trap in Visgun shows localization to both female germline ring canals and puncta in post-mitotic follicle cells. Ani and Pav KLP localize to cleavage furrows, and retain their punctate localization in post-mitotic follicle cells. We have found that neither Ani, Pav KLP, nor Vsg co-localize with Nas or Phl, indicating that Nas and Phl are not somatic ring canal components. Co-staining of Pav KLP and Vsg with markers for septate junctions, gap junctions, adherens junctions, and endosomes shows no co-localization. We are confirming the localization of Ani, Pav KLP and Vsg to somatic ring canals using ImmunoEM. Because Ani and Pav KLP have essential roles in the cleavage furrow, we are currently using several strategies to disrupt protein function post-mitotically. We are also generating mutations in Vsg in order to study its function in ring canals. A major question that remains is how many follicle cells are in a syncytia, and if that number is variable. We are using two strategies to address this question: activation of a photo-activatible GFP or Florescence Loss In Photobleaching (FLIP) performed on single cells. In conjunction with these studies we have found that GFP traps in ribosomal proteins, as well as Gal4 drivers expressed in follicle cells, exhibit mosaic patterns of expression. Incorporation of BrdU in post-mitotic cells also occurs in a mosaic fashion. We are examining whether these mosaic staining patterns are all marking the same cells, and whether the marked cells are in a syncytium. Through these methods we hope to elucidate the structure and function of somatic ring canals.

500B

The Tudor domain protein Montecristo is required for meiotic progression and microtubule-based transport to the Drosophila oocyte. Vitor Barbosa, Caryn Navarro, Ruth Lehmann. Developmental Genetics, NYU, Skirball Inst, New York, NY.

spindle-class mutations link karyosome structure to oocyte polarity through the activation of a DNA damage checkpoint. Checkpoint activation leads to decreased levels of the TGF?-like molecule Gurken (Grk) within the oocyte resulting in eggshell defects. Some of the *spindle*-class mutations reside in genes necessary for double strand break (DSB) repair during meiotic recombination. In addition to checkpoint activation in these mutant ovaries, persistent DSBs are thought to delay meiotic restriction, which could be the cause of defects in the karyosome. We used the *spindle* phenotype of the eggshell to identify Montecristo as a Tudor domain-protein. Montecristo is required for meiotic progression because *montecristo* mutants delay the restriction of synaptonemal complex and cause abnormal karyosome morphology in the later stages of oogenesis. Despite these meiotic defects, elimination of the Mei-41 dependent DNA damage checkpoint does not suppress the *montecristo* eggshell phenotype, suggesting that Montecristo functions independently or downstream of this checkpoint. A lack of DSBs is also unable to suppress the eggshell polarity defect in *montecristo* indicating that Montecristo functions independently of DSB formation. We found that Montecristo is necessary for the transport of the motor-associated protein Bicaudal D (BicD) and the dsRNA binding protein Staufen both to and within the oocyte. Consistent with the previous genetic interactions, the defect in BicD localization in *montecristo* egg chambers is not suppressed by *mei-41* mutants or by the lack of DSBs. We thus discuss the nature of the localization defect and the role of the Montecristo Tudor domain in the transport of oocyte determinants.

501C

Using an UV cross-linking assay to identify new components involved in regulating *dfmr* translation during oogenesis. Rebecca W Beerman, Thomas A Jongens. Genetics, University of Pennsylvania, Philadelphia, PA.

Specification of polarity in the *Drosophila* embryo relies on a tight translational regulation of maternal transcripts during oogenesis. The regulation of localization and translation of several transcripts have been studied in detail, including *bicoid*, *oskar*, and *gurken*. However, several other proteins and transcripts exist that also show asymmetric distribution in the developing oocyte.

We are interested in studying the specific translational regulation of one such protein, the Drosophila homologue of the fragile X mental retardation protein (dFMRP). Recent studies have shown that dFMRP is required for proper Orb expression and that dFMRP partially co-localizes with Orb, another post-trasncriptionally regulated gene required for proper specification of the embryo axes (Costa et al. 2005, Christerson et al. 1994). To investigate how dFMRP expression is regulated during oogenesis, we are carrying out an unbiased UV cross-linking experiment to identify proteins that bind to the *dfmr* 3'UTR. Once we have identified potential proteins that bind to *dfmr* mRNA, we will verify the biological relevance of these interactions using the genetically tractable system of *Drosophila*. We hope to identify new protein complexes involved in regulating dFMRP expression during oogenesis and hopefully gain insight into general mechanisms by which maternal transcripts are regulated at the level of translation.

502A

Shaping cells and finding paths: The heterotrimeric G-protein subunit *Gβ13F* during dorsal appendage morphogenesis. Michael J. Boyle[1,2], Celeste A. Berg[1,2]. 1) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

The formation of the dorsal appendages (DAs) of the *Drosophila melanogaster* eggshell provides an opportunity to discover how cells cooperate to form a tissue. The DAs form as tubes of follicle cells into which they secrete chorion. Subsets of cells within the initially flat epithelium must execute a program of shape changes, migrations, and rearrangements in order to become the roof and floor of the final tubular structure. Females homozygous for the P-element mutation *tramtrack*$^{twin\ peaks}$ *(ttk*^{twk}*)* produce egg chambers in which the DAs are correctly patterned but form a short tube. Although the basal surfaces of the DA-forming follicle cells migrate normally, the apices (lining the lumen of the tube) remain at their initial positions, leading to shortened DAs. Genetic evidence suggests that heterotrimeric G-protein signaling may be involved in this process: *Gγ1*^{k0817} suppresses the *ttk*^{twk} phenotype.

To determine whether G-protein signaling is required for DA morphogenesis we generated egg chambers mosaic for a null allele of the β subunit, *Gβ13F*$^{\Delta 1\text{-}96A}$. When the leading most row of roof cells lacked *Gβ13F*, they exhibited failures in convergent extension and anterior migration, leading to short DA lumens. When the floor cells lacked *Gβ13F*, the resulting lumen was overly wide. Thus while *ttk* is required to widen the constricted cell apices and allow lateral constriction, *Gβ13F* is required in the floor to constrict the lumen. This result may explain the observation that the G-protein mutant *Gγ1*^{k0817} dominantly suppresses *ttk*^{twk}: the roof cell apices must expand to fill the space defined by the floor. A wider floor would result in increased pressure to widen the roof cell apices, suppressing the overly constricted *ttk*^{twk} phenotype. Further study will test this hypothesis and determine by what mechanism *ttk* and G-protein signaling regulate cell shape.

503B

Visualization of Gurken distribution in follicle cells. Wei-Ling Chang[1], Willisa Liou[2], Hsiao-Chun Peng[1], Yu-Wei Chang[1], He-Yen Chou[1], Li-Mei Pai[1]. 1) Dept. of Biochem & Molecular Biol., Chang Gung University, Tao-Yuan, Taiwan; 2) Dept. of Anatomy, Chang Gung University, Tao-Yuan, Taiwan.

The asymmetrically localized Gurken - a TGF- α homolog, acts as a morphogen to activate the Egfr in follicle cells for the axes establishment in eggs and embryos. During early stages of oogenesis, Gurken could be easily detected in the posterior follicle cells. However, Gurken is hardly detected in follicle cells during middle stages of oogenesis. Here, we have generated a HRP-Gurken fusion protein which facilitated the examination of Gurken in the endocytic route inside follicle cells. The HRP-Gurken fusion protein is functional as it rescues the phenotype of *gurken* null mutant. During the middle stages of oogenesis, the HRP-Gurken fusion protein is detected not only in the dorsal but also in the ventral side of follicle cells using confocal microscopy and transmission electronic microscopy. The HRP-Gurken fusion protein is internalized into follicle cells mediated by Shibire and through Rab5-associated endocytic pathways. Furthermore, the HRP-Gurken fusion protein was detected in the endocytic vesicles in follicle cells under transmission electronic microscopy. These results indicate that Gurken-Egfr complex are likely internalized into follicle cells and processed through endocytic pathways to terminate Egfr signaling. Moreover, Egfr signaling activated by Gurken was, to our knowledge, the first time visualized in the ventral follicle cells, which is consistent with hypothetic and genetic evidence. It has been well known that Cbl acts as an endocytic adaptor to mediate internalization of Egfr. Here, we document that D-Cbl facilitates the internalization of Gurken-Egfr complex into follicle cells since no HRP-Gurken was detected in *D-cbl* mutant cells and yet an accumulation of HRP-Gurken- positive vesicles are observed in the adjacent normal follicle cells. Taken together, these results suggest that the gradient of Gurken morphogen is regulated by the internalization of Gurken-Egfr complex through *D-cbl*.

504C

The functional analysis of Endophilin B in EGFR signaling. Yu-Wei Chang, Yi-Chen Li, Pei-Yu Wang, Li-Mei Pai. Dept. Biochemistry & Molecular Biology, Chang-Gung Univ, Tao-Yuan, TW.

Endophilin is thought to be involved in clathrin-mediated endocytosis by inducing membrane curvature. While associating with CIN85, it can induce EGFR internalization through Cbl. There are two family of Endophilin : Endophilin A and Endophilin B. Although mammalian Endophilin A has been studied thoroughly, the other member of this family, Endophilin B's function remains unclear. Here, we have generated an EndoB null mutant allele, which is an Endophilin B homologue in Drosophila. Homozygote *endoB*^{54} mutant only causes mild lethality. Ectopic expressing D-Cbl L down-regulating EGFR signaling ability is reduced in heterozygote *endoB*^{54} mutant background. Implying the D-EndoB is required for D-Cbl L to regulate EGFR signaling. We have identified two Proline-rich domains in D-Cbl L, which are responsible for the formation of D-Cbl L-CIN85-Endo B complex. The interaction domains of these three components are testing in yeast two hybrid system. Since homozygote *endoB*^{54} mutant can not entirely block D-Cbl L effect, supposing the D-Cbl L has other route independent of D-EndoB. Indeed, reduction of Drk, the Drosophila homolog of Grb2, does markedly reduce the effect of D-Cbl L. Taken together, these results suggest that multiple-pathways are involved in the EGFR downregulation.

505A

Histone methylation is required for oogenesis. Emily Clough, Tulle Hazelrigg. Dept Biol Sci, Columbia Univ, New York, NY.

eggless (egg) was discovered in an EMS mutagenesis screen for lethal and female sterile mutations uncovered by Df(2R)Dll-Mp and encodes a SET domain protein similar to human SETDB1. We have shown that in vivo Egg functions as a histone methyltransferase with specificity for tri-methylation of H3K9 in both the germ and somatic cells of the Drosophila ovary. Strong egg alleles arrest oogenesis very early. In mutant ovaries, germline cysts are not fully encapsulated by somatic cells and do not bud off normally from the germarium. The presence of excess spectrosome-bearing cells along with decreased BamC expression suggests that the differentiation of germ stem cell daughters may be perturbed in mutant germaria. We analyzed the proliferative capacities of both the germ and somatic cells in egg germaria and found that the somatic cells show a marked decrease in proliferation compared to wildtype ovaries. Apoptosis is observed in both germ and somatic cells of egg germaria, but predominantly occurs in posterior somatic cells. These results reveal that trimethylation of H3K9 mediated by Egg is a critical mark required for proliferation of the somatic cells and viability of both the germ and somatic cells in the Drosophila ovary. Clonal analysis and rescue experiments with Gal4-regulated egg transgenes are currently being undertaken to determine if egg functions cell autonomously during oogenesis, or if some aspects of the egg phenotype are due to inductive interactions between different types of cells. *egglesseggDf(2R)Dll-MpDrosophila*.

506B

Analysis of an Orb related gene, orb2, during development of the germline and the nervous system in Drosophila. Nathaniel Hafer, Shuwa Xu, Paul Schedl. Dept Molecular Biol, Princeton Univ, Princeton, NJ.

Orb2 is a novel Drosophila member of the CPEB family of proteins. CPEB proteins bind to mRNA and have been shown to play a role in translational control and mRNA localization. Members of our lab have focused on the study of Orb in the germline and its role in translational regulation. Recent studies in mouse and rat suggest that the machinery that controls polyadenylation and translational activation is highly conserved in different tissues, in particular the nervous system. Previous members of the lab have attempted to detect orb message or protein in nervous tissue, however in all cases orb has only been found in the germline. A search of the complete Drosophila genome reveals that a novel orb homolog exists. We are currently studying this gene, orb2, to determine its expression pattern throughout development and function. In situ hybridization experiments reveal that this gene is expressed in the embryonic central nervous system and the female ovary. Monoclonal antibodies have been made against Orb2 protein. To our surprise, Orb2 protein is found not only in the in the embryonic nervous system but also in the germline of both sexes. To determine the function of Orb2 we are using RNAi to knock down Orb2 levels in specifc tissues. We predict that this gene has a role in the polyadenylation and translational control of transcripts in the Drosophila nervous system and germline.

507C

The role of Notch regulators in *Drosophila* oogenesis. Yvonne Hung, Tanveer Akbar, Martin Baron. Faculty Life Sciences, University of Manchester, Manchester, GB.

The Notch signalling pathway is a key mechanism required throughout development in determining a variety of cell fate decisions in both invertebrates and vertebrates. It plays a number of roles in *Drosophila* oogenesis, including cell differentiation and cell-cycle regulation. The *Drosophila* egg chamber consists of a 16 germ cell cyst surrounded by somatic follicle cells. The latter differentiate into a variety of specialised cells during egg production which is modulated by a number of different signalling pathways including Notch. Suppressor of deltex [Su(dx)] and Deltex are ubiquitin ligases that negatively and positively regulate Notch signalling respectively. However, their role in oogenesis, if any, is not known. Loss-of-function mutations of both genes result in a range of ovary phenotypes, which worsen with age, but with opposite effects in the germarium. While *deltex* mutations result in fewer 16 cell cysts, *Su(dx)* caused an accumulation of cysts in region 2b of the germarium suggesting a defect in pinching off of egg chambers. Investigations are in progress to determine the nature of the somatic or germline defects underlying these phenotypes.

508A

Antagonistic roles between the Drosophila C/EBP and CDP homologues Slbo and Cut regulate centripetal migration. Benjamin D. Levine, Leonard Dobens, Jennifer Hackney, Angela Truesdale. Molecular Biology, University of Missouri KC, Kansas City, MO.

In the ovary, the process of centripetal migration is dependent on the differentiation of the anterior centripetal migrating follicle cells (CMFC), which are derived from the more posterior columnar follicle cells (CFC). We have shown previously that the CFC/CMFC cell fate choice is directed by the bunched (bun) gene, which establishes a sharp boundary of high Notch activity in the CMFC so as to limit the number of precursor cells that contribute to the CMFC fate. At stage 10B, the CMFC undergo mass cell migration coincident with nurse cell dumping to cover the anterior of the egg chamber and produce the operculum eggshell specialization. At the same stage, the CFCs are marked by increased chorion protein loci amplification required for the rapid production of chorion proteins necessary for eggshell development. In contrast, we will show that in the CMFC chorion protein gene amplification as measured by BrDU incorporation is undetectable. Here we focus on CMFC differentiation and migration, which requires dynamic changes in the levels of two transcription factors, Slow Border Cells and Cut. Using gain- and loss-of-function approaches, we will present data to show that: (1) at 10A Notch signaling activates Slbo and represses Cut resulting in increased DE-cadherin levels in the centripetal FC; (3) at the 10A/10B transition, a rapid switch from Slbo to Cut expression is accompanied by decreasing Notch signaling and Slbo autorepression; (3) at stage 10B, Cut represses expression of Slbo and DE-cadherin to trigger centripetal migration. In addition to the opposing effects of Slbo and Cut on DE-cadherin levels, we show that Cut and Slbo both block of chorion gene amplification in the centripetal FC.

509B

Identification and genetic analysis of fusome-localized proteins. . Daniel Lighthouse[1,2], Michael Buszczak[1], Allan Spradling[1,2]. 1) Carnegie Institution/HHMI, Baltimore, MD; 2) Biology Dept, Johns Hopkins University, Baltimore, MD.

The *Drosophila* fusome is a germline-specific organelle that is required for oogenesis. It is necessary for the incomplete mitotic divisions that produce the 16-cell germline cyst, as well as for the selection of one of these 16 cells as the oocyte. Classes of proteins known to show localization to the fusome include cytoskeletal elements, microtubule-binding proteins, cell cycle regulatory proteins, and components of the protein degradation machinery. Additionally, ultrastructural studies have shown that the fusome is enriched in endoplasmic reticulum-like vesicles. A major question remaining about the fusome is the function of this vesicle component.

We have employed a bipartite strategy for further elucidation of fusome structure and function. First, we utilized a protein trap collection generated in our lab to screen for fusome-localized proteins. We screened through 1200 lines, and fifteen genes were found to have protein trap expression in the fusome. Interestingly, 13 of the 15 fusome traps were of genes with products predicted or known to reside within the endoplasmic reticulum, supporting the idea that the observed vesicles of the fusome represent a subdomain of the ER. Furthermore, the tight co-localization of a trap of the tubular ER component Rtnl1 with the fusome suggests that the fusome is comprised of tubular ER.

The second approach we are utilizing is germline clonal analysis of the fusome components identified in our screen. Germline clones of the membrane trafficking protein Rab11 arrest early in oogenesis. A marker for the oocyte, Orb protein, fails to localize to the oocyte in these cysts, and the presumptive oocyte does not localize to the posterior of the egg chamber. These preliminary results suggest a role of the vesicle component of the fusome in early germline cyst development.

510C

Growl, a novel gurken RNA binding protein essential for dorsoventral patterning. Shengyin Lin, Sui Zhang, Robert S. Cohen. Molecular Bioscience, University of Kansas, Lawrence, KS.

We describe the molecular cloning and characterization of Growl, a novel regulator of dorsoventral patterning in the Drosophila oocyte. We identified Growl by mass-spectroscopy following purification from Drosophila extracts on affinity columns containing the GLS, a conserved RNA sequence element previously shown to mediate gurken-like RNA localization when injected into living oocytes (Van De Bor et al., Dev. Cell, 2005). Growl also binds to the GLS of the D. virilis gurken gene, but not to a mutated element that lacks RNA localization activity. Expression and genetic analyses are consistent with the idea that Growl is an in vivo regulator of gurken RNA localization and/or translation. First, we find that a GFP fusion protein, produced by a homozygous viable growl::GFP gene trap allele, accumulates to high levels in nurse cells and along the oocyte's anterior cortex. No obvious enrichment of the protein is seen at the oocyte's anterodorsal corner, consistent with our finding that the GLS possess anterior, but not anterodorsal, localization activity. Second, we find that homozygousity of growl::GFP enhances the mutant eggshell phenotype of gurken heterozygotes, i.e., growl::GFP/growl::GFP; grk-/+ females produce 2-3 times more ventralized eggs than do grk-/+ females. Partially ventralized eggs are also produced by females homozygous for an FRT/Flipase-generated growl deletion allele, called growl[FRT]. Consistent with the partially ventralized eggshell phenotype, homozygous growl[FRT] mutants accumulate reduced amounts of Grk protein at the oocyte's anterodorsal corner. No obvious defects in gurken RNA accumulation or localization are seen in the growl[FRT] homozygotes, but this may simply reflect the likely hypomorphic nature of the allele, which carries a small deletion that is only expected to alter the expression of one of two encoded growl transcripts. The name growl denotes the gurken RNA binding activity of the protein and the owl ear-like appearance of homozygous growl[FRT] eggs.

511A

Investigating the role of *stonewall* in ovarian germline stem cell maintenance. Jean Maines, Tiana Endicott, Tanya Robinson, Dennis McKearin. Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX.

During Drosophila oogenesis, germline stem cell (GSC) identity is maintained largely by preventing the expression of factors that promote differentiation. This is accomplished via the activity of several genes acting either in the GSC or its niche. The translational repressors, Nanos and Pumilio, act in GSCs to prevent differentiation, likely by inhibiting translation of early differentiation factors, while niche signals prevent differentiation by silencing transcription of the differentiation factor Bam. We have found that the DNA-associated protein Stonewall (Stwl) is also required for GSC maintenance. *stwl* is required cell-autonomously; clones of *stwl*- germ cells are lost by differentiation, and ectopic Stwl causes an expansion of GSCs. *stwl* mutants act as Suppressors of Variegation, indicating *stwl* normally acts in chromatin-dependent gene repression. Stwl-dependent transcriptional repression does not target *bam*, but rather Stwl represses the expression of many genes, including those that may be targeted by Nanos/Pumilio translational inhibition.

We are currently exploring the mechanism of Stwl action by identifying transcriptional targets of Stwl as well as by identifying Stwl binding partners. We will report on the progress of yeast two-hybrid and co-immunoprecipitation assays aimed at identifying Stwl binding partners. We are also examining the role of a group of genes whose expression is dependent on Stwl and will report on our functional analysis of these genes in the ovary.

512B

Drosophila germline stem cell maintenance controlled by Loquacious-dependent miRNAs. Joseph Park[1], Xiang Liu[2], Tamara Strauss[2], Qinghua Liu[2], Dennis McKearin[1]. 1) Molecular Biology, UT Southwestern, Dallas, TX; 2) Biochemistry, UT Southwestern, Dallas, TX.

The Drosophila female germline stem cell (GSC) divides asymmetrically to produce two distinct daughter cells, a new GSC and a differentiated daughter cystoblast (CB). The translational repressors Nanos and Pumilio are required intrinsically for germline stem cell (GSC) maintenance. Recent studies suggest that GSC maintenance might also be controlled by microRNAs, a family of 21- to 25-nucleotide cellular RNAs that repress translation of cognate mRNA (Jiang et al., 2005; Forstemann et al 2005; Hatfield et al., 2005). In *Drosophila melanogaster*, the RNase III enzyme Dicer-1 (Dcr-1) functions together with the dsRNA-binding protein Loquacious (Loqs) to catalyze miRNA biogenesis. Hypomorphic *loqs* mutants are viable but characterized as displaying defects in GSC maintenance. Here we further our investigation of Loqs requirement for GSC maintenance by generating *loqs* knockout (KO) flies by ends-out homologous recombination. Analysis of $loqs^{KO}$ flies shows that Loqs proteins are maternally loaded and required for early embryogenesis. Both developmental and GSC maintenance defects of $loqs^{KO}$ flies are rescued by transgenic expression of Loqs. Moreover, GSC maintenance is fully restored in *loqs* mutant ovaries by germline-specific, but not somatic-specific, expression of Loqs. Consistently, mosaic analysis indicates that Loqs is required intrinsically for ovarian GSC maintenance. Interestingly, Loqs is only required for biogenesis of a subset of miRNAs. Dcr-1, but not Loqs, is critical for loading miRNAs onto the RNA-induced silencing complex (miRISC). Taken together, these results demonstrate that Loqs is necessary and sufficient for the *Drosophila* miRNA pathway and development. Specifically, our studies suggest that Loqs-dependent miRNAs play a critical and cell-autonomous role in female GSC maintenance.

513C

Template DNA strand retention in the stem cells of the *Drosophila* ovary. Laura Ponting, Martin Baron. Faculty of Life Sciences,, University of Manchester, Manchester, GB.

Stem cells are able to divide asymmetrically, giving rise to a daughter stem cell and a differentiated cell. It has been suggested that, as a mechanism for preventing error, the daughter stem cell retains the template strand of DNA, whilst the synthesised strand is passed on to the differentiated cell. This has been shown to occur in a number of systems including mouse muscle satellite cells and intestinal stem cells. It has not yet been shown to occur in *Drosophila*. The *Drosophila* ovary contains 3 stem cell population, germline stem cells (GSCs) and 2 somatic stem cell populations. The GSCs are very well characterised and are an ideal system in which to study template strand retention as they divide regularly and continuously throughout the lifetime of the fly. BrdU was used to label the template strand of DNA and my results suggest that the GSCs are not retaining this label. However, BrdU retention was observed in other cells in the ovary and work is currently being carried out to determine their identity.

514A

Missing oocyte and Nup44A/Seh-1 physically interact in the drosophila germ line. Stefania Senger, Mary Lilly. CBMB, NICHD/NIH, Bethesda, MD.

We are examining the role of the missing oocyte gene in the regulation of early oocyte differentiation and meiotic progression. Egg chambers from mio mutant females frequently develop with 16 polyploid nurse cells and no oocyte. Initially mio ovarian cysts enter meiosis, form synaptonemal complexes and localize oocyte specific marker. However, instead of arresting in prophase of meiosis I, in mio mutants the oocyte inappropriately follows the nurse cells into the endocycle and the oocyte fate is gradually lost. Egg chambers from mio mutant female rarely develop beyond stage 5 of oogenesis. We are interested in understanding how mio influences meiotic progression and the maintenance of the oocyte fate. Towards this end we are working to identify proteins that physically interact with Mio. Using tandem immunoprecipitation and mass spectrometry we have determined that Mio protein physically interacts with the highly conserved nucleoporin Nup44A/Seh1 in both S2 tissue culture cells and the ovary. Seh1 is a component of the Nup107-160 complex, which is thought to be a structural component of the nuclear pore. Intriguingly, in yeast recent evidence implicates the Nup107-160 complex in the tethering of telomeres to the nuclear periphery and to the repair of double-stranded breaks in subtelomeric regions of the genome. Moreover, it has been demonstrated that this complex is targeted to the kinetochore during mitosis. These data suggest multiple functions associated to the Nup 107-160 sub-complex not yet fully understood. Two alleles of nup44A were retrieved from Bloomington Stock center. Preliminary analysis indicates that mutations in nup44A can dominantly suppress the mio mutant phenotype. Results from ongoing experiments further characterizing the relationship between mio and nup44A during early oogenesis will be presented. *mio nup44A*.

515B

Mastermind, a member of the Notch signaling pathway, regulates somatic stem cells in the Drosophila ovary. Cynthia Vied, Daniel Kalderon. Dept Biological Sci, Columbia Univ, New York, NY.

In the Drosophila ovary 2-3 somatic stem cells (SSC) give rise to several specialized cell types, including border cells, polar cells, stalk cells, and follicle cells. The SSCs reside in a niche in contact with inner germarial sheath cells. This contact appears to be important for maintenance of the SSC. Additionally,they receive instructive signals from cells at the tip of the germarium, cap cells and terminal filament cells. Three signaling pathways [Hedgehog (Hh), DPP and Wingless (Wg) signaling pathways] have been implicated in directly regulating normal SSC behavior. SSC clones of Hh, Wg, or DPP signaling components result in progressive SSC loss. Interestingly, constitutive activation of the Wg pathway in the SSCs also results in SSC loss. Uniquely, constitutive activation of the Hh pathway through loss of Patched (Hh receptor) in the SSCs induces the formation of extra SSCs. To further these studies we have devised a deficiency screen to identify modifiers of the Hh somatic cell over-proliferation phenotype. We found that mastermind (mam) can dominantly suppress the Hh SSC overproliferation phenotype. Mam is part of the Notch signaling pathway. It is a transcriptional coactivator that is essential to bridge the Notch intracellular domain with Suppressor of Hairless [Su(H)] to make a functional transcriptional complex. Clones of a mam loss of function mutation result in progressive loss of SSCs. Using positively marked patched and mam double mutant clones we were able to directly visualize loss of the somatic stem cell lineage over time, demonstrating that mam is epistatic to patched in SSC regulation. In contrast to mam mutant clones, null mutations of Notch or Su(H) do not cause a dramatic loss of SSCs. Overexpression of the Notch pathway using a consitutively active form of Notch or Su(H)VP16 results in SSC loss (and excess follicle cells, as previously shown). We are currently attempting to clarify the role of the Notch pathway in SSC development and whether the Hh and Notch pathways work together in this process.

516C

The golgin Lava Lamp is required for Drosophila oogenesis. H Wang[1], K Monzo[1], J.T. Warren[2], L.I. Gilbert[2], J.C. Sisson[1]. 1) The Section of MCD Biology and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

Golgins are a structurally diverse family of Golgi-associated filamentous proteins important for Golgi function. They were originally identified as autoantigens associated with a variety of human autoimmune disorders. Studies conducted in mammalian cells have suggested that golgins function as tethers for membrane vesicles and cytoskeletal proteins and maintain the integrity of Golgi structure. Despite their importance in animal cell function, their role in animal development has been understudied. We have previously shown that Lava Lamp (LVA), a novel *Drosophila* golgin, functions as an adaptor on the surface of Golgi bodies for microtubule motility factors dynein, dynactin, and CLIP-190, and serves to facilitate efficient membrane secretion required for cleavage furrow formation in early embryos. In order to address whether LVA functions at other times in development, we depleted endogenous LVA using the GAL4-UAS system to drive the expression of double-stranded *lva* RNA in flies.

Somatic cell depletion of LVA causes female specific sterility. Analysis of dissected ovarioles reveals a decrease in vitellogenic stages (8-14) and higher frequencies of degenerating egg chambers, revealing a block in vitellogenesis. Indirect immunoflourescence demonstrates that LVA is eliminated in follicle cells (FCs) by stage 8 while expression in germline cells is unaffected. While FCs themselves do not display any discernable abnormalities in size, number, and morphology in LVA depleted egg chambers, a significant decrease in ecdysone titre is observed by radioimmunoassay, consistent with the possibility that depletion of LVA disrupts ecdysone synthesis in FCs, a primary source of ecdysone in adult females, which is required for vitellogenesis. Information gained from these studies should enhance our understanding of the role of LVA and the Golgi apparatus during oogenesis.

517A

The role of Rab11 and endocytic recycling in maintaining cell polarity and suppressing cell motility in Drosophila epithelia. Jiang Xu, Robert S. Cohen. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

The formation and maintenance of epithelial cell layers is essential for organ formation and growth, wound repair and many other aspects of normal animal development. Here, we show that the rab11 and sec15 genes, which function critically in endocytic recycling, play essential roles in the maintenance of epithelial cell layers in the Drosophila ovary. Upon loss of rab11 or sec15 gene activity, epithelial cells lose their apical-basal polarity, delaminate from the epithelial layer and "move" into neighboring germ cell clusters. Phospho-histone3 expression studies indicate that rab11 and sec15 mutant cells are non-dividing. Thus their "movement" into the germ cell cluster appears to represent true migratory or invasive activity, reminiscent of metastatic carcinoma cells. We propose from these findings that endocytic recycling—the trafficking of internalized proteins and membranes back to the cell surface—plays an essential role in the maintenance of epithelial cell polarity and the integrity of epithelial cell layers.

518B

Requirements for the transcriptional regulator *lola* in adult stem cell maintenance. Erin Davies[1], Leanne Jones[1,2], Margaret Fuller[1]. 1) Developmental Biology, Stanford University, Stanford, CA; 2) Laboratory of Genetics, Salk Institute La Jolla, CA.

Adult stem cells are required for the long-term maintenance and repair of many tissues, including blood, intestinal epithelia and sperm. We use *Drosophila* spermatogenesis as a model system to study the mechanisms that promote stem cell identity *in vivo*. To identify downstream effectors of a stem cell self-renewal pathway in germline stem cells (GSCs), as well as factors that promote GSC maintenance via parallel pathways, we screened for genes that are differentially expressed in stem cells and their immediate progeny versus differentiating cells. Here, we present progress on the functional characterization of one candidate gene, *longitudinals lacking (lola)*. The *lola* locus encodes a family of 19 BTB domain-zinc finger proteins via alternate splicing that act as transcriptional regulators. All Lola isoforms share a common N-terminus, whereas the C-termini are unique. There is growing evidence that *lola* isoforms execute nonredundant functions, and that Lola variants partner with transcription factors and chromatin modifying enzymes. Using a pan anti-Lola antibody, we have shown that Lola localizes to the nuclei of germline and somatic cells in the testis. Using null alleles of *lola*, we have shown that *lola* is required cell autonomously in the germline for GSC maintenance: *lola* mutant GSCs do not self-renew, and are rapidly lost to differentiation. Mosaic analysis using isoform-specific alleles of *lola* indicates that at least two isoforms are required for GSC maintenance. We are currently investigating whether other *lola* isoform(s) are necessary for GSC maintenance by knocking down individual isoforms in early germ cells. Conversely, we are assaying which isoform(s) are sufficient for GSC self-renewal by performing rescue experiments in a mosaic *lola* null background. Finally, we are addressing whether *lola* operates downstream or in parallel to pathways that promote stem cell self-renewal in the testis.

519C

Testing the Role of the Maternal Pronucleus in Wolbachia-induced Cytoplasmic Incompatibility in Drosophila melanogaster. Patrick Ferree[1], William Sullivan[2]. 1) Dept of Mol Biol and Genetics, Cornell University, Ithaca, NY; 2) Dept of Mol Cell and Developmental Biology, UC Santa Cruz.

Wolbachia are widespread endoparasitic bacteria that manipulate the reproduction of their insect hosts for their own benefit. The most common Wolbachia-induced manipulation is a form of male sterility known as cytoplasmic incompatibility (CI). In CI, host progeny die as early embryos if Wolbachia-infected males fertilize uninfected females. However, if females carry the same Wolbachia strain, embryonic lethality is reversed or suppressed and development occurs normally. It is widely held that CI lethality is the result of an unknown Wolbachia-induced modification of paternal nuclear material in the host testes. However, the mechanism by which this modification kills host embryos is currently not known. Previous cytological studies suggested that CI lethality results from asynchrony in cell cycle timing between paternal and maternal pronuclei. Under this hypothesis, the Wolbachia-modified paternal pronucleus should support androgenetic development (i.e., solely from the paternal pronucleus). Taking advantage of mutations in *Drosophila melanogaster* that produce exceptional androgenetic progeny, we have demonstrated that the Wolbachia-induced modification inhibits androgenetic development. This result suggests that CI does not involve the maternal pronucleus and argues against pronuclear asynchrony as the primary cause of CI. We propose that CI occurs instead as the result of either a developmentally incompetent paternal pronucleus or asynchrony between the paternal pronucleus and the cell cycle of the egg cytoplasm.

520A

Mating induces morphological changes in the *Drosophila* female reproductive tract. Anat Kapelnikov[1], Patricia Rivlin[2], Ronald Hoy[2], Yael Heifetz[1]. 1) Dept Entomology, Hebrew Univ, Rehovot, IL; 2) Dept. of Neurobiology and Behavior, Cornell Univ., Ithaca, NY, USA.

The female reproductive tract is comprised of three tissues: muscle, nerve and epithelium. Our previous genomic/proteomic analysis reveals that mating induces changes in the reproductive tract. More specifically, mating induces changes in muscle and epithelial specific proteins that contribute to the establishment of the muscle and epithelial functional identity, and changes in transcription of genes that play key roles in development. These studies suggest that mating may play a role in the final development/differentiation of the reproductive tract. To address this question, we are conducting a morphological investigation of the reproductive tract in unmated and mated three day-old females. Preliminary studies at the light and electron microscopic (EM) levels revealed changes in muscle, nerve and epithelium after mating. EM studies of both mated and unmated females suggest that the oviduct musculature is in a state of differentiation. Using synaptic markers and confocal imaging, we observed a 36-42% increase in the number of synaptic terminals innervating the oviduct in mated females. Taken together, these results suggest that mating induces nerve-muscle interactions that may play important roles in the final maturation of the reproductive tract. Changes in epithelium are also observed after mating. Our EM study reveals that the apical surface of the lateral oviduct epithelium gives rise to extensive microvilli that are coated in a thin layer of cuticle. In the mated female, the space between the overlying cuticle and microvilli is expanded and filled with an electron dense, filamentous material, thus suggesting that mating may stimulate epithelial secretion in this region of the oviduct. The morphological changes observed here may play an important role in determining reproductive success.

521B

Ecdysone signalling during the development of the *Drosophila* female reproductive tract. Vidya Nagalakshmi[1], Paul Mack[2], Anat Kapelnikov[1], Michael Bender[2], Yael Heifetz[1]. 1) Department of Entomology, The Hebrew University, Rehovot, IL; 2) Department of Genetics, University of Georgia, Athens, GA, USA.

The insect female reproductive tract provides a favorable environment for the activation and transport of gametes, two aspects critical for successful fertilization. In *Drosophila*, the female genital disc undergoes extensive differentiation and development to become a functional reproductive tract. Confocal analyses of the female reproductive tract from different developmental stages using markers for muscular, epithelial and neuronal proteins revealed significant morphological differences in these tissues during metamorphosis and during the first three days post-eclosion. Microarray studies conducted on oviduct and sperm storage organs of three-day-old unmated and mated females showed that mating results in extensive gene regulation and indicate that some previously unexpressed transcripts are first synthesized in the reproductive tract at the time of mating. These unique post-mating gene expression signatures suggest that the reproductive tract continues to develop subsequent to mating possibly to facilitate later reproductive processes. We have also found that the *ecdysone receptor* (*EcR*) gene and ecdysone responsive genes are expressed in the reproductive tract of unmated females and that mating induces a change in the expression level of ecdysone responsive genes. Based on these results we hypothesize that ecdysteroid signalling is involved in mediating the differentiation of reproductive tract tissues at different developmental and reproductive stages of the fly. In this model, ecdysteroids would regulate reproductive tract formation in the developing female and also act to prime the reproductive tract to attain its functional maturity both before and after mating. To examine ecdysone involvement in reproductive tract development, we are now analyzing the spatial and temporal distribution of *EcR* and ecdysone responsive genes in the reproductive tract at different developmental stages. The functional significance of these results will be discussed.

522C

Specific microRNAs regulate GSCs through Dacapo/p21:. Steven Reynolds. Dept Biochemistry, Univ Washington, Seattle, WA.

We have shown previously that the microRNA pathway plays an essential role in Drosophila germline stem cells where in the absence of microRNA, up regulated Dacapo/p21 inhibits the G1/S transition. We have now turned our attention to determining which of the many microRNAs found in Drosophila participate in the regulation of Dacapo/p21 and how their function may vary. Several microRNAs are computationally predicted to target Dacapo for translational repression and we have showed different levels regulatory effect for miR-7, miR-8, miR-309, and bantam on Dacapo's 3'UTR in S2 cells. These regulatory effects have motivated our examination of separate microRNAs effect on Dacapo in vivo where we made loss and gain of function studies of these microRNAs in flies as well as detecting them with in vivo sensors. We have also examined the effect of molecular dissection of the Dacapo 3'UTR to show in both S2 cells and in vivo that microRNA dependent regulation operates through multiple sites in Dacapo mRNA. Next we will address the question of what regulates the expression of miR-7, miR-8, miR-309, and bantam.

523A

Functional analysis of *CG3056*, the closest paralog of *Sex-lethal* in *Drosophila*. Sha Sun, Thomas W. Cline. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Gene duplication is considered a major factor in evolutionary change. A relatively recent duplication event gave rise to the current *Drosophila* master sex-determination gene, *Sex-lethal* (*Sxl*), and its closest paralog, *CG3056*. We are attempting to determine the functional relationship between *Sxl* and this paralog in *D. melanogaster* in order to discover something about the ancestral role of *Sxl* and the possible relevance of this most recent duplication event to the recruitment of *Sxl* to the sex-determination pathway. This information may help explain why *Sxl*, a feminizing switch gene, would also generate male-specific proteins and non-sex-specific mRNAs, despite the fact that deleting the gene from males causes no obvious phenotypic abnormalities. Although *Sxl* orthologs have been identified in species as distant as the honey bee, the fact that sex-specific expression of *Sxl* is limited to the genus *Drosophila* suggests that *Sxl* only participates in sex determination in this genus. Sequence alignments indicate that the duplication event that gave rise to *Sxl* and *CG3056* occurred in the *Schizophora* division, and hence likely predated *Sxl*'s acquisition of a role in sex determination. We recovered three different imprecise excisions of a *P*-element in *CG3056* in our effort to determine the null phenotype for this gene. The longest deletion removed nearly all of *CG3056*, including the two RRMs (RNA binding motifs) - the only long continuous region with strong homology to *Sxl*. All three deletions eliminate the neighboring gene, *CG14770*. Nevertheless, all three deletions are viable and fertile in both sexes, and display no obvious visible phenotype. We are currently generating double-mutant combinations of these *CG3056* deletions with *Sxl* mutant alleles of various sorts, and with other genes known to interact with *Sxl*. The phenotypes of these double mutants should reveal whether functional redundancy between *Sxl* and its closest paralog, perhaps for functions that predate *Sxl*'s recruitment to the sex determination pathway, accounts for the ability of males to tolerate loss of *Sxl* gene.

524B

Sperm of male sterile mutation *sheepish* fail to be stored in females. Masatoshi Tomaru, Takashi Ohsako, Naoto Juni, Hiroshi Matsubayashi, Hiromi Sato, Masa-Toshi Yamamoto. Drosophila Genetic Resource Center, Kyoto Institute of Technology, Kyoto, Japan.

Mature sperm are transferred from males to females during copulation, stored in the sperm storage organs of females, seminal receptacle and spermathecae, and then utilized for fertilization. A male sterile mutation, *sheepish*, was induced by ethylmethane sulfonate. The *sheepish* male produces motile sperm, which are transferred to females during copulation. Although the amount of *sheepish* sperm in the uterus was the same level as the control (*sheepish* heterozygotes) five minutes after copulation, the amount stored in seminal receptacle and spermatheca was significantly fewer than the controls 1 h or 24 h after copulation. Females mated with *sheepish* males laid inseminated eggs significantly less than the control. However, more than 90% eggs inseminated by the *sheepish* sperm as well as the control sperm initiated nuclear divisions, suggesting that the *sheepish* sperm are able to fertilize if enter eggs. We concluded that a low efficiency of sperm entry into the female sperm storage organs is the primary cause of the sterility of *sheepish*.

Mating plugs, which are formed as a product of interaction between seminal fluid and substances in uterus, were found in females that mated with *sheepish* males, as well as in those of females that mated with *sheepish* heterozygotes, suggesting that the seminal fluid of *sheepish* is able to form mating plug. About 30% of females that mated with *XO; sheepish* males mated again as did those mated with *XO; sheepish/TM3* males as the first males. Since XO males do not produce motile sperm but do produce seminal fluid, the seminal fluid of *sheepish* suppresses remating as the control. It seems less likely that the seminal fluid of *sheepish* has any defects. The *sheepish* was mapped within 95F on the 3R by deficiency mapping.

525C

PPS, a novel protein required for establishment of the *Sxl* autoregulatory splicing loop. Matthew Johnson[1], Alexis Nagengast[2], Helen Salz[1]. 1) Case Western Reserve University, Cleveland, OH; 2) Widener University, Chester, PA.

Female-specific expression of *Sex-lethal (Sxl)* is maintained by an autoregulatory splicing loop. A number of additional gene products are required for SXL to control its own splicing. Interestingly, all of these genes, *snf*, *snRNP70K*, *U2af50*, *U2af38*, *spf45*, *vir* and *fl(2)d*, encode proteins whose orthologues purify with the human spliceosome. Here, we describe a new member of the *Sxl* splicing pathway, *pps* (*protein partner of snf*, CG6525) that stands apart due to its absence from any of the multiple purified splicing complex preparations. *pps*, which was identified in a yeast two hybrid screen for SNF interacting proteins and confirmed via co-IP assays, is a 222 kD nuclear protein that is expressed throughout development. Two different classes of *pps* mutant alleles have been isolated: a null non-sex specific lethal allele and an antimorphic, female specific lethal allele. Analysis of both of these mutations reveals that the maternal contribution of *pps* is important for the establishment of *Sxl* activity in embryos. Although important for establishment, *pps* does not appear to be required for maintaining *Sxl* splicing as animals homozygous for the deletion null allele die during the third instar larval stage without any apparent defects in *Sxl* regulation. In contrast, animals homozygous for the female-lethal antimorphic allele arrest development early in embryogenesis (~1-2 hours after egg deposition), the timing of which coincides with the stable establishment of the autoregulatory splicing loop. These data, together with our finding that CG6525 associates with the U1 snRNP via a direct interaction with SNF, suggests that *pps* is required for the establishment of the autoregulatory loop early in development. Additionally, we show *pps* is required for *Sxl* splicing in the female germline. Paradoxically, protein motifs present in CG6525 suggest functions in both RNA processing and transcriptional control. Finding a *Sxl* regulatory factor with mixed motifs is intriguing in light of the current view that transcription and splicing are mechanistically coupled.

526A

Evidence that *transformer* is not the only gene target of *Sex-lethal* that directs female sexual differentiation in *D. melanogaster*. Meghan R. Jones, Daniel S. Evans, Melissa M. Burns, Thomas W. Cline. Dept. Molecular and Cell Biology, Univ. of California, Berkeley, CA.

The master sex-determination switch gene *Sex-lethal* (*Sxl*) was thought to control all aspects of female somatic sexual differentiation by acting on its switch-gene target, *transformer*. However, our work with a new constitutively feminizing *tra* transgene, *P{U2af-tra^F}*, which rescues *tra*$^-$ mutant females to fertility, shows that additional *Sxl* gene targets remain to be discovered in this part of the sex-determination pathway. If *tra* were the only feminizing target of *Sxl*, then *P{U2af-tra^F}* should rescue *Sxl* mutant females whose only defect is in somatic sex differentiation. While some such females are rescued to fertility by *P{U2af-tra^F}*, others are not. The latter make functional eggs and mate, but fail to ovulate. These results argue for the existence of a gene target of *Sxl* that is required for egg-laying behavior, operating in a previously unknown branch of the sex determination pathway. We refer to this as the "*tra*-insufficient feminization" (TIF) branch. Two observations made us concerned that the ovulation defect arguing for TIF might be an artifact of a failure of *P{U2af-tra^F}* to express properly in particular *Sxl* mutant backgrounds. We found that expression of *Sxl*$^+$ in neurons that sex-specifically express *fruitless* (*fru*) under the control of *tra* rescues ovulation in TIF$^-$ females, and that TIF$^-$ females have an intersexual Muscle of Lawrence (MOL) indicating sexually inappropriate expression of *fru*. We used feminizing *fru* mutant alleles and an analysis of intersexual MOL formation in females to eliminate this concern. We show that the level of *tra* insufficiency indicated by this MOL intersexuality is compatible with fertility and that *P{U2af-tra^F}* expression is not influenced by the *Sxl* genotype. Moreover, we show *Sxl* mutant females carrying a constitutively feminizing point mutation in the endogenous *tra* locus still display the TIF$^-$ defect. We conclude that *tra* and the TIF pathway target work together in *fru* neurons to control sex-specific behavior.

527B

Deciphering the functions of *Drosophila melanogaster* male accessory gland proteins using RNA interference. Kristipati Ravi Ram, Mariana F. Wolfner. Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY. 14853 USA.

Seminal proteins are important modulators of female mating behavior and physiology. In Drosophila, majority of these male derived proteins are contributed by the accessory gland. To date, 69 accessory gland proteins (Acps) that fall into different protein classes have been identified. We used RNAi interference to systematically knockdown 26 of these Acps, individually, in vivo and tested for their roles in reproduction. We found that five Acps are essential for increased egg production and receptivity in mated females. We have identified four new Acps that are required for increased egg production in female after mating. One of these Acps shows very short-term effect whereas the remaining ones are necessary for long-term effect, which is usually referred to as sperm effect and one of these is also vital for the release of sperm from storage. The findings from the present study further validate Drosophila as an excellent and rapid molecular model for elucidating the functions of conserved seminal protein classes.

528C

Kokopelli: a novel, pleiotropic cyclin required for germline stem cell maintenance. James D. Baker, Maurice J. Kernan. Dept Neurobiology and Behavior & Center for Developmental Genetics, Stony Brook University, Stony Brook, NY.

Flies lacking *kokopelli*, a conserved but uncharacterized Drosophila cyclin, have few male germline stem cells at eclosion. Mutant larvae have a near-full complement of germline stem cells, but these are lost steadily during development. *kokopelli* mutants have a reduced rate of stem cell division suggesting that the loss of stem cells may be the consequence of a mitotic defect. A rescue construct expressing YFP-tagged Kokopelli shows that the protein is expressed in stem cells, gonial cells and spermatocytes, and is weakly expressed in somatic cells. The loss of stem cells could result, nonexclusively, from defects in growth factor signaling, from a failure to initiate or complete cell division, or from a change in stem cell identity. We are working to distinguish between these possibilities; initial results implicate *kokopelli* in the TGFβ signaling pathway. In support of this connection, misexpression experiments can produce a tumorous testis phenotype, reminiscent of the loss-of-function phenotype of *bag of marbles*, a TGFβ regulated gene. *kokopelli* mutants also have defects in wing, thorax, eye and ovary development. The common features of these phenotypes will define the cellular/molecular functions of this cyclin.

529A

Direct Regulation of Germline and Somatic Stem Cell Maintenance by Jak-STAT Signaling in the Drosophila Testis. Crista Brawley, Maggie de Cuevas, Erika Matunis. Dept Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

In the Drosophila testis there are two types of stem cells, germline stem cells (GSCs) and somatic stem cells (SSCs). Both are maintained via the Jak-STAT signaling pathway, and mosaic analysis has shown that Stat92E is directly required in the GSCs to prevent differentiation. Here, we generated MARCM clones to remove Stat92E from SSCs, and found that Jak-STAT signaling is directly required in SSCs for their maintenance. Therefore, in this niche two stem cell populations directly receive one signal, and this signal ensures that each population is maintained within the niche. This finding also suggests that GSCs and SSCs can be maintained independently. Consistent with this hypothesis, SSCs can be maintained in the absence of GSCs (Gonczy and Dinardo. Development 122(8), 1996). Here, we determine what happens to GSCs when SSCs are depleted by conditionally removing Stat92E from all the cells while restoring it to the germline using the Gal4-UAS system. These experiments revealed that a wild type number of GSCs is still present even with a two-fold reduction in the pool of SSCs. These GSCs also still made oriented divisions. However, the spermatogonial zone within these testes became lost over time likely because differentiation depends on the somatic lineage. Thus, the Jak-STAT signaling pathway directly and independently maintains two different stem cell populations in one niche. Identifying the specific Stat92E targets in each stem cell lineage will reveal mechanisms of how these cells coexist within this niche.

530B

Characterization of *nmd* and its paralog in mitochondrial morphogenesis in *Drosophila* spermatogenesis. Bevin C English, Sarah D. Durnbaugh, Kara M. Koehrn, Amanda C. Aldridge, Sara H. Holmberg, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

The *no mitochondrial derivative* (*nmd*) gene is required for mitochondrial aggregation in *Drosophila* spermatogenesis. Flies homozygous for mutations in *nmd* either fail to survive past the early pupal stages or are male sterile due to improper mitochondrial aggregation. Male homozygous for nmd^{ry4} mutants are sterile; this hypomorphic mutation is due to the insertion of *P{ry11}* in the 5' UTR of *nmd*. Nmd is a member of a subfamily of the AAA ATPase superfamily that also includes spastin and katanin, proteins known to have effects on microtubule integrity and arrangement. Using TubulinTracker Green, we determined that mutations in *nmd* do not affect microtubule integrity or arrangement during different stages of spermatogenesis. In order to determine the subcellular location of Nmd, we performed immunofluorescence in both wild type and mutant testes using polyclonal antibodies we raised against an Nmd fusion protein. The antibodies associated with the nuclei in primary spermatocytes. However, the same fluorescence pattern (and a more intense band in immunoblotting experiments) was seen in homozygous nmd^{ry4} mutants. Decreasing the dosage of an *nmd* paralog had no effect on the intensity of antibody staining. To test whether there is a cryptic promoter at the end of *P{ry11}* driving expression, we used RT-PCR; preliminary results are negative. Since the antibodies may be non-specific, we are creating a *GFP-nmd* fusion that we will use to assess Nmd subcellular localization in transgenic flies. To determine the molecular function of an uncharacterized paralog of *nmd* (which is represented only among testis ESTs), we performed a chemical mutagenesis screen in the paralog's genomic region, which resulted in two allelic male sterile mutants with mitochondrial defects; further tests will determine whether these strains carry mutations in the *nmd* paralog. This work was supported by the National Science Foundation under grant 0133335 to K.G.H.

531C

Loss of Off-schedule, an eIF4G-like protein, causes arrest of meiosis and spermatid differentiation in the testis. Tina Franklin-Dumont[1], Chandrima Chatterjee[1], Steve Wasserman[2], Steve DiNardo[1]. 1) Cell & Developmental Biol, Univ Pennsylvania Sch Med, Philadelphia, PA; 2) Cell & Developmental Biol, Univ of California at San Diego, La Jolla, CA.

During spermatogenesis, cells must coordinate complex differentiation with the mitotic and meiotic cell cycles to generate functional gametes. Recently, a transcriptional regulatory circuit has been defined that controls both meiosis and differentiation in males. We have identified *off-schedule* (*ofs*) as a new gene essential for this coordinated control. In *ofs* mutants, the mitotic divisions and entry into meiosis, including the premeiotic S phase, all appear normal. Yet during the meiotic G2 phase, the mutant germ cells do not grow in size at the same rate as their wildtype counterparts, and we observe no evidence of meiotic division or significant differentiation. While phenotypically similar to the transcription regulatory mutants, *ofs* is not a part of the known circuit, as it neither controls genes of that set, nor is controlled by them. Moreover, Boule and Twine, proteins appearing just prior to Meiosis I, are still expressed in *ofs* spermatocytes but are delayed. Cyclin A protein localization is also dramatically altered due to overexpression of Rough-ex, a cyclin kinase inhibitor. These changes are all distinct from those observed in mutants affecting the transcriptional circuit and confirm that *ofs* reveals a novel pathway controlling meiosis and differentiation. *ofs* is encoded by CG10192, the C terminus of which has striking homology to the eukaryotic translation initiation factor, eIF4G. Since *ofs* is abundantly expressed in spermatocytes, while the eIF4G ortholog (CG10811) is not, we suggest that Ofs acts as the male germline eIF4G. Consistent with this hypothesis, dsRNA knockdown and subsequent rescue experiments conducted in S2R+ cells demonstrate that Ofs can indeed act redundantly with the eIF4G ortholog. The arrest phenotype in *ofs* mutants and Ofs' role in translation initiation suggest that monitoring G2 spermatocyte growth contributes to the decision of when to initiate the meiotic divisions and the elaborate differentiation program.

532A

Roles for *mitoshell* in mitochondrial aggregation and meiotic cytokinesis during *Drosophila* spermatogenesis. Karen G. Hales, Sarah E. Coffey, Sheena E. Favors, Amanda C. Aldridge. Department of Biology, Davidson College, Davidson, NC.

Mitochondrial aggregation and fusion beside the spermatid nucleus are hallmarks of early post-meiotic morphogenesis during wild type *Drosophila* spermatogenesis. Male flies homozygous for the *mitoshell* mutation are sterile and show dramatic defects in the timing and nature of mitochondrial aggregation. Mitochondria in *mitoshell* mutants prematurely aggregate in primary spermatocytes shortly before meiotic entry. Instead of gathering beside the nucleus, these mitochondria surround the spermatocyte nucleus in a shell-like configuration. Spermatocyte nuclei subsequently undergo meiotic karyokinesis within the mitochondrial shell; the four early post-meiotic nuclei remain together in the mitochondrial shell unless subjected to the pressure of a cover slip in live squashed preparation. Meiotic cytokinesis is not initiated. Microtubule staining indicates that a flagellar axoneme still forms for each post-meiotic nucleus. The mitochondria in the perinuclear shell eventually dissociate and elongate beside the axoneme; spermatids appear to contain many small elongating mitochondria, suggesting that mitochondrial fusion did not occur. We mapped the *mitoshell* mutation by deficiency to a narrow chromosomal region containing twenty-three genes, of which five are represented among testis expressed sequence tags. We will present our analysis of candidate genes. This work was supported by the National Science Foundation under grant 0133335 to K.G.H.

533B

Candidate genes for elongated sperm, a microarray approach. Sarah Kingan[1], Daniel Hartl[1], Scott Pitnick[2]. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Department of Biology, Syracuse University, Syracuse, NY.

Many species in the genus Drosophila have unusual sperm morphology including extremely long sperm and sperm heteromorphism. Investment in long or sterile sperm represents an evolutionary paradox because males of most species produce many small gametes, most of which are capable of fertilization. The genetic basis of variation in sperm length was examined using strains of *Drosophila melanogaster* that were artificially selected for longer sperm. cDNA microarrays were used to compare testes-specific patterns of gene expression in “high” versus control lines. Genes that were up or down regulation in lines with longer sperm were selected for further analysis. The molecular evolution, transcriptional regulation, and functional importance of these candidates is discussed.

534C

The D. melanogaster SUN protein Giacomo is required for male fertility and links axonemal microtubules (MTs) to the spermatid tail plasma membrane. M. Kracklauer[1], X. Chen[2], H. Wiora[1], J. Fischer[1], M. Fuller[2]. 1) Dept MCD Biol, UT Austin, Austin, TX; 2) Dept Dev Biol, Stanford U School Med, Stanford, CA.

Spermatogenesis in D. melanogaster involves 4 mitoses of a gonial blast resulting in 16 spermatogonia, followed by 4 meioses generating 64 round spermatids. These then undergo major morphological changes including nuclear condensation/elongation, formation of a flagellum with axonemal MTs, and investment of spermatids in plasma membrane (PM). The D. melanogaster gene CG6589 encodes an uncharacterized SUN protein. Many SUN proteins localize to the inner nuclear membrane (INM), where they interact in the intermembrane space with KASH proteins in the outer nuclear membrane (ONM). By RT-PCR, we showed that CG6589 is transcribed only in males. CG6589 knockout flies were generated using ends-out homologous recombination, and CG6589 males were completely sterile. Inspired by the existing male-sterile mutant casanova (csn), we call CG6589 giacomo (giac). Preliminary immunofluorescence (IF) experiments show that Giac localizes at 2 foci at round spermatid nuclei, and at basal bodies and axonemal MTs in elongating spermatids. Phase contrast microscopy showed that giac males have normal mitotic and meiotic divisions, nuclear/axonemal elongations, and membrane investment. However, 4-5 day old adult males have seminal vesicles devoid of motile sperm compared with wild-type males of the same age. In addition, elongating sperm tails show flagellar membrane swelling, suggesting that Giac links axonemal MTs to flagellar PM. These results suggest that Giac has functions other than tethering KASH proteins in developing male gametes that are important for sperm motility. Future experiments using tagged rescuing giac transgenes will verify the IF results. We also plan structure/function analyses by generating partial Giac proteins and testing for their rescuing abilities. In addition, the proteins’ subcellular localization will be investigated by IF. Finally, we will use immunoprecipitation to identify Giac-interacting proteins in developing sperm cells.

535A

Male-specific neurotransmitter transporter essential for spermiogenesis. Janet Rollins, Nabanita Chatterjee, Chris Bazinet. Biological Sciences, St. John's University, Queens, NY.

Neurotransmitter transporters play a critical role in synaptic transmission and are the targets of many psychoactive drugs. A highly conserved family of Na Cl cotransporters mediates reuptake by neurons and glial cells of neurotransmitters such as serotonin and dopamine; prokaryotic homologues are known to transport amino acids. The *D mel* gene CG7075 encodes a highly-conserved predicted member of this family. RT-PCR studies show that the gene is expressed much more highly in males than in females. Expression is limited to the testes, as previously shown by D. Bigler, although a low level of expression in the head has not been ruled out. Mobilization of a P{EPgy2} element inserted in the 3' end of the gene by the BDGP gene disruption project yields male-sterile mutants defining a single complementation group. Early stages of sperm development appear normal, but formation or unfurling of the nebenkern seems to be defective. Preliminary analysis of mutant sperm individualization using rhodamine-phalloidin in a don juan-GFP expressing line indicates normal formation and movement of the actin investment cones, although mitochondrial dynamics appear abnormal, with little or no "leading whorls" of mitochondrial material ahead of the actin cones. To our knowledge, these *ntl* (Neurotransmitter Transporter-Like) mutants represent a previously unknown phenotypic class, in which the actin dynamics of sperm individualization are dissociated from the mitochondrial dynamics.

536B

Dedifferentiation of Spermatogonia in the *Drosophila* Germline Stem Cell Niche Involves Extensive Cellular Rearrangements. Xuting Sheng, Crista Brawley, Erika Matunis. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

Cellular differentiation is commonly viewed as an irreversible process where an unspecialized progenitor cell becomes a specialized cell of a certain tissue type. However, when tissue injury occurs and the initial stem cell pool is depleted, differentiated stem cell daughters may revert back into stem cells in a process called dedifferentiation. Dedifferentiation has been observed in both the Drosophila testis and ovary, and may also be a conserved mechanism for stem cell regeneration in other systems. When germline stem cells (GSCs) in the testis are first depleted by manipulating the stem cell maintenance gene STAT92E, dedifferentiation of transit amplifying spermatogonia occurs to replace lost GSCs. Here, we have established an alternative method to induce dedifferentiation using ectopic expression of Bag-of-marbles (Bam), a differentiation gene expressed exclusively in spermatogonia, driven by the heat-shock promoter. When these flies were heat-shocked, we observed a progressive loss of GSCs without the loss of somatic stem cells (SSCs), a phenomenon that has not been observed in previous studies of spermatogonial dedifferentiation. Remarkably, SSCs occupied much of the space adjacent to the hub that GSCs normally occupied. When heat-shocked flies with lost GSCs were allowed to recover, they were able to regenerate GSCs in almost all testes observed. We found that germline cells did not need to contact the hub before recovery in order to regenerate GSCs, and that 4- and 8-cell spermatogonia displayed actin-rich protrusions during dedifferentiation. These data suggest that spermatogonia may become motile during dedifferentiation, and that cellular rearrangements are required to position newly formed GSCs in their normal niche space.

537C

A Novel Role for Integrins in Hub Cell Morphogenesis. Guy Tanentzapf[1,2], Danelle Devenport[2,3], Nicholas H. Brown[2]. 1) CSB Department, University of Toronto, 25 Harbord Street, Toronto, Ontario, M5S 3G5; 2) The Gurdon Institute and Department of Physiology, Development, & Neuroscience,University of Cambridge, Cambridge, UK, CB2 1QN; 3) Laboratory of Mammalian Genetics and Development, Rockefeller University, Box 300, 1240 York Avenue, New York NY 10021.

Stem cell self-renewal and differentiation are coordinated through interactions with the stem cell niche, a microenvironment of specialized regulatory cells that govern stem cell behaviour. Currently little is known about how stem cell niches are formed during development. The Drosophila testis contains a well-documented niche, referred to as the 'hub', which regulates stem cell fate by secreting signalling molecules and influences the orientation of stem cell divisions. Hub cells arise during late embryogenesis at the anterior tip of the gonad. We found that integrin-mediated adhesion to the extracellular matrix (ECM) that surrounds the gonad is essential for hub cell positioning. In the absence of integrins the hub cells sort to the center of the gonad and become surrounded by germ cells. Germ cell divisions were oriented perpendicular to the hub, even when it was in the center of the gonad, indicating that the hub cells have already initiated their niche activity in the embryo. Our work demonstrates a role for integrin-mediated cell adhesion in establishing the proper organization of the hub cell stem cell niche in the Drosophila testis.

538A

The link between acrosome function and sperm plasma membrane breakdown during Drosophla fertilization. Kathleen L. Wilson, Michelle K. Smith, Barbara T. Wakimoto. Dept Biol, Univ Washington, Seattle, WA.

We performed a large-scale screen of the Zuker collection to identify EMS-induced male sterile and paternal effect mutations. The largest class of mutations that affected sperm function after entry into the egg caused arrest shortly after sperm entry and before sperm nuclear decondensation. This defect in sperm activation was characteristic of mutations in five different genes. As we previously reported, the defect caused by *sneaky* (*snky*) mutations is a failure in sperm plasma membrane breakdown (PMBD), an early step which normally occurs in the egg cytoplasm. Our finding that Snky is an acrosomal membrane protein establishes a link between acrosome function and PMBD. Here we report the role of four additional genes in the sperm activation pathway. Using markers, including Snky-GFP, to track acrosome formation and fate, we show that mutations in *aghino*, *kugi*, and *popsickle* affect biogenesis and morphology of the acrosome and either reduce or eliminate the quantity of Snky protein in the acrosome of mature sperm. In contrast, *misfire* mutations have no apparent effect on acrosome morphology or Snky localization but still fail in sperm PMBD. From these data, we propose a genetic pathway for these genes during spermatogenesis and fertilization. We also confirm a non-conventional role for the acrosome, exclusive of exocytosis, in contributing to sperm membrane dynamics during fertilization.

539B

The LIM-HD gene *tailup* and *Iro-C* cooperate in *Drosophila* dorsal mesothorax specification. Joaquin de Navascues, Juan Modolell. Centro de Biología Molecular "Severo Ochoa" (UAM-CSIC). Cantoblanco, Madrid (SPAIN).

Much is known about the formation of the wing territory of the wing imaginal disc. However, our conception of the specification of the notum, the other main region of the dorsal mesothorax, is a somehow linear plot in which the EGFR signalling pathway activates the genes of the *iroquois* complex (*Iro-C*) in the proximal part of the disc, and these genes in turn instruct cells to become notum. The LIM-homeodomain gene *tailup* (*tup* = *islet*), has been categorized as a prepattern gene that antagonizes formation of sensory bristles on the notum of *Drosophila* by downregulating the expression of the proneural genes *achaete* and *scute*. Here we show that *tup* has an earlier function in the development of the imaginal wing disc, namely, the specification of the notum territory. Absence of *tup* function causes cells of this anlage to upregulate different wing-hinge genes and to lose expression of some notum genes. Consistently, these cells differentiate hinge structures or modified notum cuticle. Overexpression of *tup*, similarly to that of *araucan* (a gene member of the *Iro-C*), has a very limited capacity to induce ectopic notum-like development, but that of both genes together is most effective to promote this transformation. Also we present data suggesting that Tup do not interact with the usual LIM-HD partners for this pronotum function. While the *Iro-C* genes are activated in the notum anlage by EGFR signalling, *tup* is positively regulated by Dpp signalling. Our data support a model in which the EGFR and Dpp signalling pathways, with their respective downstream genes *Iro-C* and *tup*, converge and cooperate to commit cells to the notum developmental fate.

540C

Senz'aria, a MAGUK family adapter, is required for tracheal morphogenesis. Katherine E. Moyer, J. Roger Jacobs. Biology, McMaster University, Hamilton, ON, CA.

Membrane associated guanylate kinases (MAGUKs) are a class of scaffolding proteins that organize macromolecular complexes at cell junctions and integrate regional specialisation of the cell surface. MAGUKs are characterised by three functional protein interaction domains: a guanylate kinase, SH3 and PDZ domains. We have characterised the structure and phenotype of a new MAGUK coding gene, *CG9326*. There are two predicted proteins encoded by *CG9326*, differing in the inclusion of a N-terminal structural motif, the L27 domain. This domain is found in a subset of MAGUKs, including Lin-2, Stardust, Pals1 and PSD95beta and is thought to associate with the L27 domain of other scaffolding proteins.

Expression analysis reveals that *CG9326* transcript appears at embryonic stage 11, and continues to hatching. Expression in diverse tissues is detected in the adult. Protein is detectable from stage 13 onwards in the embryonic ectoderm. Other embryonic epithelia also express *CG9326*, in particular the hindgut and trachea. In all cell types, protein is restricted to the sub-apical membrane domain. We have generated a deletion mutant of *CG9326*, which is characterised by late embryonic lethality. The trachea fail to inflate in the embryo; we have correspondingly named the *CG9326* mutant *senz'aria* (*szar*), Italian for airless. The dorsal trunk of the uninflated trachea are enlarged, and varicose. Many secondary trunks fail to connect to the dorsal trunk. Our analysis of the role of *szar* in the differentiation of the submembrane scaffold will be presented. Supported by NSERC.

541A

Spatio-temporal coordination of epithelial cell internalization by EGFR signaling. Mayuko Nishimura[1,2], Yoshiko Inoue[1], Shigeo Hayashi[1,2]. 1) Riken CDB, Kobe, JP; 2) Kobe Univ., Grad. Sch. Sci.Tech.

Invagination of epithelial sheets is a key morphogenetic process of organogenesis . Apical constriction and cell movements has been thought to play important roles in epithelial invagination. But the exact events leading to invagination are not well known. To understand the mechanisms involved, we investigated invagination of the tracheal primordium in *Drosophila*. First, we performed detailed analyses of invagination manners such as cell shape changes by time-lapse imaging and staining embyos. Upon invagination, 10-12 cells at the dorsal position of the tracheal primordium constricted their apices and became flask-shaped to shift basally and internalized, followed by internalization of the surrounding cells. We next used the anti-double phosphorylated MAPK (dpMAPK) antibody to follow the activation of Epidermal Growth Factor Receptor (EGFR) signaling that was known to be involved in tracheal invagination. Prior to the change in cell morphology, dpMAPK was detected in a circular pattern that spread from the prospective invagination site to the periphery. While cells at the periphery in the activated circular domain accumulated dpMAPK in the nucleus and cytosol, dpMAPK was lacalized apically and excluded from the nucleus in the central cells of the domain. After internalization, the level of dpMAPK was rapidly decreased. Mutations of EGFR signaling components (*rhomboid , egfr , Dsor1*) severely inhibited dpMAPK and apical constriction. In those embryos, internalization of the tracheal primordium still occurred, but the onset of invagination was delayed and the position of invagination was often misplaced. From these results, we conclude that EGFR signaling is required for apical constriction and specifies the timing and the position to initiate tracheal invagination, which may allow orderly internalization of tracheal precursor cells having competence to invaginate.

542B

Control of epidermal differentiation. Francois Payre[1], Helene Chanut-Delalande[1,2], Isabelle Fernandes[1], Philippe Valenti[1], Severine Viala[1], Serge Plaza[1]. 1) Centre de Biologie du Developpement, Toulouse, FRANCE; 2) Biozentrum der Universitat Basel, Basel, SWITZERLAND.

It is well established that developmental programs act during embryogenesis to determine animal morphogenesis. How these developmental cues produce specific cell shape during morphogenesis, however, has remained elusive. We addressed this question by studying the morphological differentiation of the Drosophila epidermis, governed by a well-known circuit of regulators leading to a stereotyped pattern of smooth cells and cells forming actin-rich extensions (trichomes). It was shown that the transcription factor Shavenbaby plays a pivotal role in the formation of trichomes and underlies all examined cases of the evolutionary diversification of their pattern. To gain insight into the mechanisms of morphological differentiation, we sought to identify shavenbaby's downstream targets. We show here that Shavenbaby controls epidermal cell shape, through the transcriptional activation of different classes of cellular effectors, directly contributing to the organization of actin filaments, regulation of the extracellular matrix, and modification of the cuticle. Individual inactivation of shavenbaby's targets produces distinct trichome defects and only their simultaneous inactivation prevent trichome formation. Our data show that shavenbaby governs an evolutionarily conserved developmental module consisting of a set of genes collectively responsible for trichome formation, shedding new light on molecular mechanisms acting during morphogenesis and the way they can influence evolution of animal forms.

543C

Imaginal hindgut development in Drosophila. Shigeo Takashima, Marianna Mkrtchyan, Volker Hartenstein. Depertment of Molecular Cell, and Developmental Biology, UCLA, Los Angeles, CA.

During metamorphosis, the larval hindgut degenerates and is replaced by adult hindgut precursor cells. The group of adult precursor cells, called the imaginal ring, resides at the anterior end of the hindgut during larval periods. To understand the development of the adult hindgut, we focused on to the imaginal ring and studied how it grows during life periods through the embryonic development to the time of metamorphosis. Next, we searched for genes which expression are related to the imaginal ring, and found that several genes are expressed in or near the imaginal ring. Since we found some growth factors are included, we studied their involvement to the growth control of imaginal ring cells.

544A

Regulation of the Notch pathway during stem cell division in the adult fly. Allison Bardin, Francois Schweisguth. Biology, Ecole Normale Superieure, Paris, FR.

The Notch pathway plays many roles throughout development to specify cell fates. Much progress has been made on our understanding of the mechanisms controlling the Notch pathway during inductive signaling, lateral inhibition, and asymmetric cell division. However, little is understood of how the Notch pathway is regulated during tissue homeostasis. By nature, tissue homeostasis must allow for dynamic regulation in response to environmental cues. Recent work has demonstrated the existence of intestinal stem cells in the midgut of the adult fly and has shown that the Notch pathway plays an important role in the growth and differentiation of the midgut stem cells and their progeny (Ohlstein and Spradling, 2006 ; Micchelli and Perrimon, 2006). This system is ideally suited to understand how the Notch pathway is controlled during tissue homeostasis. To this end, we have analyzed which ligand is responsible for Notch activation and determined from which cell ligand signaling occurs. Additionally, we have investigated whether the E3 ubiquitin ligases Neuralized and Mindbomb1 play a role in this system. Our data indicate that activity of the Notch pathway is regulated both at the level of ligand expression and ligand activity. Our ongoing work will be presented.

545B

FoxK, a new Fork Head protein required for Dpp-dependent midgut specification. Sergio Casas-Tinto[1,2], Pedro Fernandez-Funez[1], Begona Granadino[2]. 1) Neurology, UTMB, Galveston, TX; 2) CIB, CSIC, Madrid, Spain.

Regulatory interactions between the visceral mesoderm and the endoderm are critical for midgut differentiation. Decapentaplegic (Dpp) plays a leading role in this developmental process by diffusing from the visceral mesoderm to the adjacent endoderm. Dpp initiates a complex signaling cascade that activates the key transcription factor D-fos in the endoderm. D-fos, in turn, regulates Labial expression in the endoderm, which is critical for midgut differentiation. However, D-fos overexpression does not activate ectopic Labial, indicating that D-fos plays a permissive role in Labial regulation. Since Dpp overexpression induces ectopic Labial activation in the endoderm, other factors, in addition to D-fos, might be involved in Labial regulation. We identified a novel transcription factor of the Fork Head family, FoxK, required for midgut development. As expected, FoxK expression in the midgut endoderm is under the control of dpp: FoxK expression is eliminated in dpp mutants, while dpp overexpression leads to ectopic FoxK activation. Additionally, we have identified functional Mad binding-sites in the FoxK promoter, supporting a direct control of FoxK by Dpp activity. FoxK mutant flies show reduced Labial expression in the endoderm and severe midgut disorganization. Similarly to D-fos, FoxK overexpression does not induce ectopic Labial expression. Since neither FoxK nor D-fos can direct ectopic Labial expression on their own, we assessed the possibility that the transcription factors FoxK and D-fos might cooperate during midgut development. We found that co-expression of FoxK and D-fos results in ectopic Labial expression. We propose a novel regulatory mechanism during midgut development that requires FoxK and D-fos cooperative activity to direct Dpp-dependent Labial expression in the endoderm.

546C

Dumpy interacts with a large number of proteins in the epithelial apical extracellular matrix. Ross MacIntyre, Jeff Chien, Amber Carmon. Dept Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

The complex *dumpy* gene encodes a huge protein located in the extracellular matrix (ECM) of epithelial cells underlying cuticular structures and on the inside lumens of tubes such as the trachea and the salivary gland duct. Dumpy is also found on the apical surfaces of dorsal and ventral epithelial cells in the developing wing disc. Prout *et al* (1997) and Walsh and Brown (1998) conducted screens for lethal genes, which caused wing blisters in somatic cell clones in adult flies, due to failure of dorsal and ventral epithelial cells to properly adhere in the pupal wing disc. Approximately 30 genes were identified including *dumpy*. We have carried out *in vivo* tests for dominant and recessive interactions between *dumpy* and a subset of these 30 genes. To assess interactions, we analyze *dumpy oblique* (wing size) expression in *dumpy* flies heterozygous for a wing blister mutant or exhibiting a blister in homozygous mutant cell patches. We also compare blister sizes in *dumpy* and wild type wings, and show that the two different scoring systems are highly correlated. To date, the following genes have been found to interact with Dumpy: *bubblwing, blisterwing, cassowary, 2RF, 2RL, auk, kitikete, moa, piopio, takahe, xenicid, 3RC, steamer duck,* and *struthio,* indicating Dumpy has at least several roles in the epithelial cells and the ECM.

547A

The Expression, Function, and Regulation of Cad74A in *Drosophila* Oogenesis. Jeremiah Zartman[1], Nir Yakoby[1], Chris Bristow[1], Trudi Schupbach[2], Stanislav Shvartsman[1]. 1) Lewis Sigler Institute and Dept Chemical Engineering, Princeton Univ, Princeton, NJ; 2) Howard Hughes Medical Institute and Department of Molecular Biology, Princeton Univ, Princeton, NJ.

During *Drosophila* oogenesis, a two-dimensional follicular epithelium gives rise to an elaborate three-dimensional eggshell (Berg, 2005). Eggshell morphogenesis critically depends on the patterning of the follicle cells, but the connection between signaling pathways, pattern formation, and eggshell morphogenesis is poorly understood. Recently we have identified the non-classical cadherin, Cad74A, as a key molecule that bridges epithelial patterning and morphogenesis in this system. Starting in mid-oogenesis, *cad74A* is expressed in all the columnar cells except for two dorsolateral patches, in the border cells, and later in the future operculum domain. Using several mutant backgrounds and related fly species, we show that this pattern is correlated with the formation of multiple structural features of the eggshell, suggesting that Cad74a plays an important functional role on oogenesis. To test this hypothesis, we performed a phenotypic analysis of a Cad74A mutant. We found that the egg laying and hatching rate of eggs from Cad74A homozygous females are significantly reduced. In addition, females homozygous for the disrupted allele of *cad74A* exhibit severe defects in late egg development. Furthermore, we show that Cad74A is required for maintaining the epithelial integrity of the egg chamber and prevents premature apoptosis in late oogenesis. Finally, the data strongly suggest that the dynamic pattern of *cad74A* expression is partially controlled by the EGFR and Dpp signaling pathways, two of the key regulators of follicle cell patterning. On the basis of these results, we propose that Cad74A provides an important link between signaling, pattern formation and morphogenesis during egg development.

Berg, C. A. (2005). The *Drosophila* shell game: patterning genes and morphological change. *Trends in Genetics* 21, 346-355.

548B

Examining the Requirement for Rac during Adult Myogenesis. Krishan Badrinath, Allison Siebert, Joyce Fernandes. Dept Zoology, Miami Univ, Oxford, OH.

The Indirect Flight Muscles (IFMs) of *Drosophila* are among several muscle groups that are adult-specific, and develop during pupation. They are divided into two sets, the Dorsal Lateral Muscles (DLMs), and the Dorso-ventral Muscles (DVMs). During the formation of these muscles, myoblasts derived from the wing imaginal discs proliferate, segregate around, and fuse with founder cells (FCs, for DVMs) or persistent larval muscles (for DLMs), to give rise to the requisite number of muscle fibers.

Innervation is known to regulate several aspects of adult myogenesis. This includes myoblast proliferation and expression of the protein, Dumbfounded (Duf). Duf is an Immunoglobulin domain-containing transmembrane protein expressed in founder cells that acts as a myoblast attractant and is required for fusion. A similar molecule, Sticks and stones (Sns), another member of the Immunoglobulin superfamily is expressed in myoblasts and is also required for fusion. It is postulated that downstream signaling from Duf and Sns acts through the GTPase, Rac, to influence the actin cytoskeleton, thus facilitating fusion.

We sought to examine the specific roles of Rac in myoblasts and founder cells during IFM myogenesis. The dominant negative $Rac1^{N17}$ protein was targeted to either myoblasts or FCs prior to, and during fusion. Our results indicate that $Rac1^{N17}$ expression in myoblasts during fusion has no adverse affect on IFM formation, while its expression in founder cells results in missing or reduced muscles. However, when $Rac1^{N17}$ is expressed in myoblasts prior to fusion, DVM fibers fail to form, and DLMs are severely reduced in number and size. This suggests that Rac has a role to play in myoblasts prior to fusion. This may involve proper cellular localization of proteins that are needed for the reception of signals from the FC, or changes in the cytoskeleton that occur as a response to such signals. Further work will seek to investigate these questions.

549C

Regulation of Myoblast Proliferation during Adult Myogenesis in *Drosophila*. Krishan Badrinath, Michael Gottlieb, Meatal Patel, Joyce Fernandes. Dept Zoology, Miami Univ, Oxford, OH.

Myogenesis in *Drosophila* occurs twice, first during embryogenesis, and again during metamorphosis, when adult-specific muscles are formed. During embryonic myogenesis, each muscle is prefigured by a founder cell (FC), to which myoblasts fuse. FCs express the Immunoglobulin domain-containing transmembrane protein, Dumbfounded (Duf), which is a myoblast attractant and is required for fusion.

During adult myogenesis, Duf expressing FCs prefigure a number of muscles, including some abdominal muscles, and the thoracic Dorso-ventral Muscles (DVMs), which are part of the Indirect Flight Muscles (IFMs). Motor innervation has prominent functions during adult myogenesis, and regulates DVM myogenesis in two ways: It is required for maintaining the size of the myoblast pool through the regulation of proliferation, and also for the maintenance of Duf expression in the FCs.

We have investigated the relationship between the FCs and the size of the myoblast pool. Ablation of the FCs by the expression of Reaper leads to a reduction in myoblast proliferation that mimics the effect of denervation. Blocking fusion by the expression of the dominant negative $Rac1^{N17}$ leads to an increase in proliferation. This suggests that the FCs can regulate the size of the myoblast pool during IFM myogenesis.

Another aspect of myogenesis is the relationship between the size of the myoblast pool and muscle size. We have examined this by targeting the cdk inhibitor, p21, and the constitutively active Ras^{V12} to myoblasts. p21 is known to inhibit mitosis, while Ras^{V12} promotes proliferation. Our results show that p21 expression results in DVMs with fewer nuclei, while Ras^{V12} expression results in an increase in nuclei, without any significant change in muscle size. Further work will investigate the mechanisms whereby the myoblast pool, the FCs, and the motor neuron interact to determine the final IFM profile.

550A

Pox meso, a paired-box transcription factor, is required for development of the ventral somatic musculature. Seth A. Brodie[1], Jonathan K. Kassel[1], Marc S. Halfon[1,2,3]. 1) Department of Biochemistry, State University of New York at Buffalo, Buffalo, NY; 2) Center of Excellence in Bioinformatics and the Life Sciences, State University of New York at Buffalo, Buffalo, NY; 3) Molecular and Cellular Biology Department, Roswell Park Cancer Institute, Buffalo NY.

Paired-box transcription factors act as regulators of development in numerous tissue types in many species. The mammalian genes *Pax1* and *Pax9* are both required for the formation of mesodermal derivatives, in particular the sclerotome. The *Drosophila* gene *Poxm (Pox meso)* is orthologous both to *Pax1* and *Pax9*. We had previously proposed that *Poxm* functions as an important tissue-specific selector gene for ventral muscle development. To determine its specific role, we created a *Poxm* null mutant by homologous recombination and are currently assessing the specific phenotypes associated with this mutation. Our initial results indicate that the ventral musculature is grossly disrupted in these embryos. However, the dorsal mesoderm appears normal. Conversely, ectopic expression of *Poxm* in the dorsal mesoderm causes extensive patterning defects in the dorsal musculature, but overexpression in the ventral mesoderm has little effect. We are currently conducting a finer-scale analysis using markers for specific muscle precursors and other mesodermal derivatives to more fully define the loss-of-function phenotypes. We have also conducted a genome-wide screen for putative transcriptional targets of *Poxm* by computational methods using a position weight matrix determined from an *in vitro* SELEX assay. Our plan is to combine these results with those from microarray-based transcriptional profiling of null mutant embryos in order to pinpoint genes directly regulated by *Poxm*. Concurrently, we are investigating how *Poxm* itself is regulated and have identified sequences 5' to the gene that drive early *Poxm* expression. Collectively, these studies will lead to a comprehensive understanding of the roles and regulation of *Poxm* in ventral mesoderm development.

551B

Founder cells regulate muscle pattern but not fiber formation during adult myogenesis in Drosophila. Joyce Fernandes, Badrinath Krishan. Zoology Dept, Miami Univ, Oxford, OH.

During insect myogenesis, myoblasts are organized into a prepattern by specialized organizer cells. The dorso-ventral muscles (DVMs) of Drosophila, are prefigured by large imaginal pioneer cells, a feature shared with grasshopper myogenesis. They express the embryonic founder cell marker, Dumbfounded, which is important for fusion. Founder cells are known to regulate muscle pattern, myoblast fusion and properties of the mature muscle. The role of DVM imaginal pioneers/founder cells in these processes has not been tested. Using the UAS/Gal4 system of targeted transgene expression, Duf-expressing cells were manipulated through ablation or by increasing their number, and the subsequent effects on myogenesis were observed using markers for myoblasts (Erect Wing), founder cells (22C10) and muscle (β-3 tubulin). Targeted expression of the cell death gene reaper resulted in the ablation of founder cells. This was followed by the recruitment of additional cells from the myoblast pool, which were capable of initiating fusion and fiber formation. The resulting alterations in fiber number resemble phenotypes previously observed upon laser ablation of larval scaffolds that give rise to a related muscle group, the Dorsal Longitudinal Muscle (DLM). Targeted expression of constitutively active rasV12 in the putative DVM founder cells resulted in the appearance of supernumerary cells, which were capable of seeding muscle fibers. Taken together, these data suggest that DVM myoblasts are capable of organizing into fibers, and that the founder cells are necessary to generate the correct number of fibers, but not for fiber formation itself.

552C

Hemangioblast Differentiation: Asymmetric Division Versus Localized Signaling. Melina Grigorian, Lolitika Mandal, Volker Hartenstein. MCDB, Univ. California, Los Angeles, Los Angeles, CA.

Since the discovery of a common progenitor for Drosophila blood cells and blood vessel, called hemangioblast (Mandal, 2004), questions have arisen about the mode of differentiation that allows the development of the two distinct cell types. The two theories that are the most probable for the demarcation of vascular cells from blood cells from a single hemangioblast include asymmetric cell division or the presence of an asymmetrically distributed signal. Our data support the second possibility. The blood cell phenotype depends on the activation of the Notch pathway. The signal responsible for Notch activation at the relevant stage is Delta (Dl), which is specifically localized in cells flanking the blood precursors. Currently, we are studying the different signaling pathways that are responsible for the spatially restricted expression of Dl. Candidate pathways include the EGFR, FGF and DPP signaling pathway. Given that Notch activation appears also to be a pivotal mechanism in activating blood progenitor fate from among hemangioblasts in vertebrate embryos, our findings will potentially shed light on early hematopoiesis in vertebrates.

553A

Investigation of the cellular dynamics of *Drosophila* myogenesis using primary cell culture. Thomas J. Metzger[1,2], Mary K. Baylies[1]. 1) Dept Development, Sloan-Kettering Inst, New York, NY; 2) BCMB program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY.

Muscles are syncytial, that is, each muscle fiber is a single cell containing multiple nuclei. In *Drosophila*, a muscle develops from the fusion of a Founder cell (FC) to multiple Fusion competent myoblasts (FCMs). Each muscle in the embryo fuses a reproducible number of times. Although much is known in this system about specification of individual muscle identities, the molecular mechanisms underlying the execution of those identities, in particular, the generation of specific sizes and shapes, remain unclear.

To further understand the cellular processes underlying myoblast fusion and muscle size, we examined the behavior of individual myoblasts in primary culture from dissociated embryos. We are able to mark all myoblasts and particularly, specific founder cells with different sets of fluorescent markers. By culturing these cells we can examine the fusion process, from a single myoblast into a syncytium. This approach eliminates interference from surrounding cells, allowing us to probe protein localization and redistribution, membrane dynamics and other sub-cellular processes in vivo at high resolution. Currently our efforts are focused on characterizing the system: we have determined the localization of the “fusion machinery” within these cells, and are examining the role of the cytoskeleton during fusion. We will test hypotheses about the role of the both the actin and microtubule cytoskeletons during myoblast fusion, and examine their relationship to vesicle trafficking during the fusion process. In addition, we will begin to examine populations of mutant myoblasts in this system, and plan to examine myoblast fusion in a mixed population of mutant and wild type cells.

554B

***Drosophila* GATA, Friend of GATA and Runx factors regulate *lozenge* expression in crystal cells.** Selen Muratoglu, R. Barry Hough, Soe T. Mon, Nancy Fossett. Center for Vascular and Inflammatory Diseases, Department of Pathology, University of Maryland, Baltimore, MD.

The *Drosophila* Runx1 homolog, Lozenge (Lz) is a DNA binding transcription factor, essential for cell fate determination in early hematopoiesis. The protein and its biological function are well conserved across taxonomic groups, ranging from flies to humans. Together with GATA and Friend of GATA (FOG) proteins, Lz regulates crystal cell lineage commitment and differentiation. Serpent (Srp), the *Drosophila* GATA homolog, is expressed in hematopoietic precursors and is required for *lz* expression in crystal cells. While *lz* expression in crystal cells and the requirement of Lz function in crystal cell development is well documented, the mechanisms underlying this process are not well understood. To better understand the temporal and spatial expression of *lz*, we examined the *cis*- and *trans*-regulation of the gene. Our analyses identified a minimal *cis*-regulatory module (CRM), which is essential for crystal cell expression. The CRM is located upstream of the transcription initiation site and directs both embryonic and larval hematopoietic expression. Mutational and genetic analyses of the CRM indicate that Lz and Srp directly activate *lz* expression in the crystal cell lineage. In addition, CRM activation is blocked by the co-expression of the canonical GATA factor, SrpNC, together with the *Drosophila* FOG protein, U-shaped. Our findings provide new insights into the *in vivo* regulation of *lz* during hematopoiesis. Considering that GATA-1, FOG-1 and Runx1 genes are co-expressed in mammalian blood lineages and they function in differentiation and development of the blood cell types, our findings may have implications for the regulation of Runx1 hematopoietic expression and its role in leukemia.

555C

Defective Dpp signaling results in heart overgrowth and reduced cardiac output in Drosophila. Stuart Newfeld, Aaron Johnson. Sch Life Sci, Arizona State Univ, Tempe, AZ.

During germ band extension Decapentaplegic (Dpp) signals from the dorsal ectoderm to maintain Tinman (Tin) expression in the underlying mesoderm. This signal specifies the cardiac field and homologous genes (BMP2/4 and Nkx2.5) perform this function in mammals. We showed previously that a second Dpp signal from the dorsal ectoderm restricts the number of pericardial cells expressing the transcription factor Zfh1. Here we report that, via Zfh1, the second Dpp signal restricts the number of Odd-skipped-expressing and the number of Tin-expressing pericardial cells. Dpp also represses Tin expression independent of Zfh1, implicating a feed forward mechanism in the regulation of Tin pericardial cell number. In the adjacent dorsal muscle Dpp has the opposite effect. Dpp maintains Kruppel and Even-skipped expression required for muscle development. Our data shows that Dpp refines the cardiac field by limiting the number of pericardial cells. This maintains the boundary between pericardial and dorsal muscle cells and defines the size of the heart. In the absence of the second Dpp signal, pericardial cells overgrow and this significantly reduces larval cardiac output. Our study suggests the existence of a second round of BMP signaling in mammalian heart development and that perhaps defects in this signal play a role in congenital heart defects and heart disease.

556A

Regulation of the transcription factor Hand in the visceral mesoderm. Dmitry Popichenko[1], Julia Sellin[1], Marek Bartkuhn[2], Achim Paululat[1]. 1) Dept. of Zoology, University of Osnabrueck, D-49069 Osnabrueck, Germany; 2) Institute for Genetics, Justus-Liebig-University of Gieβen, D-35390 Gieβen, Germany.

We have identified regulatory regions in the bHLH transcription factor gene hand gene that are essential for the orchestrated expression in the visceral mesoderm during embryogenesis. A 300 bp element, which is highly conserved between different Drosophila species turned out to be necessary and sufficient to activate hand expression in visceral cells. The enhancer region harbors several putative binding sites for transcriptional regulators. Among them, we identified the FoxF transcription factor Biniou as being a positive regulator of hand expression in this tissue.

557B

Fusion competent myoblasts require Loner activity for myoblast fusion. Kate M. Rochlin[1], David Soffar[2], Mary K. Baylies[2]. 1) Weill Graduate School of Medical Sciences at Cornell University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Myoblast fusion is essential for the formation of skeletal muscle. In *Drosophila*, each muscle is seeded by a special myoblast, the founder cell (FC), which contains all the information to direct the formation of a specific muscle with a particular size, shape and orientation. Founder cells then fuse to surrounding naïve fusion competent myoblasts (FCMs) to achieve a particular muscle size. The *loner* gene is required for myoblast fusion and encodes a guanine nucleotide exchange factor (GEF) for Arf6, a member of the Ras superfamily of small GTPases. Arf6 is thought to regulate endosomal- membrane traffic and structural organization at the cell surface. Arf6 activity also leads to the membrane localization of Rac, another small GTPase important in cytoskeletal dynamics, during myoblast fusion.

Loner was previously reported to be present only in founder cells. However, our data from a yeast two-hybrid analysis performed with cytoplasmic domains of Hibris and Sticks and Stones, two adhesion proteins exclusively localized to FCMs and critical for the FC-FCM recognition and adhesion, led us to question whether Loner activity was solely required in FCs. Using immunofluoresence we find that Loner is not asymmetrically distributed into founder cells, but is present in both founder cells and fusion competent myoblasts. Moreover, we have demonstrated that overexpression of dominant negative forms of Loner only in FCMs leads to a disruption in fusion. Reduction in Loner function in FCMs also leads to muscle attachment defects, suggesting an additional role for Loner during muscle maturation. Taken together, it appears that Loner is required both in FC and FCMs during multiple steps for fusion and maturation to occur. Future work will address the mechanism of Loner function in FCMs during myoblast fusion and in muscle morphogenesis.

558C

The Gli-like transcription factor Lame Duck is essential for correct cell fate decisions in the dorsal mesoderm of *Drosophila melanogaster*. Julia Sellin, Maik Drechsler, Achim Paululat. Zoology Department, University of Osnabrueck, Osnabrueck, Germany.

Lame Duck (lmd) is a Gli-like transcription factor expressed in fusion competent myoblasts (fcm) in the *Drosophila* embryo. Lmd loss of function leads to a severe muscle fusion phenotype which is well described and results from the disability of fcm's to specify in the absence of *lmd* function.

The observation of a yet undescribed heart phenotype in *lmd* mutant embryos led us to the conclusion that fcm's are not just lost when unspecified, but are recruited by other mesodermal tissues. Examples are an increased number of pericardial cells and adult muscle precursors, though both cell types never express *lmd* in the wildtype and therefore are unlikely to accumulate through overproliferation in *lmd* mutants.

Since *lmd* is expressed in the somatic mesoderm only after stage 11, these findings seem to indicate an elasticity of certain mesodermal derivatives after this stage, which is further substantiated by gain of function studies we conducted with UAS-lmd and UAS-zfh1.

559A

N-linked glycosylation requirements for SNS-Duf mediated cell adhesion. Claude Shelton IV[1,2], Sandra Berger[3], Susan Abmayr[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS; 3) Institut National Agronomique Paris-Grignon, France.

Formation of the somatic musculature in the *Drosophila* embryo is dependent on interaction between SNS expressing fusion-competent cells and Duf expressing Founder cells. Previous studies show that S2 cells can be used as a model system for interactions directed by these cell adhesion molecules. We are now using this system to address the importance of N-linked glycosylation in SNS and Duf mediated cell interactions. Both are modified by N-linked glycosylation when expressed in S2 cells, with 15 predicted sites in SNS and 6 in Duf. Cell aggregation does not occur in the presence of tunicamycin, indicating that N-linked glycosylation of SNS, Duf, or both is essential. We note that elimination of N-linked glycosylation by site-directed mutagenesis has no impact on the normal membrane localization of SNS or Duf, in contrast to the effect of glycosylation on the SNS ortholog Nephrin (Yan et al, J Am Soc Nephrol, 2002). SNS proteins in which combinations of the predicted sites are mutated show reduced levels of glycosylation, and exhibit a proportional reduction in their ability to direct heterotypic interaction with Duf expressing cells. No heterotypic cell interaction is observed in the absence of SNS glycosylation. Interestingly, however, elimination of Duf glycosylation by similar site-directed mutagenesis has no effect on heterotypic cell interaction, but abolishes its ability to direct homotypic interaction with other mutant Duf expressing cells. Thus glycosylation of SNS, but not Duf is essential for heterotypic cell interaction in S2 cells. And yet, in this same system, Duf glycosylation is necessary for homotypic cell interaction. Intriguingly, as in S2 cells, SNS is glycosylated in the embryo, and the absence of glycosylation has no impact on SNS localization to the myoblast cell membrane. Experiments to examine whether glycosylation deficient SNS or Duf can function in embryonic myoblasts are ongoing.

560B

Organogenesis of the wing circulatory organs in *Drosophila*. Markus Tögel[1,2], Günther Pass[2], Achim Paululat[1]. 1) Department of Zoology, University of Osnabrueck, 49069 Osnabrueck, Germany; 2) Department of Evolutionary Biology, University of Vienna, 1190 Vienna, Austria.

In insects, circulation is mainly achieved by the contractions of the tubular heart (dorsal vessel). However, additional accessory autonomous pumps maintain the hemolymph supply of the long body appendages such as antennae, wings, legs, and cerci. In *Drosophila*, the wing circulatory organs (WCO) consist of two muscular diaphragms which are situated bilaterally in the scutellum. They suck hemolymph out of the posterior wing veins by phases of rhythmic contractions which are independent from that of the heart. Until now, the origin and development of these additional circulatory organs was completely unknown. We have therefore investigated the organogenesis by live cell imaging techniques utilizing the first available transgenic reporter fly line that mediates GFP expression in the cells of the WCO throughout development. With the help of this reporter we were able to identify four pairs of cells as the embryonic precursors, which arise in a segmental pattern and come to lie in front of the heart. The precursors start to proliferate during the larval stages and undergo two subsequent relocation events during metamorphosis, one in the prepupa and one in the pupa, which finally result in their lateral location in the scutellum. In initial experiments we have also identified factors that play a role in WCO formation during development.

561C

Identifying the mechanisms of fusion and morphogenesis during myogenesis of *Drosophila melanogaster*. Mu Xu, Mary Baylies. Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Larval muscle development of *Drosophila melanogaster* involves a series of fusion events and subsequent morphogenic processes, during which founder cells (FCs) fuse with fusion competent myoblasts (FCMs) multiple times to achieve the final muscle structure. Each muscle has a unique number of nuclei depending on its muscle identity. Although the prerequisites for fusion, such as myoblast identify and fusion machinery, have been intensively studied, the intrinsic mechanisms governing the number of fusion events and the subsequent morphogenic processes remain remain elusive. Using an EMS mutagenesis screen, we seek to identify components involved in muscle cell fusion and morphogenic processes. To facilitate our screening process, we have employed live visualization of muscles. We have generated transgenic flies that express fluorescence markers in the nuclei of a subset of muscles. Of 2,000 lines screened so far, we have identified 38 mutants with aberrant nuclei numbers and/or muscle morphology, including several mutants with decreased nuclei number and one mutant with increased nuclei number. We have also used deficiency mapping to pinpoint the location of one mutant with distinctive morphology defect, *swoosh* to a small interval on the left arm of the third chromosome. Preliminary data suggest that *swoosh* has a general muscle defects that affect all body wall muscles. We are currently in the process of further mapping the mutation to a smaller interval and eventually identifying the corresponding gene.

562A

The Mevalonate Pathway Controls Heart Formation in Drosophila by Isoprenylation of Gγ1. Peng Yi[1], Zhe Han[2], Xiumin Li[1], Eric Olson[1]. 1) Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept. of Internal Medicine, Dept. of Cell and Developmental Biology, Univ Michigan, Ann Arbor, MI.

The early morphogenetic mechanisms involved in heart formation are evolutionarily conserved. A screen for genes that control Drosophila heart development revealed a cardiac defect in which pericardial and cardial cells dissociate, which causes loss of cardiac function and embryonic lethality. This phenotype resulted from mutations in the genes encoding HMG-CoA reductase, downstream enzymes in the mevalonate pathway, and G protein Gγ1, which is geranylgeranylated, thus representing an end point of isoprenoid biosynthesis. Our findings reveal a cardial cell-autonomous requirement of Gγ1 geranylgeranylation for heart formation and suggest the involvement of the mevalonate pathway in congenital heart disease.

563B

The role of Nedd4 family genes in Drosophila melanogaster. Tanveer Akbar, Ann Marie Carbery, Yvonne Hung, Martin Baron. Faculty Life Sci, Univ Manchester, Manchester, GB.

Nedd4 family proteins are ubiquitin ligases, with a characteristic structure consisting of a C2 domain, three to four WW domains and a HECT domain. There are three members of the Nedd4 family in Drosophila melanogaster called DNedd4, Su(dx) and Dsmurf. These genes are evolutionary conserved from insects to human. Studies in the Drosophila wing have shown that Su(dx) and DNedd4 behave as negative regulators of Notch signalling. Su(dx) and Dsmurf have a redundant role in Drosophila wing, eye and leg development. We have found that Su(dx) has a role in Drosophila ovary development and egg production. Contrary to the other tissues, in Drosophila ovary, Su(dx) has an antagonistic role to that of Dsmurf. We are characterising DNedd4 mutants and aim to see the effect of all the members of Nedd4 family genes in the development of Drosophila ovary.

564C

Functional analysis of the Iroquois complex genes. Esther González-Pérez[1], Natalia Barrios[1], Annalisa Letizia[1,2], Sonsoles Campuzano[1]. 1) Centro Biología Molecular SO , CSIC-UAM, Madrid, Spain; 2) Instituto Biología Molecular, CSIC, Barcelona, Spain.

The *Iroquois* complex (Iro-C) of *Drosophila* contains three genes, *araucan* (*ara*), *caupolican* (*caup*) and *mirror* (*mirr*), which encode highly related homeodomain-containing proteins (reviewed in Cavodeassi, Modolell and Gómez-Skarmeta (2001) *Development* 128, 2847-28551). In addition, these genes share *cis*-regulatory elements that drive their expression in overlapping though distinct patterns. These genes are thought to act redundantly in several developmental contexts such as notum specification and dorso/ventral compartimentalization of the eye disc. However, since all existing *ara* and *caup* mutants are associated to chromosomal inversions or deficiencies affecting regulatory sequences common to both genes, this prevents a thorough analysis of their functional redundancy. To address this question we have generated, by FLP-FRT mediated recombination, novel Iro-C mutations. Analysis of a null allele of *caup*, associated to a small deletion that does not modify *ara* expression, shows a crucial role of Caup in eye and maxillary palp development. The putative redundant nature of Ara, Caup and Mirr is further being analyzed by means of rescue and overexpression experiments. The Iro-C harbours in addition two apparently non-coding transcription units, *lincoyan* (*linc*) and *quilapan*. Interestingly, these genes are transcribed from regions of the Iro-C genomic DNA containing wing disc-specific enhancers (Letizia, A., Barrio, R and S. Campuzano, *Development*, submitted). Thus, the possibility exists that transcription of these non-coding genes may affect the activity of the Iro-C enhancers located in the region. However, as far as *linc* is concerned, its deletion has no effect on the expression of the Iro-C genes.

565A

The Liprin-α binding site, but not LAR phosphatase activity, is essential for LAR mediated R7 photoreceptor targeting. Kerstin D Hofmeyer[1], Corinne Maurel-Zaffran[2], Jessica E Treisman[1]. 1) Skirball Institute, NYU Medical Center, New York, NY; 2) Institut de Biologie du Developpment de Marseille, Marseille.

Photoreceptor (PR) axons of the *Drosophila* eye project to different target regions in the adult brain. R1-R6 axons terminate in the lamina, while R7 and R8 grow into the medulla, where they separate into a distal layer (R8) and a proximal layer (R7) of termini. Mutations in the *LAR* and *Liprin-α* genes show an identical mistargeting of the R7 axon to the R8 layer. In vertebrates and *Drosophila*, the Liprin-α protein binds to the catalytically inactive D2 tyrosine phosphatase (PTP) domain of LAR. We have found this domain to be required for LAR function in R7 targeting. In COS cells, Liprin-α is required to localize human LAR to focal adhesions. Although LAR and Liprin-α co-localize in PR growth cones as well as at focal adhesions in S2R+ cells, we found that LAR transport to growth cones or localization in S2R+ cells is independent of Liprin-α. Thus, Liprin-α is likely to act downstream of LAR, which is supported by our finding that overexpression of Liprin-α can partially rescue R7 targeting in the absence of LAR. Liprin-α is tyrosine phosphorylated and therefore a potential substrate for LAR. In order to understand the role Liprin-α might play downstream of LAR, we tested whether LAR acts as a phosphatase in R7 targeting. A mutant form of LAR with reduced in vitro phosphatase activity was still able to rescue R7 targeting in *LAR* null mutants. We are currently investigating whether LAR-phosphatase activity is truly dispensable, or if it is substituted by a redundant RPTP, such as PTP69D. Similar R7 mistargeting has been reported for *PTP69D* mutants. PTP69D is able to act on the appropriate substrates, since a LAR-PTP69D chimeric protein containing the intracellular domain of PTP69D is able to rescue R7 targeting in *LAR* null mutants. We show that LAR and PTP69D co-immunoprecipitate from S2R+ cells, suggesting that these proteins might form a complex in which either intracellular domain can transmit the signal.

566B

A screen for dominant enhancers of a *trio* mutant phenotype. Eric Liebl[1], Colan Baldyga[1], Lindsay Bickel[1], Kathryn Dean[1], Morgan Kopeke[1], Rohan Manohar[1], Julianne McCall[1], Jenna McCroskey[1], Jessica Smith[1], Mark Seeger[2]. 1) Dept Biol, Denison Univ, Granville, OH; 2) Department of Molecular Genetics and Center for Molecular Neurobiology, The Ohio State University, Columbus, OH.

Trio is a GEF involved in axon pathfinding. The allelic combination of a mis-sense allele within trio's GEF-1 domain, $trio^{M89}$, and a P-insertion allele of *trio*, $trio^{P0368/10}$, yields ~80% of the expected viable adults. Our previous work had shown that either heterozygous mutations in *abl*, a gene encoding a cytoplasmic tyrosine kinase, or heterozygous mutations in *failed axon connections* (*fax*) could dominantly enhance this *trio* mutant phenotype[a]. We have therefore undertaken a screen to identify additional dominant enhancers of this *trio* semi-lethality phenotype on both the second and third chromosomes. To date we have isolated thirteen independent dominant enhancers. None of these appear to be new *fax* alleles. At least one of these appears to be a new null allele of *abl* and another appears to be a new null allele of *trio*. One of the enahncers has been mapped to the second chromosome. Our continued progress towards the molecular and phenotypic characterization of these dominant enhancers of the *trio* mutant phenotype will be presented. a. Liebl et al., Neuron 26:107-118 (2000).

567C

Collapsin response mediator protein and dihydropyrimidinase are functionally divergent, alternatively splice products of the *crmp* gene. Deanna Morris, John Rawls. Department of Biology, University of Kentucky, Lexington, KY.

The CRMP protein has been shown to mediate growth cone dynamics in cultured mammalian neurons and biochemical studies have shown associations with a variety of signal transduction components and cytoskeletal elements. Interestingly, CRMP is a member of a protein family including the presumably ancestral DHP protein that carries out the second step in pyrimidine degradation. To better understand the entirely divergent roles of these very similar proteins, we have carried out studies to resolve their relationship and the role of CRMP in neurogenesis. In *Drosophila*, CRMP and DHP proteins are produced by alternatively spliced transcripts of the *crmp* gene and are 91% identical, suggesting that the distinct functions of the two proteins are derived from differences in a small region of the protein. Experiments with GFP-fusion transgene constructs show that CRMP RNA is found exclusively in neuronal tissues, whereas DHP is ubiquitously expressed in non-neuronal tissues. Loss-of-function mutations of *crmp* that lack both proteins have been isolated. Homozygous animals display DHP-null phenotypes but exhibit no overt developmental or neurological defects. Mis-expression of CRMP results in specific developmental blocks as well as morphological abnormalities. We have isolated genetic suppressors of those defects and subsequent analyses are underway to identify *crmp*-interactive genes.

568A

Functional analysis of Golden goal, a novel transmembrane protein involved in photoreceptor axon guidance. Tatiana Tomasi, Satoko Hakeda-Suzuki, Stephan Ohler, Takashi Suzuki. Max-Planck-Institute of Neurobiology, Martinsried, Germany.

In the *Drosophila* visual system, the eight photoreceptor (R) axons target specific layers of the optic lobe. The axons of R1-R6 terminate in the lamina, while R7 and R8 axons pass through the lamina to terminate in two separate layers of the medulla. Using the FLP/FRT system, a large-scale mutagenesis screen was performed to identify regulators of axon guidance in the visual system. Mutagenized mosaic larvae were analyzed for visual system connectivity defects (Newsome et al., *2000*). In the screen, 3 alleles of a novel gene, named *gogo* (***go****lden* ***go****al*) were isolated (T.S., J. Berger, and B. J. Dickson, unpublished data). *gogo* encodes a novel transmembrane protein with two conserved extracellular domains, a TSP (Thromospondin) and a CUB (Complement subcomponents Clr/Cls, Uegf, Bmpl) domain, which both have been shown to be present in receptors involved in axon guidance. We set out for a detailed analysis of *gogo*'s function. Eye-specific mosaic larvae of *gogo* show an abnormal overall structure with clumpings of axons bundles and the presence of gaps in the lamina and the medulla. In the adult fly pathfinding of R8s is disrupted, as neighboring R8s cross each other. In addition, target recognition is affected, as R8s inappropriately target the R7 layer. *gogo* is expressed in the R neurons as well as in some of the medulla neurons. For the R neurons, we could show by *in situ* staining of *gogo* mRNA and by promoter bashing, that *gogo* seems to be specifically expressed in R8 neurons. Consistent with this, Gogo antibody staining shows that during larval development the protein is localized to R8 axons and is enriched in their growth cones. Trans-heterozygous animals can be rescued by expressing Gogo under the control of the eye specific GMR promoter. This indicates that Gogo function is only required in the R neurons and not in the target cells in the brain. We are currently analyzing Gogo's function in terms of cell-cell communication among R axons. Results of the further studies assessing the functional role of Gogo in the R axon guidance will be presented.

569B

PRECISE CONTROL OF *FASCICLIN2* EXPRESSION IS REQUIRED FOR THE ADULT MUSHROOM BODY DEVELOPMENT IN *DROSOPHILA*. Hidenobu Tsujimura, Kazuma Fushima. Dept Developmental Biol, Tokyo Univ Agric & Technology, Tokyo, JP.

fas2 is a cell adhesion molecule that work in axonal pathfinding, fasciclation and divergence in the *Drosophila* nervous system. Here, we examined how precisely its expression must be controlled for the development of adult mushroom body (MB), a center for olfactory learning in the brain. *fas2* expression is controlled rigidly in the development of MB. Among three types of MB neurons, γ, α'/β' and α/β, born sequentially from the same four MB neuroblasts, *fas2* expression is restricted only to γ and α/β neurons. α'/β' neurons never express *fas2*. Results showed that suppression of *fas2* expression in α'/β' neurons was essential for the formation of divergent pattern of adult α'/β' and α/β lobes. When *fas2* was mis-expressed in the developing mushroom body neurons, there occurred loss or reduction of adult α'/β' lobes and concurrent misdirection of α/β lobes, even when no anatomical defects occurred in the larval MB lobes. Ectopic expression of *fas2* in α'/β' neurons was essential for these defects because no defects were caused when *fas2* was expressed only in γ and α/β neurons. Results also suggested pioneering role of α'/β' lobes in the pathfiding of α/β axons.

570C

Regulation of Thoracic Neuroblast Proliferation by Bnl, Hh, and Trol in the Drosophila CNS. Jonathan Lindner, Paul Hillman, Youngji Park, Sumana Datta. Dept Biochemistry/Biophysics, Texas A&M Univ, College Station, TX.

Proper patterning of the central nervous system during development requires the regulation of stem cell division. Distinct populations of stem cells, differing in developmental fate, timing, and location, may respond to different growth factor cues. In the *Drosophila* central nervous system, the developing larval brain has separate pools of mitotically quiescent neural stem cells, or neuroblasts, that resume proliferation at various times. Neuroblasts in the thoracic region resume the cell cycle during early second instar. Hedgehog (Hh) and Branchless (Bnl) are growth factors that have roles in the reactivation of optic lobe and central brain neuroblasts during first instar. The signaling of these growth factors is mediated by the heparan sulfate proteoglycan Trol in the brain lobe. We wanted to examine whether Hh and Bnl play a role in the reactivation of the spatially and temporally distinct set of neuroblasts in the thoracic region, and whether Trol mediates this signaling and proliferation. The expression of *hh* and *bnl* was examined in the thoracic region using *lacZ* insertions. Hh signaling was followed using a response gene, *ptc*, and activation of the Bnl pathway was followed using a reporter for MAPK activity. Hh and Bnl overexpression and loss-of-function mutations were investigated separately to determine the effects of ligand levels on neuroblast proliferation. *trol* expression was visualized using a *trol-GFP* construct, and genetic interaction studies were used to determine whether Trol modulates Hh and Bnl signaling in the thoracic region. To examine more closely how signaling was leading to neuroblast reactivation, brains were double labeled for dividing neuroblasts using BrdU and for either Hh or Bnl signaling. Cells that were responding to Hh or Bnl signaling were then characterized using double staining techniques to identify specific cell types. Our results suggest that the distinct population of thoracic neuroblasts responds indirectly to Hh and Bnl signaling, and the signaling is modulated by the heparan sulfate proteoglycan Trol.

571A

***Drosophila* Insulin Receptor Signaling in the Embryonic Central Nervous System.** Tamar R. Sterling[1], Ronald A. Kohanski[2], Leslie Pick[1]. 1) Department of Entomology, University of Maryland, College Park, MD; 2) Departments of Pediatrics and of Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD 21205.

The *Drosophila* insulin receptor (DInR) is required for axon targeting in the developing adult visual system. DInR interacts directly with the adaptor protein Dock to regulate the targeting of axons from the eye to the brain (1). It is not known if DInR functions upstream of Dock in other tissues. Previous studies have identified a role for Dock in axon guidance in the embryonic central nervous system (CNS) (2). We tested the hypothesis that DInR functions upstream of Dock in the embryonic CNS by analyzing *dinr* mutant embryos with antibodies that recognize specific embryonic CNS components: 22C10; BP102, to visualize axonal tracts; and Fasciclin II to visualize the connectives and commisures of the ventral nerve cord. We observed similar CNS defects in *dock* and *dinr* mutant embryos. The clearest defects were evident in the third bundle of the connectives, which were arched and/or discontinuous in *dinr* mutant embryos. As *dinr* is maternally deposited, we sought to assess the contribution of the maternally deposited gene product in the development of the embryonic CNS. Although *dinr* is required for oogenesis, analysis of a series of *dinr* alleles revealed that females harboring some hypomorphic *dinr* alleles do produce eggs. Using these, we found severe CNS defects in embryos with maternal and zygotic loss of *dinr* function. These results suggest that DInR functions upstream of Dock in the CNS, as it does in the developing adult visual system. References 1. Song, J., Wu L., Chen Z., Kohanski R.A., Pick L. (2003) Science 300: 502-505. 2. Desai, C., Garrity, P., Keshishian, H., Zipursky, S.L., Zinn, K. (1999) Development 126: 1527-1535.

572B

Genes required for Drosophila midline cell development during late embryogenesis. Yi Zhang, Warren Perry II, Kelly Daigle, Patricia Estes. Department of Genetics, NC State University, Raleigh, NC. 27695.

Midline cells within the central nervous system play a number of developmental roles, including regulating ventral ectoderm development via the Spitz signaling pathway and axon guidance through the secretion of attractant and repellent signals. While the role of the midline has been extensively studied in a number of organisms, the Drosophila midline provides a simple system to study many aspects of central nervous system development and function. It consists of a small number cells that differentiate into several types of glia and neurons, and many genes needed for the specification and early differentiation of midline cells have been previously identified. We have recently initiated an EMS mutagenesis screen to identify genes involved in Drosophila midline development during late embryogenesis. Single-minded (sim), a bHLH-PAS protein, is the master regulator of Drosophila midline development and is expressed in all midline precursor cells at early embryonic stages. With the use of a sim:GFP marker, we can examine both midline glia and neurons in late stage embryos, a time of development when it is more difficult to examine these cells using antibody staining techniques. We have screened 1037 mutagenized second chromosomes and identified 29 mutations which cause altered midline GFP expression. These mutations fall into three general classes: mutations in 1) genes involved in segmentation patterning and developmental signaling pathways previously shown to affect midline development, 2) genes required for lateral central nervous system development and 3) novel genes. We are presently characterizing a member of the last class of mutations. This mutation causes the production of extra midline cells in the lateral central nervous system, as well as the ventral displacement of all midline cells. These studies will identify developmental mechanisms required for the formation of functional midline neurons and glia during late embryogenesis.

573C

Regulation of gliogenesis by Rap/Fzr, an activator of the Anaphase Promoting Complex and Loco, an RGS protein. Margarita Kaplow, Adam Korayem, Tadmiri Venkatesh. Dept Biol, CCNY, New York, NY.

Despite their immense importance in neurobiology, glia are remarkably understudied and the molecular genetic mechanisms that direct the differentiation of glia are poorly understood. The developing nervous system of Drosophila offers a superb experimental system to understand these mechanisms. Rap/Fzr is the Drosophila homolog of the mammalian Cdh1, a regulatory subunit of the Anaphase Promoting Complex, APC. APC is an E3 Ubiquitin ligase complex of about 12 polypeptide subunits and primarily characterized for its role in cell cycle progression. During cell cycle, APC targets key mitotic regulators such as cyclin A, cyclin B, cdks and Securin for destruction by the 26S proteosome and thus regulates the timely exit from cell cycle. Rap/Fzr facilitates the targeting of protein substrates to the APC and regulates progression through mitosis. We present data that demonstrate a novel cellular role for Rap/Fzr. Our data show that Rap/Fzr is required for normal glia differentiation in the developing nervous system. Loss-of-function of *rap/fzr* leads to a marked increase in the number of glia in the nervous system of third instar larvae. Conversely, ectopic expression of *UAS-rap/fzr* using *repo-GAL4* results in the drastic reduction of glia. Our results show that this reduction in glia number is not due to apoptosis in *UAS-rap/fzr;repo-GAL4* flies. The pattern of mitosis in the eye imaginal disk is also maintained when *rap/fzr* is ectopically expressed in glia cells. Our study suggests that *rap/fzr* regulates glia differentiation through its interaction with Loco, a RGS(regulator of G protein signaling) protein and a key effector of glia specification. We will present genetic and biochemical results which show that Rap/Fzr and Loco interact. We propose that in the developing nervous system, Rap/Fzr targets Loco for ubiquitination through the APC and thus regulates glia differentation.

574A

Nonstop and Rap/Fzr/Cdh1 interact to regulate cell cycle progression and retinal axon targeting. Margarita Kaplow, Tania Moin, Eliana Mino, Tadmiri Venkatesh. Dept Biol, CCNY, New York, NY.

The *nonstop (not)* gene encodes a ubiquitin specific protease (UBP) which is required for proper glia migration and axon targeting in the developing nervous system (Poeck et.al,2001). The molecular mechanisms by which Nonstop, a de-ubiquitinating enzyme, regulates glia migration and axon targeting are not well understood. UBPs function to disassemble multi-ubiquitin chains from proteins destined to the 26S proteosome. UBPs have birectional control in the rate of protein degradation, accelerating degradation by allowing the recycling of free ubiquitin or inhibiting proteolyis by removing ubiquitin tags from proteins and therefore preventing further degradation. Rap/Fzr is the Drosophila homolog of the mammalian Cdh1, an activator of the Anaphase Promoting Complex(APC), a ubiquitin ligase complex. APC regulates mitotic progression by catalyzing the ubiquitination of key mitotic regulators such as cyclins and targeting them to the 26S proteosome. We previously showed that Rap/Fzr regulates mitotic progression by targeting cyclins and promoting cell cycle exit in the developing eye and the embryo (Jacobs et. al, 2002; Pimentel and Venkatesh, 2005). In the following study, we present data which suggest a novel functional role for Rap/Fzr and Nonstop. Nonstop acts as a dominant suppressor of the *rap/fzr* loss-of-function phenotype. Our data also show that Nonstop regulates APC activity and plays a novel role in cell cycle progression. Co-immunoprecipitation studies show that Nonstop physically interacts with Rap/Fzr. In addition, in the developing optic lobe, we show that loss-of-function *rap/fzr* mutants lead to mis-targeting of R1-R6 axons similar to *nonstop* null mutants. These results suggest that Rap/Fzr and Nonstop interact and regulate both axon targeting and cell cycle progression.

575B

Role of Glia in the Organization and Function of the Visual Nervous System of Drosophila. Rosa Mino[1], Johanna Palacio[1], Margarita Kaplow[1], Jorge Morales[1], Peter O'Day[2], Tadmiri Venkatesh[1]. 1) Department of Biology, City College Of New York, New York, NY 10031; 2) Institute of Neuroscience, Huestis Hall, University of Oregon, Eugene, OR 97403.

In recent years, it has become increasingly clear that glia are pivotal for proper neuronal development and function. Glia mediate a remarkable array of cellular functions including axon ensheathment, establishment of blood brain barrier, trophic response, ionic equilibrium, synaptogenesis, axon pruning, engulfment and neuronal plasticity. Studies made in our laboratory have demonstrated that Rap/Fzr an activator of the ubiquitin ligase complex, APC (Anaphase promoting complex), regulates gliogenesis in the developing nervous system. Loss-of-function mutations in *rap/fzr* lead to an increase in number of glia in the developing nervous system and conversely, *rap/fzr* gain-of-function results in the drastic reduction of the number of glial cells. We have investigated the role of glia in the structural organization and function of the Drosophila visual nervous system using *rap/fzr* loss-of-function and gain-of-function mutations. We will present anatomical, physiological and behavioral data which suggest that glia play an important role, not only in the proper organization of the nervous system, but also in establishing a functional synaptic connection in the adult brain. Increase in the number of glia leads to abnormal axon projections in the optic ganglia, while loss of glia leads to abnormal phototaxis, electrophysiological responses and neurodegeneration. Together, these results indicate that normal number and organization of glia are critical to prevent neurodegeneration in adult flies. Data from light and electron microscope studies, phototaxis behavior, and electrophysiology will be presented. Supported by NIH-SCORE RCMI, MBRS-RISE grants and the City College Fellowship.

576C

The *Drosophila* sialylation genes are expressed in a subset of neurons during embryonic development. Ilhan Akan, Sundeep Singh, Chris Riling, Sheba Mathew, Karen Palter. Department of Biology, Temple University, 1900 N. 12th St., Philadelphia, PA 19122.

Sialic acids are generally expressed as terminal carbohydrates on glycoconjugates of eukaryotic cells. The regulated expression of the homopolymer form, polysialic acid (PSA) on the neural cell adhesion molecule of vertebrates is required for axonal targeting and synaptic plasticity, such as long term potentiation, of specific regions of the adult brain. Our laboratory in collaboration with M. Betenbaugh and Y.C. Lee (Johns Hopkins University) and the Panin laboratory have characterized functional sialylation enzymes in *Drosophila*. We have created a null mutation in the sialic acid synthase gene and have shown that homozygous mutant flies exhibit partial embryonic lethality, behavioral defects and significantly reduced longevity. In agreement with a previous report (Koles et al. (2004) J. Biol. Chem. 279, 4346-4357), we observe expression of the sialyltransferase gene and also the sialic acid synthase in a subset of central nervous system neurons starting at stage 16 using FISH. We show that while FasII is a homolog of vertebrate NCAM, the neuroblasts that express FasII are distinct from those expressing the sialyltransferase gene. By performing double label FISH with known neuronal markers we are attempting to identify which neurons express the sialylation genes. Experiments are in progress to identify their expression pattern in the adult brain in an effort to elucidate the role of sialylation in the *Drosophila* nervous system.

577A

The role of Acj6 in odor receptor gene choice. Lei Bai, Aaron Goldman, John Carlson. MCDB, Yale University, New Haven, CT.

The selected expression of odor receptor (*Or*) genes underlies the odor-specificities of olfactory receptor neurons. The *Or* gene family in *Drosophila* consists of 60 members, most of which are expressed in either the larval olfactory organ or one of the adult olfactory organs in a non-overlapping pattern. Little is known about how individual neurons select which *Or* genes to express. *Abnormal chemosensory jump 6*, which encodes a POU-domain transcription factor, has been implicated in this process. Physiological analysis of individual olfactory neurons shows that in *acj6* mutants, some neurons appear normal, some lose response to all tested odors, and some acquire a different response profile. We characterized the expression of a subset of *Or* genes in the adult *acj6* mutants. Of the *Or* genes that normally are expressed in neurons of the maxillary palp or the large basiconic sensilla of the antenna, a majority are not expressed in *acj6* mutants, resulting in the loss of odor sensitivities of many neurons. A subset of neurons, however, chooses to express an odor receptor that normally is expressed in larval, but not adult, olfactory neurons. The switch in receptor expression accounts for the change in the odor response profile previously characterized. Thus Acj6 acts positively on some *Or* genes, and negatively on another. Using an in vitro binding selection assay, we characterized the binding specificities of Acj6 to short oligonucleotides. Based on the results, we identified predicted Acj6 binding sites in the promoters of a subset of *Or* genes. Mutation of some of these sites greatly reduces reporter gene expression driven by *Or* gene promoters, suggesting that Acj6 regulates a subset of *Or* genes by directly binding to their promoter sequences.

578B

Role of extra macrochaetae (emc) gene during retinal development. Abhishek Bhattacharya, Hui Zhang, Nicholas E. Baker. Dept Molecular Genetics, AECOM, Bronx, NY.

Drosophila extra macrochaetae (emc) gene encodes a Helix-Loop-Helix transcription factor that antagonizes bHLH transcription factors by forming non-functional heterodimers with them. Emc is the drosophila homologue of mammalian Id [Inhibitor of differentiation] proteins. Emc has been shown to positively regulate cell proliferation and negatively regulate differentiation. During retinal development Emc is expressed in all cells, but in different levels and its expression in the eye imaginal disc is regulated by notch signaling. Our data also suggests that during eye development emc is required only for cell proliferation anterior to the morphogenetic furrow but not required for the second mitotic wave. We also found that emc is required for multiple aspects of retinal differentiation. Details of the results will be presented in the poster.

579C

Identification of novel genes involved in external sensory organ formation. Nikolaos Giagtzoglou[1,2,4], Hillary Andrews[3,4], Karen L. Schulze[1,2], Shinya Yamamoto[3], Hugo Bellen[1,2,3]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas; 2) Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, Texas; 3) Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas; 4) equal contirbution.

The bristles on the thorax of the adult fly are External Sensory Organs (ESO), each developing from a single precursor cell. ESOs consist of multiple cell types that arise from iterative asymmetric divisions of their respective precursor cells. The spatiotemporal pattern of emergence of both the precursor cell as well as their progeny occurs in a highly stereotyped manner. These features render ESOs an excellent experimental system for exploring a wide range of developmental mechanisms, such as cell to cell signaling, cell fate specification and maintenance of the differentiated state. In order to identify novel genes that are involved in these processes, we performed an adult mosaic genetic screen of chromosome arm 2R to isolate mutations leading to bristle formation defects. We screened 55000 chromosomes and established 163 stocks, all of which have bristle development defects. Among these we identified new alleles of O-fut and phyllopod, two genes previously shown to be involved in Notch signaling. After complementation analysis of 153 stocks, we have identified 27 complementation groups, each containining an average of 4 alleles. We report the results of the screen and of the preliminary phenotypic characterization of the mutants.

580A

Uncovering developmental gene regulatory networks in the *Drosophila* CNS midline. Amaris Guardiola, Stephen Crews. Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC.

Understanding the generation of neuronal and glial diversity is one of the major goals of developmental neuroscience. The *Drosophila* CNS midline cells constitute a simple model system to study neurogenesis, cell fate acquisition, and neuronal function. We have identified and determined the developmental expression profiles of 290 midline-expressed genes. The expression of 70 of these genes including transcription factors, signaling proteins, and neural function genes was analyzed using multi-label confocal imaging, and the expression patterns were mapped at the single-cell level at multiple stages of CNS development. These maps uniquely identify individual cells and predict potential regulatory events and combinatorial protein interactions that may occur in each midline cell type during their development. Large-scale genetic analysis is being performed to uncover gene regulatory networks predicted by these maps.

We show genetic analysis of *islet*, a LIM homeodomain transcription factor that is important for neuronal development in both vertebrates and invertebrates. In the *Drosophila* CNS, *islet* is required for proper axon pathfinding of a subset of motor neurons and for dopamine and serotonin synthesis. We show that *islet* is expressed in a single midline neuron, the H cell. The H cell is a dopamine-producing neuron that receives serotonergic, glutamatergic and peptidergic inputs. We have identified additional *islet* target genes that are involved in dopamine synthesis and transport. Additionally, *islet* represses *neuropeptide F receptor* expression in abdominal segments. Experiments are in progress to determine the role of *islet* in H cell axonogenesis and how *islet* expression is regulated. Together with the genetic analysis of islet these experiments will help identify developmental gene regulatory networks that will likely be evolutionarily conserved.

581B

Initial insights into the mechanism controlling stochastic *spineless* expression required for the color vision retinal mosaic. Robert Johnston, Claude Desplan. Dept of Biology, New York University, New York, NY.

Stochastic events play an important role in many biological processes. In Drosophila, the R7 photoreceptor makes a cell autonomous stochastic fate choice that determines the formation of two distinct subtypes of ommatidia involved in color vision. Expression of specific rhodopsin pairs in R7 and R8 define ommatidia as either pale (p) or yellow (y) type. R7 cells make the p/y ommatidial choice and then impose fate onto R8. These ommatidial subtypes are distributed stochastically in the retina. The Dioxin receptor transcription factor *spineless* (*ss*) plays a critical role in this process. *ss* is expressed for a short burst during mid-pupation in a large subset of R7 cells preceding rhodopsin expression by more than one day. Loss of *ss* function results in all R7 and most R8 adopting the p fate whereas over-expression of *ss* induces y-R7 fate. The stochastic expression of *ss* in R7 cells therefore controls the entire mosaic of ommatidia in the fly retina.

Though *ss* has been identified as a crucial factor controlling R7 subtype cell fate, the mechanisms controlling *ss* expression remain a mystery. A first inkling comes from the observation that introduction of extra copies of non-productive *ss* promoter yields a reduction in the percentage of y fate ommatidia, presumably resulting from a reduction in the level of *ss* expression. This observation is consistent with the existence of a specific upstream limiting factor. What is this factor? Does it act as part of a biochemical threshold such as a gradient of transcription factor activity? Or is this factor permissively required for *ss* activation while promoter specific variation (noise) is the underlying mechanism? Here, we present an analysis of the cis-regulatory logic controlling *ss* expression targeted to identify key regulatory elements and trans-acting factors. We also present initial results from a screen for new factors involved in p/y ommatidial determination. Finally, we quantitate aspects of *ss* expression in order to address if *ss* is controlled through variable, noise-based mechanisms.

582C

Merlin/NF-2 and the Warts/Hpo/Sav pathway are required to specify R8 photorecepter subtypes. David Jukam, Claude Desplan. Dept Biology, 1009 Main Bldg, New York Univ, New York, NY.

Color vision relies on an organism's ability to detect color contrasts. The Drosophila eye consists of ~800 ommatidia which each contain 8 photoreceptor cells that respond to light. The two innermost photoreceptors, R7 and R8, respond to UV and colored wavelengths, respectively, by expressing one of four rhodopsins (*rh3*, *rh4*, *rh5*, *rh6*). The R7 and R8 photoreceptors are paired within each ommatidium in two subtypes: 'yellow', where R7 expresses *rh4* and R8 expresses *rh6*, or 'pale', where R7 expresses *rh3* and R8 expresses *rh5*. The ommatidial subtypes are found in a yellow:pale ratio of 70:30, randomly distributed in the retina.
Recently our lab showed that a tumor suppressor, *warts*/D-lats, and a growth regulator, *melted*, control the post-mitotic specification of R8 subtype fate. *warts* and *melted* repress each other's transcription to form a bistable feedback loop that directs expression of either Rh5 or Rh6 in R8. Warts, and its co-factors, Hippo and Salvador, are necessary for the yellow-R8 subtype and expression of Rh6. Whether the Warts/Hpo/Sav complex specifies photoreceptor fate with its canonical signaling partners or through a distinct signaling pathway is unknown.

The Drosophila NF-2 homolog, Merlin, acts upstream of Warts in the tumor suppressor function. Here we show that *merlin* is required for proper R8 subtype specification (yellowR8 and Rh6). We have also determined when the Warts/Hpo/Sav pathway is required to instruct yR8 subtype specification using a temperature sensitive *merlin* allele. Finally, we present our genetic interaction analyses of other known Warts pathway members (*expanded*, *yorkie*, *fat*, etc.) and a model for how the Warts pathway controls photorecepter fate.

583A

Regulation of Notch endosomal routing and γ-secretase function in various neurogenic backgrounds. Ritu Kanwar, Mark Fortini. CDBL, National Cancer Institute, NIH, Frederick, MD 21702.

The γ-secretase complex plays a critical role in cleaving the transmembrane receptor Notch during Drosophila development. Functional inactivation of the γ-secretase complex or other neurogenic genes including Notch results in over-proliferation of neuronal lineages and other cell differentiation defects. We have studied the localization and function of epitope-tagged full-length Notch and a membrane-tethered activated form in loss-of-function mutants of the γ-secretase complex and other neurogenic genes. In these studies, we found that proper endosomal routing of the activated form of Notch is dependent upon an intact γ-secretase complex and is also altered in certain neurogenic mutant backgrounds. Our results indicate that Notch intracellular trafficking and γ-secretase-dependent Notch activation are modulated by other neurogenic gene functions. We are currently investigating the effect of these mutant backgrounds on the assembly and maturation of the γ-secretase complex itself.

584B

CONTROL OF NEURONAL CELL FATE SPECIFICATION BY COMBINATORIAL PATTERNING MECHANISMS. Daniel Karlsson, Magnus Baumgardt, Stefan Thor. Molecular Genetics, Linkoping University, Linkoping, SE.

In the developing Drosophila ventral nerve cord, about 100 neurons express the LIM-HD gene apterous (ap). An easily distinguishable subclass of these, the Ap cluster neurons, consists of clusters of 4 cells located specifically in the lateral thoracic segments. In the Ap cluster, one cell, the Tv neuron, expresses the neuropeptide gene FMRFa, while another, the Tvb, expresses the Nplp1 neuropeptide gene. Studies from several labs have led to the elucidation of a highly cell-specific regulatory cascade acting to ensure proper Ap cluster specification, and to activate the cell-specific expression of FMRFa and Nplp1. Recent studies from our lab have determined that the origin of these Ap cluster cells is the neuroblast 5-6 (NB 5-6). With this information at hand, we are now asking the important question of which upstream regulatory events act to ensure that only the thoracic NB 5-6, and no other nerve cord neuroblast, activates the complex genetic cascade leading to the formation of the Ap cluster. We are finding that both positive and negative input, from all four major nerve cord determination axes i.e. thoracic-abdominal, segment-polarity, mediolateral, and temporal, act to ensure the activation of the genetic cascade leading to Ap cluster specification in NB 5-6. Some of these upstream regulators appear to act in concert, while others act largely independetly to trigger Ap cluster specification. The implications of these findings with respect to neuronal cell fate specification in general will be discussed.

585C

Interaction Patterns of Echinoid Homologous Protein, Friend of Echinoid(Fred). Woongki Kim, Susan Spencer. Biology, Saint Louis university, 3507 Laclede ave St.Louis, MO. 63103.

The Epidemal Growth Factor receptor (EGFR) and Notch signalling cascades represent crucial pathways that transmit information during development between cells. Echinoid(*ed*) and Friend of Echinoid (*fred*) are highly homologous adhesion proteins which regulate EGFR and Notch signaling cascades. Ed has been shown to undergo homophilic interaction which is essential for its localization on the plasma membrane. To determine whether Fred behaves similarly, undergoing homophilic binding, we have created constructs of Fred with C-terminal myc- and FLAG epitopes under control of the heat shock promoter. After transfecting these Fred constructs either to the same S2 cells, allowing both cis and trans binding, or to separate populations of the cells and then mixing the populations together,allowing only trans binding, we are able to check whether or not these Fred constructs are co-precipitated with each other. Because of the strong homology between Ed and Fred extracellular domains, we are also investigating the ability of these two molecules to bind one another. We will present results from our binding assays and suggest a model for Ed and Fred interaction.

586A

Charecterizing the role of Dbx in the embryonic CNS development. Haluk Lacin, Heather Broihier, Yi Zhu, Beth Wilson, Hemi Mistry, James Skeath. Dept Genetics, Washington Univ, St Louis, St Louis, MO.

Homeobox transcription factors play important roles in determining cell fates in higher metazoans. Dbx is in the H20-like Homeobox gene family which is highly divergent from other homeobox proteins. In vertebrates, dbx is required for the development of a subset of interneuorons. Due to its role in verteberate CNS development and our interest in understanding the genetic regulatory hierarchy that controls the fate of different classes of neurons in the CNS, we decided to characterize the role of dbx in the Drosophila CNS. To date our work has focused on (i) descriptive analysis of dbx expression and (ii) an initial functional characterization of the role of Dbx in neuronal cell fate specification in the CNS. We find that dbx is expressed in the embryonic, larval, and adult CNS. Using FLP/FRT based lineage tracing system we have mapped most Dbx+ neurons to individual progenitor neuroblasts and followed the axonal projections of these cells. dbx is expressed only in neurons found in the posterior of each hemisegment suggesting regulatory input from segment polarity genes. Moreover, most of Dbx+ cells are interneurons suggesting evolutionary conserved roles for dbx in vertebrate and invertebrate CNS development. To elucidate the function of Dbx, we generated a null allele of dbx using P element mediated imprecise excision. This deletion removes 2 kb of genomic DNA including the dbx transcriptional start site. In mutant embryos, we find that RP2 sibs inappropriately retain Eve expression. As the expression of Eve and Dbx transiently overlap in RP2 sibs, these results indicate that Dbx inhibits Eve expression in RP2 sib neurons. The mild expressivity of dbx phenotype in RP2 sibs suggests other proteins collaborate with Dbx to repress Eve expression. Thus, dbx is expressed in interneurons like its vertebrate homolog and its function is required to repress Eve expression in flies. In the future we plan to assess the role of Dbx in controlling the differentiation of its expressing cells and to investigate the regulatory relationship between segment polarity genes and Dbx.

587B

Expression and functional requirements for the bangsensitive gene *easily shocked*. Elaine R Reynolds, Arda Hotz, Kristen Balsamo, Stephanie Cote. Program in Neuroscience and Biol Dept, Lafayette Col, Easton, PA.

Bang-sensitive mutants are most notable for an abnormal behavioral response to mechanical stress. With age, this behavioral phenotype worsens and the brain degenerates resulting in a shortened lifespan for most bs mutants. The relationship between the behavioral phenotype and age-related phenotypes is unclear since anticonvulsants that reduce seizures fail to rescue age-related phenotypes. One of the bs genes, *easily shocked (eas)*, encodes ethanolamine kinase, an enzyme involved in de novo synthesis of phosphotidylethanolamine. Expression of this gene is tissue specific and is found in nervous system precursors in the embryo and larva. In the larva, *eas* expression has been linked to mitotic progression and proper development of the adult mushroom bodies (Pascual et. al, 2005). In adults, specific sensory neurons associated with the antenna and head bristles, brain neuropil, and specific neurons within the central brain also express the gene. Similar patterns of expression are seen in other bs mutants.

Adult expression information from enhancer trap insertions in *eas* is being combined with functional information from expression of a UAS eas^{+} construct to map the requirements for the *eas* product for both phenotypes. A progress report from this work will be presented. In experiments that utilize Gal4 drivers that express in neuronal precursors and adult brain, it appears that expression in the adult mushroom bodies and its precursors partially rescue the bang-sensitive phenotype. Additional experiments looking at the role of *eas* in the mushroom body will also be presented.

588C

Ero1L, a protein involved in disulfide bond formation, affects Notch signaling. An-Chi Tien[1,4], Akhila Rajan[2,4], Karen L. Schulze[3], Hugo J. Bellen[1,2,3]. 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics, Baylor College of Medicine; 3) HHMI, Houston, TX; 4) Equal contribution.

Cell-cell signaling mediated by receptor of Notch is involved in a wide variety of developmental processes. In *Drosophila*, we use the external sensory organ (ESO) to understand the molecular mechanism of Notch signaling. During development, Notch signaling is required for lateral inhibition, in which one sensory organ precursor of the ESO is selected from a field of equivalent cells. In a genetic screen to isolate mutations affecting ESO development, we identified mutations that cause bristle tufting phenotypes in notum clones, indicating a defect of lateral inhibition. Mapping identified *Endoplasmic reticulum oxidoreductin-1L* (*Ero1L*) as the culprit. This gene encodes a conserved enzyme which plays a role in formation of disulfide bonds (DBs). In yeast, formation of DBs occurs through oxidation of substrate proteins by protein-disulfide isomerases (PDI). Subsequently, PDI is oxidized by Ero1L. Loss of *Ero1* in yeast results in retention of misfolded proteins in the ER and induction of the unfolded protein response (UPR). Surprisingly, although loss of Ero1L in *Drosophila* induces UPR, it shows specific *Notch* loss-of-function phenotypes without affecting other signaling pathways. Notch, Delta and Serrate have many EGF repeats and each has three pairs of DBs. Furthermore, Notch has a novel LNR (lin-12 Notch repeat) domain with a unique DB structure. In *Ero1L* clones, Notch is highly upregulated within the ER but is unable to reach the membrane. While full-length Notch requires Ero1L to traffic to membrane, the ligand Delta and the intracellular domain of Notch can still function in *Ero1L* clones, suggesting that the extracellular domain of Notch is the target of Ero1L. In addition, we reason that the EGF repeats of Notch may not be the target of Ero1L since they are found in Delta and Egfr, which are not affected in *Ero1L* clones. Because Notch is the only protein in the genome which has the LNR domain, we are testing if this domain is the target of Ero1L.

589A

Functional analysis of the homeodomain protein Ind during embryonic CNS development. Tonia L. Von Ohlen[1], Dervla M. Mellerick[2], Canda Harvey[1], Li-Jun Syu[2]. 1) Division of Biology, Kansas State University, Manhattan, KS; 2) Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI.

Dorsoventral (DV) patterning within the *Drosophila* embryos and CNS is a two-step process. The first step involves the subdivision of the embryo into specific tissue types along the dorsoventral axis. The second step involves the further subdivision of each tissue into more precise DV domains. For example, the neuroectoderm expresses three homeobox genes in adjacent DV columns: the ventral column expresses *ventral nervous system defective (vnd)*, the *intermediate column expresses intermediate neuroblasts defective (ind)*, and the dorsal column expresses *muscle segment homeobox (msh)*. Expression of these homeodomain proteins ultimately results in specification of the neurons and glia within the mature CNS. The mechanism by which each of these genes controls transcription is only partially understood. Here we investigate the function of Ind and its interaction with co-factors known to play key roles in development. When we aligned sequences of Ind proteins from different insects we found that outside to homeodomain there were two additional regions of conserved sequence similarity. These domains included a putative engrailed homology (Eh1) domain, suggesting interaction with the Groucho co-repressor and a novel domain not seen in other proteins. Our data shows that Ind binds to Groucho, however, its Eh1 domain is necessary, but not sufficient, for this interaction. In addition, we found that deletion of the C-terminus also reduces Groucho binding efficiency. When we compared the repression activity of Ind lacking the Eh1 domain to that of wild-type Ind in transgenic embryos, the capacity of the mutant Ind to repress gene expression was compromised relative to the wild-type protein. Deletion of the C-terminal region did not have a significant effect on the ability of Ind to repress target gene expression in vivo. However, deletion of the second conserved domain showed marked effects on the ability of Ind to repress target gene expression.

590B

The Role of Histone Deacetylase 1 (*Rpd3*) in Dendritic Targeting of *Drosophila* Olfactory Projection Neurons. Takahiro Chihara[1,3,4], Joy S. Wu[1,2,4], Liqun Luo[1,2]. 1) Howard Hughes Medical Institute, Department of Biological Sciences; 2) Neurosciences Program, Stanford University, Stanford, CA 94305-5020, USA; 3) Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; 4) These authors contributed equally to this work.

The complex neural network in the brain results primarily from simple connections between axons and dendrites. Although axon targeting has been widely investigated, little is known about the mechanisms that regulate dendritic targeting. To reveal the molecular mechanisms for dendritic targeting, we utilize *Drosophila* olfactory projection neurons (PNs) whose dendrites precisely target one of the ~50 glomeruli in the antennal lobe. We performed a MARCM-based genetic mosaic screen with EMS mutants and identified several mutants affecting different aspects of dendritic morphogenesis, including dendritic targeting. Among these, one mutant has a missense mutation in the *Rpd3* gene encoding *Drosophila* Histone Deacetylase 1 (HDAC1). Single-cell PN clones homozygous for *Rpd3* show a fully penetrant mistargeting phenotype from the laterally located glomerulus DL1 to more medial glomeruli, such as DM5 and DM6. Postmitotic expression of a UAS-Rpd3-V5 transgene can fully rescue the dendritic mistargeting phenotype of *Rpd3* PN clones. MARCM analysis with several PN-specific GAL4 drivers has yielded similar dendritic mistargeting phenotypes. These results indicate an important and specific role for Rpd3 in determining neuronal connectivity in postmitotic neurons. The phenomenon of a ubiquitously expressed chromotin remodeling factor in controlling specific targeting decisions is of particular interest in elucidating the molecular mechanisms of connection specificity. To further address this question, we are searching for genes that may be regulated by Rpd3 to control this wiring specificity in the *Drosophila* antennal lobe.

591C

Analysis of the Role of Rab11 in Nuclear Translocation in Drosophila. Tarek Houalla, Yong Rao. Dept Neurology & Neurosurgery, McGill Univ, Montreal, PQ, CA.

Nuclear translocation is a key step in neuronal migration during the development of the nervous system. Defects in nuclear migration affect eye morphogenesis in Drosophila and cortical neuronal migration in lissencephaly patients. However, the molecular mechanism underlying the developmental control of nuclear migration remains largely unknown. We have previously revealed a novel signaling pathway in which the Ste20-like kinase Msn regulates the function of Bic-D and the motor protein dynein to drive the apical migration of photoreceptor nuclei in the fly visual system. Msn increases the phosphorylation of Bic-D. Loss of Msn causes the mis-localization of both Bic-D and dynein, thus disrupting the apical migration of photoreceptor nuclei. Our recent results suggest that integrin and Rab family proteins are also required for photoreceptor nuclear migration. We are currently investigating if these proteins are components of the Msn signaling pathway in regulating nuclear migration during development.

592A

Drosophila models of human developmental brain disorders: comparative and cell culture approaches. Katherine Olson[1,2], Robert Kraft[2], Jennifer Inlow[3], Linda Restifo[1,2]. 1) Graduate Interdisciplinary Program in Genetics, University of Arizona, Tucson, AZ; 2) ARL Division of Neurobiology, University of Arizona, Tucson, AZ; 3) Dept. of Chemistry, Indiana State University, Terra Haute, IN.

In previous studies, we demonstrated striking phylogenetic conservation of human mental retardation (MR) genes in Drosophila, with 75% having a candidate functional ortholog in the fruit fly (Inlow & Restifo, 2004, Genetics 166:835-881). Since then, new MR genes have been published at a rapid pace, with more than 360 MR genes now molecularly identified. An interesting small subset of these have both autism and MR as phenotypes. We will present an updated analysis of the recently identified human MR genes and their Drosophila homologs and orthologs, focusing on several of particular interest.

Using our established primary cell culture methods (Kraft et al, 2006, J Neurosci 26:8734-8747) and newly developed semi-automated image-analysis software (Narro et al, 2006, Brain Res, in press), we have begun to screen Drosophila MR-gene mutants for neuronal phenotypes. The genes include *dfmr1* (human: *FMR1*, fragile X syndrome), *nejire* (human: *CREBBP*, Rubinstein-Taybi syndrome), and *rsk2* (human: *RSK2*, Coffin-Lowry syndrome). For some of the mutations, the phenotype of Drosophila neurons *in vitro* is qualitatively similar to the neuropathological defects of human patients. Our comparative genetic analyses and experimental data further support the use of the Drosophila genetic system for understanding the biology of human developmental brain disorders (Restifo, 2005, Ment Retard Dev Disabil Res Rev 11:286-294). Funded by NIH grants NS28495 (Pr 4) and NS055774 to LLR, NSF IGERT fellowship (DGE 0114420) to KO, and an American Philosophical Society Franklin Research Grant to JKI.

593B

CONTROL OF PROXIMAL-DISTAL DISTRIBUTION OF BRANCHING POINTS IN DENDRITIC TREES OF DROSOPHILA SENSORY NEURONS. Daisuke Satoh[1], Daichi Sato[2], Taiichi Tsuyama[2], Motoki Saito[2], Fuyuki Ishikawa[2], Melissa M. Rolls[3], Chris Q. Doe[3], Hiroyuki Ohkura[4], Tadashi Uemura[2]. 1) Graduate School of Science, Kyoto University, Kyoto, JP; 2) Graduate School of Biostudies, Kyoto University, Kyoto, JP; 3) HHMI, University of Oregon, Eugene, OR; 4) The University of Edinburgh, Edinburgh, UK.

Precise patterning of the dendritic field is essential for neuronal function. One class of Drosophila dendritic arborization (da) neurons, which are thought to receive mechanical stimuli, develop large and highly elaborate dendritic trees, and only 3 cells cover the entire epidermis of each abdominal hemisegment. In order to acquire such a wide receptive field, the da neuron adds more new branches to the distal region of each dendritic tree than to the proximal region. How does the da neuron coordinate its branch distribution?

We conducted a genetic screen to hunt genes that affected dendritic morphology, and isolated *dandelion clock* (*dlic*) mutants, which formed smaller receptive fields. Time-lapse recordings of the mutant neurons showed that the characteristic regional difference in branch formation was dramatically altered. Terminals of major branches kept extending without further branching and some of pre-existing side branches retracted; in contrast, the proximal region became bushy over time. This result suggested that *dlic* regulates the distribution of branching points. *dlic* encoded the dynein light intermediate chain that is an accessory subunit of a microtubule minus-end motor, cytoplasmic dynein. Mutant neurons of either *lis-1* or *khc* (*kinesin heavy chain*) displayed similar phenotypes. We interpreted these dendrite phenotypes as being due to dysfunction of intracellular transport of components that are necessary for branching in the dendritic tree. We are attempting to identify those components/cargos.

594C

Role of Wnt Signaling Pathway during Neuronal Remodeling. Ajeet P. Singh[1], Bidisha Roy[2], VijayRaghavan K.[2], Veronica Rodrigues[1,2]. 1) Dept. of Biological Sciences, TIFR, Mumbai, India; 2) National Center for Biological Sciences, TIFR, Banglore, India.

During insects metamorphosis several larval neurons prune their branches and grow new processes to attain adult-specific morphologies. Regulation of pruning and re- growth, in a mature cell, poses a fascinating developmental problem. A pair of contra-laterally projecting, Serotonin- immunoreactive deutocerebral (CSD) neurons, in the CNS of Drosophila melanogaster, undergo developmentally programmed pruning and remodeling and the mechanism of their identification also allows their genetic manipulation. Each CSD neuron undergoes stereotypic pruning during early metamorphosis and later elaborates adult specific processes. This identified neuron provides us with an opportunity to study the regulatory mechanisms underlying neuronal remodeling in the central brain. Signaling pathways that play a major role during early development have recently been shown to be involved in regulation of neuronal morphogenesis. Our preliminary observations suggest a key role for Wnt signaling pathway in CSD neuron metamorphosis. Ectopic expression of a dominant negative form of the Wnt effector- TCF- affects the elaboration of adult arbors and not the pruning of the larval form. We are using loss- and gain- of function genetics to demarcate which of the three pathways downstream of Wnt signaling is involved in neuronal re-modeling. Mutations in the Disheveled (Dsh) that allow us to distinguish between three different modes of Wnt signaling are available. We are using these alleles as well as other genes that are active in canonical and non-canonical Wnt signaling to dissect the effect of their roles in neuron re-modeling.

595A

Restructuring of the abdominal neuromuscular system during metamorphosis. Joyce Fernandes, Meredith Dorr, Camilo Molina, Sarita Hebbar, Aswati Subramanian. Zoology Dept, Miami Univ, Oxford, OH.

During its life cycle, Drosophila makes two sets of neuromuscular junctions (NMJs), embryonic/larval and adult, which serve distinct stage-specific functions. During metamorphosis, the larval NMJs are restructured to give rise to their adult counterparts, a process that is integrated into the overall remodeling of the nervous system. Previous work in our laboratory has characterized the neuromuscular junctions of abdominal muscles (Hebbar, et al, J. Neurobio., 2006). These muscles are arranged in sets of ventral lateral and dorsal muscles. The NMJs formed on these muscles have boutons that are comparable to larval NMJs and are much larger than those on the thoracic flight muscles. We have examined morphological attributes of the ventral abdominal NMJ and show that an embryonic motor neuron identity gene, dHb9, is expressed at these adult junctions. In order to correlate the remodeling of motor neurons in the central nervous system with the changes in innervation and motor units at the periphery, motor neuron specific Gal4 drivers are being used to identify the location of cell bodies in the CNS as well as their characteristic projections in the periphery. We hope to address questions relating to the number and identity of motor neurons that innervate the adult abdominal muscles. We will also present our studies to examine how NMJ remodeling is related to the overall change in the shape of the nervous system, particularly the number of nerves.

596B

The Function of Bällchen in Neuronal Stem Cell Maintenance. Ufuk Gunesdogan, Herbert Jackle, Alf Herzig. Developmental Biology, MPI for Biophysical Chemistry, Goettingen, DE.

Stem cells have the remarkable capability to generate both differentiating and self-renewing daughter cells. *Drosophila* neuroblasts divide asymmetrically to self-renew, generating another neuroblast and a differentiating daughter cell. However, the mechanisms that control this process are poorly understood. The *Drosophila* Vaccinia Related Kinase ortholog Bällchen/NHK-1, a histone modifying kinase, is involved in the maintenance of stem cell identity. We have shown that *bällchen* (*ball*) is required for the maintenance of germline stem cells (Herzig et al., submitted). BALL expression during embryogenesis becomes restricted to the germ cells and neuronal stem cells. Loss-of-function *ball* mutants show severe defects in larval gonads, because stem cell self-renewal is impaired. In addition, larval *ball* mutant brains are reduced and lack neuronal stem cells suggesting that self-renewal in this stem cell population also depends on BALL activity. In order to assess the function of ball in neuronal stem cell maintenance in detail we are carrying out clonal analysis in larval brains of *Drosophila*. Our results provide evidence that Bällchen is involved in both germ line and neuronal stem cell maintenance. This dual role of Bällchen is in contrast to most of the known stem cell factors in *Drosophila* that are required in a tissue specific manner.

597C

Targeting of Sanpodo to asymmetric pericentrosomal early endosomes regulates Notch signaling in sensory organ precursor cells. Fabrice Roegiers, Xin Tong, Diana Zitserman. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA.

Development of the Drosophila adult peripheral nervous system is a powerful in vivo model to study binary cell fate decisions based on activation or inhibition of Notch activity. In dividing sensory organ precursor cells Numb protein is asymmetrically localized and antagonizes Notch signaling in the pIIb daughter cell. Notch signaling in sensory organ progenitors requires Sanpodo, a transmembrane protein that is regulated Numb. Using in vivo imaging of Sanpodo-GFP fusion protein and GFP reporters for endosomal compartments we show that a localized early endosome compartment, containing Sanpodo, forms specifically in the Notch-inhibited daughter cell. Sanpodo-positive early endosomes form around the pIIb cell centrosome within ten minutes of sensory organ precursor cell mitosis. Blocking formation of the early endosome compartment by inhibiting endocytic vesicle fusion causes cell fate switching, apparently due to increased Notch activity. We are current exploring the composition of this early endosome compartment and analyzing the genetic requirements for its pericentrosomal targeting in pIIb cells. We hypothesize that Notch signaling is regulated shortly after progenitor cell mitosis by sorting of Notch pathway components, such as Sanpodo, from the plasma membrane into an early endocytic node which forms in only one daughter cell.

598A

Molecular mechanisms that underlie the transition of neuroepithelial cells to neuroblasts in the Drosophila optic lobe. Daiki Umetsu, Tetsuo Yasugi, Makoto Sato, Tetsuya Tabata. University of Tokyo, IMCB, Tokyo, JP.

Molecular mechanisms regulating neurogenesis have been extensively studied in the embryonic development of Drosophila. The transition from epithelial cells to neuroblasts (NBs) is explained to progress in a stochastic manner. In contrast, the process appeared to be regulated in a deterministic mechanism in the optic lobe development. We are studying molecular mechanisms underlying transition of neuroepithelial cells to NBs in the optic lobe as an experimental model for postembryonic neurogenesis. The groups of cells called proneural clusters are allocated from the epithelial cells at the first step of the neurogenesis. Within each proneural cluster, the cells compete with each other and only one cell is singling out to develop into a sensory organ precursor or a NB. The process is mediated by lateral inhibition through the Notch signaling. In contrast to the embryonic development, temporal progression of the neurogenesis can be observed in a spatially organized manner in the optic lobe development. Drosophila visual system is composed of the retina and the optic lobe. The latter contains three optic ganglia: the lamina, the medulla and the lobula complex. The neurons of the lamina and the medulla are derived from neuroepithelium. It provides neuroblasts (NBs) for medulla neurons and lamina precursor cells at the outer and the inner edge, respectively. Neurogenesis of medulla neurons proceeds as follows: 1. NE cells proliferate by symmetric cell division until they differentiate into neuronal progenitor cells. 2. Cells in outer edge of the neuroepithelium progressively differentiate into medulla NBs, and thus differentiation of NE cells to medulla NBs progresses outer to inner direction. 3. Medulla NBs undergo asymmetric division and produce ganglion mother cells, which divide again and become medulla neurons. Here, we discuss the molecular mechanisms that underlie the transition of neuroepithelium to medulla NBs in the optic lobe.

599B

Role for the JAK/STAT signaling pathway in the optic lobe development. Tetsuo Yasugi[1], Daiki Umetsu[1], Satoshi Murakami[1], Kuniaki Takahashi[2], Kaoru Saigo[3], Ryu Ueda[2], Shoko Yoshida[1], Makoto Sato[1], Tetsuya Tabata[1]. 1) IMCB, Univ. Tokyo, Tokyo, Japan; 2) NIG, Shizuoka, Japan; 3) Dept. Biophys. Biochem., Grad. Sch. Sci., Univ. Tokyo, Tokyo, Japan.

The *Drosophila* visual system is composed of the compound eye and the optic lobe in the brain. The latter contains three neural ganglia, namely, lamina, medulla, and lobula complex. These optic ganglia are derived from two neuroepithelial ectoderms, the outer optic anlage (OOA) and the inner optic anlage (IOA). The lamina neurons and a part of the medulla neurons differentiate from OOA cells. In this study, we demonstrate that the Drosophila JAK/STAT signaling is required for regulating the timing of producing medulla neuroblasts. In the wild type flies, the number of neuroepithelial cells (NE cells) of the OOA increases by repetitive symmetric cell divisions until early third instar. During mid-third instar, the NE cells located at the outer edge start to differentiate into medulla neuroblasts. Next, medulla neuroblasts undergo asymmetric division and produce ganglion mother cells, which divide again and become medulla neurons. Differentiation of NE cells to medulla neuroblasts progresses outer to inner direction. On the other hand, NE cells located at the inner side receive signals from innerevating retinal axons and differentiate into lamina neurons. The Drosophila JAK/STAT signaling pathway is composed of four major factors, the ligand Unpaired (Upd), the transmembrane receptor Domeless (Dome), the JAK homolog Hopscotch (Hop), and the STAT homolog Stat92E. The number of NE cells was fewer in *hop* mutants and this defect resulted in fewer medulla neurons and the loss of lamina neurons. NE cells in the *Stat92E* clone began to differentiate into medulla neuroblasts earlier than the surrounding WT tissue. These results suggest that the JAK/STAT signal regulates growth and maintenance of the NE cells. We also discuss the relation between JAK/STAT signaling pathway and Notch signaling pathway in the OOA development and the mechanisms of the NE cells to differentiate into the medulla neuroblasts.

600C

Two types of *Drosophila* R7 photoreceptor cells are arranged randomly: A model for stochastic cell-fate determination. Steven G. Britt[1], Melanie L. Bell[2], James B. Earl[1]. 1) Department of Cell & Developmental Biology, University of Colorado at Denver & Health Sciences Center, Aurora, CO; 2) Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand.

The R7 photoreceptor cells of the *Drosophila* retina are sensitive to ultraviolet light and are thought to mediate color discrimination and polarized light detection. Color vision in many organisms is based on the patterned expression of different visual pigments, rhodopsins, in different photoreceptor cells. In *Drosophila*, there is growing evidence that the color sensitivity of the R8 cell within an individual ommatidium is regulated by a genetic switch that depends on the type of R7 cell adjacent to it. Here we examine the organization of the two major types of R7 cells by rigorous statistical methods, and present evidence that they are arranged randomly. We show that the identity of neighboring R7 cells is not significantly different from that obtained in a random simulation, suggesting that R7 cells in neighboring ommatidia are unlikely to interact and influence each other's identity. This is consistent with the idea that R7 cell subtypes are arranged and determined stochastically in a cell autonomous manner. As compared to traditional lineage or inductive mechanisms, this may represent a novel mechanism of cell fate determination that is based upon noisy or stochastic gene expression in which the differentiation of an individual R7 cell is a random event, but the proportions of R7 cell subtypes are regulated.

601A

The intraflagellar transport protein REMPA/IFT140 is a component of the chordotonal ciliary dilation. Eugene Lee[1,2], Elena Sivan-Loukianova[3], Daniel F. Eberl[3], Maurice Kernan[1]. 1) Dept of Neurobiology; 2) Program in Neuroscience, SUNY, stony brook, NY; 3) Department of Biology, University of Iowa, Iowa City, IA.

In flies, mechanosensory and chemosensory transduction take place in specialized cilia at the tips of neuronal sensory processes. Ciliary assembly requires a conserved intraflagellar transport (IFT) complex, which is transported out from the cell body by kinesin-2, and returned by dynein; components of the IFT-B and IFT-A subcomplexes are required for outward and retrograde movement each. Drosophila mutants lacking the IFT-B protein NOMPB/IFT88 or subunits of the IFT kinesin-2 lack sensory cilia, resulting in a complete loss of sensory transduction. We now find that reduced mechanoreceptor potential A (rempA) mutants lack the fly homolog of IFT140, an IFT-A protein. rempA mutants have reduced mechanotransduction in bristles, but completely lack mechanotransduction in chordotonal organs. Cilia are still present but are shortened, and the ciliary dilation (CD), a characteristic feature of chordotonal cilia, is absent. Consistent with a specific defect in retrograde IFT, rempA and another IFT-A mutant, oseg1/IFT122, both accumulate the IFT-B protein NOMPB in shortened cilia. To find the location of REMPA, we expressed a REMPA-yellow fluorescent protein fusion from the native promoter, in transgenic flies. Rescue of the rempA1 homozygotes by rempA-YFP showed that it retains wild type function. In chordotonal organs of transgenic pupae, the YFP signal initially appears in the sensory neurons, distributed along the cilia and diffusely in the neuronal cytoplasm. As the organs complete differentation, the signal concentrates at paired foci, at the exact position of the CDs. Each CD is a discrete, electron-dense lattice, enclosed within the microtubules of the axoneme at about ¾ the length of the cilium. CDs are also disorganized in some other transduction-defective mutants but their molecular makeup was unknown. The unexpected localization of REMPA/IFT140 to this structure suggests that IFT-A proteins may have a post-differentation role in mechanotransduction.

602B

Feedback from Rhodopsin 6 protein is required to maintain pR8 identity through inhibition of Rh5 expression. Daniel Vasiliauskas[1], Esteban O. Mazzoni[2], Claude Desplan[1]. 1) Department of Biology, New York University, New York, NY; 2) Department of Pathology College of Physicians and Surgeons, Columbia University, New York, NY.

Generally, an individual sensory neuron expresses a single sensory receptor to avoid sensory confusion. This is known as a one neuron-one receptor rule. Neurons accomplish this by choosing to express one of a number of genes encoding alternative receptors, while repressing expression of the rest. Studies of olfaction in mice, raised an intriguing possibility that a feedback signal from the sensory receptor protein itself plays a role in the choice mechanism, possibly by directing repression of alternative genes. Photoreceptor cells (PR) in the adult Drosophila eye express one of five different rhodopsins, photon capturing G-coupled seven trans-membrane proteins and thus, follow the one neuron-one receptor rule. Each unit, omatidium, of the eye contains 2 inner PRs, R7 and R8, surrounded by 6 outer PRs which express Rh1. Excluding the dorsal rim area, 30% of the eye is populated by “pale” (p) omatidia which express Rh3 in R7 and Rh5 in R8, and 70% of the eye is populated by “yellow” (y) omatidia which express Rh4 in R7 and Rh6 in R8. p and y omatidia are randomly distributed throughout the eye. This pattern is set up through a stochastic decision in R7 cells, which then signal and determine R8 rhodopsin expression. By the end of pupation, robust, stable and exclusive rhodospin expression in the fly eye has been established. Here we ask whether feedback signals from rhodopsin proteins participate in regulating the choice of rhodopin gene expression. We find that in rh6 mutants, yR8 type is specified normally. However, in older adults yR8 cells start to express Rh5, which normally is only expressed in pR8. This phenotype can be reverted by expression of the rh6 ORF in the mutant background under the control of rh6 promoter. Thus, Rh6 does indeed generate a signal which controls transcription. However, this signal does not participate in the initial rhodopsin choice, but rather acts to maintain yR8 identity by suppressing Rh5 expression in the adult.

603C

The Role of Serine Protease Inhibitors in Nervous System Development. You-Seung Kim, Thomas Osterwalder, Haig Keshishian. Dept MCDB, Yale Univ, New Haven, CT.

Proteolytic events and the regulation of its activity are critical for the proper development and function of nervous system. Serine proteases participate from remodeling of the extracellular matrix to regulating synaptic growth and plasticity. Neuroserpin is a serine protease inhibitor that regulates the activity of tissue plasminogen activator (tPA) in both synaptic development and function. Both neuroserpin and tPA were also known to be critical for long term potentiation (LTP) at the hippocampus. Previously we identified Neuroserpin homolog, Drosophila Serine Protease inhibitor 4 (Spn4) that is expressed widely in the larval CNS, including motoneurons and putative peptidergic neurons. Here we found that Spn4 is an important regulator for the development of neuromuscular junction (NMJ). Spn4 loss-of-function (LOF) mutants have increased number of synaptic boutons at NMJ. However overexpression of Spn4.1, an isoform that bears C-terminal ER retention signal, reduces the number of synaptic boutons and reduced larval locomotive speed. Such Spn4.1 overexpression phenotype is rescued by the co-expression of an Spn4.1 RNAi construct. Our data suggest that Drosophila neuroserpin may involve in the regulation of synaptic development and function.

604A

Posttranslational Regulation of the *Drosophila* Circadian Clock Requires Protein Phosphatase 1 (PP1). Yanshan Fang, Sriram Sathyanarayanan, Amita Sehgal. Howard Hughes Medical Institute, Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

Circadian rhythms in *Drosophila* require the cycling of the protein products of two major clock genes *timeless (tim)* and *period (per)*. The cyclic expression of *tim* and *per* is executed by a delayed feedback loop, in which TIM and PER proteins repress their own transcription while timely degradation of TIM and PER relieves the repression and allows the transcription of *tim* and *per*. In the absence of rhythmic transcription, however, TIM and PER abundance still oscillate. It indicates that timekeeping mechanism requires posttranslational regulations such as phosphorylation, which has been closely studied at the level of the kinases involved. Here, we demonstrate an essential role for protein phosphatase 1 (PP1) in the *Drosophila* clock.

PP1 interacts and dephosphorylates TIM, and inhibition of PP1 renders TIM unstable in both S2R+ cells and clock neurons. Unlike the phenotype of flies overexpressing SGG (the kinase phosphorylating TIM), inhibition of PP1 in flies decreases TIM abundance, lengthens circadian period and reduces amplitude of behavioral rhythms. Moreover, inhibition of PP1 in flies shows additive effects on lengthening period with the tim^{UL} mutation, but incompletely antagonizes the period-shortening efffect of SGG overexpression. This suggests that, PP1 and SGG do not act in a simple antagonistic manner and the precise control of the clock involves crosstalk between kinases and phosphatases.

Finally, we found that TIM and PER have different sensitivity to phosphatase inhibitors, and TIM protects PER from inhibition of PP1 but not that of PP2A, suggesting that these two major protein phosphatases function differently in the clock. Thus, the regulation of the clock by protein phosphatases is as important and complex, if not more, as that of kinases.

605B

JETLAG resets the *Drosophila* circadian clock by promoting light-induced degradation of TIMELESS. Kyunghee Koh, Xiangzhong Zheng, Amita Sehgal. HHMI, Neuroscience, University of Pennsylvania, Philadelphia, PA.

Travel across time zones often produces jet lag because it takes some time to resynchronize internal circadian clocks to the new day and night cycle. Organisms ranging from bacteria to humans synchronize their internal clocks to daily cycles of light and dark. Photic entrainment in *Drosophila* can be mediated by the visual system and by CRYPTOCHROME (CRY), a circadian blue-light photoreceptor. When the fly is exposed to light, CRY binds a core clock protein, TIMELESS (TIM), which leads to subsequent ubiquitination and degradation of TIM by the proteasome pathway. Rapid, light-dependent degradation of TIM underlies the fly's ability to reset the circadian phase to reflect environmental fluctuations in light levels. However, the specific signals that drive the TIM response to light are not known. We have identified mutations in *jetlag (jet)* - a gene coding for an F-box protein with leucine-rich repeats - that result in reduced light sensitivity of the circadian clock. Mutant flies show rhythmic behavior in constant light, reduced phase shifts in response to light pulses, and reduced light-dependent degradation of TIM. Transgenic expression of wild-type JET in *jet* mutants restores their ability to reset the clock in response to light pulses. Furthermore, expression of JET in cultured S2R+ cells confers light-dependent degradation onto TIM, thereby reconstituting the acute response of the circadian clock to light in a cell culture system. JET physically associates with TIM, and the association is stronger in light than in dark. Our results suggest that JET is essential for resetting the clock by transmitting light signals from CRY to TIM. Ongoing experiments examine the effects of various combinations of *tim* and *jet* alleles on light sensitivity, and results will be discussed.

606C

An oenocyte clock regulates the expression of *desat1*, a gene required for sex pheromone biosynthesis. Joshua J. Krupp, Clement Kent, Julia A. Schonfeld, Joel D. Levine. Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario, CA.

The circadian system of *Drosophila melanogaster* has been shown to regulate the temporal pattern of mating. Mating success relies heavily upon chemical courtship cues provided in the form of cuticular hydrocarbon molecules. Specialized adult cells called oenocytes are a primary site for the production of these courtship cues. Given the circadian regulation of mating, we wished to determine whether a circadian clock mechanism exists within the oenocytes, and whether this peripheral clock could be functionally linked to the production of cuticular hydrocarbon molecules. We show by quantitative PCR performed on dissected oenocyte preparations that the core clock genes *period* (*per*), *timeless* (*tim*), and *clock* (*clk*) are cyclically expressed in Canton-S, a laboratory wild-type strain. Oscillations in clock gene expression persist in constant darkness, and are lost in the arrhythmic mutant, per^0. By the second day of constant darkness, the time of peak expression of each clock gene is temporally advanced by approximately 7.8hr in the oenocyte clock, as compared to the sum of the various head oscillators. Furthermore, we show that the expression of *desaturase1* (*desat1*), a gene encoding an enzyme required for the synthesis of courtship cues, oscillates within the oenocytes. Cyclic expression of *desat1* is *per*-dependent and may be regulated by the oenocyte clock. Thus we have determined the existence of a circadian clock in the oenocytes, and provide a potential mechanism whereby the temporal pattern of mating may be regulated through the circadian production of courtship cues.

607A

***logjam* is expressed in a small number of cells in the CNS and is required for egg laying behavior.** Ginger Carney, Kara Boltz, Lisa Ellis. Dept Biol, Texas A&M Univ, College Station, TX.

Reproductive behaviors in flies are interesting due to their stereotypical nature, complexity and importance for the animal's reproductive success. Flies use sight, sound, smell, taste and touch to identify and choose mates. The major female reproductive behaviors are receptivity to (or rejection of) male courtship, followed by copulation, increased egg ovulation, and oviposition. Females resist mating for the first day of their lives, while physiological changes occur to prepare the female for reproduction. Once the female mates, her behavior changes. Compounds in the male ejaculate increase the female's rate of ovulation and oviposition and decrease her receptivity to mating. One goal of our laboratory is to identify and characterize the genes and neural circuits that underlie these female behaviors.

One example of a gene necessary for oviposition is the *logjam (loj)* locus, which encodes a p24 protein that localizes to the early secretory pathway in Drosophila. Females that lack *loj* are unable to lay their eggs, while male fertility is not affected. Our recent data show that *loj* expression in the central nervous system is necessary for egg laying and that only a small number of CNS cells appear to express the protein. Interestingly, there is a sexual dimorphism in Loj protein expression; male flies express Loj in a crescent-shaped pattern in the central brain, while female flies lack this expression. We are working to determine the identity of the Loj-expressing cells in both sexes and to determine if Loj has a required function in male behavior.

608B

***Drosophila melanogaster* males can respond rapidly at the genetic level to courtship interactions.** Lisa L. Ellis, Ginger E. Carney. Dept Biol, Texas A&M Univ, College Station, TX.

Behaviors are mediated by complex genetic interactions and are influenced by the environment and experiences. We can better understand the genetic underpinnings of behavior by studying the stereotypical courtship behavior of *Drosophila melanogaster* males. The sex-determination pathway mediates reproductive behaviors in males and females through the transcription factors, *fruitless, dissatisfaction* and *doublesex*. However, only a few targets of this pathway are known.

We have taken a genome-wide approach to determine courtship-responsive genes and targets of the sex-determination pathway. Expression profiles of males that courted females were compared to unexposed males by use of Affymetrix *Drosophila* 2.0 microarrays. A small group of genes, including sex-determination targets, showed significant transcript level changes as a result of courtship. These loci may be involved in learned courtship behaviors, e.g. male-male habituation, or could play a role in motivating subsequent courtship activity.

609C

The effects of interspecific courtship on mating success and learning in Drosophila melanogaster. Scott McRobert, Rebecca Dawson. Dept Biol, St Joseph's Univ, Philadelphia, PA.

Sexual interactions between two sympatric, distantly related species, Drosophila melanogaster and Drosophila affinis, were analyzed. Mature D. melanogaster males performed vigorous courtship in response to both virgin and mated D. affinis females, but performed no courtship in response to virgin females of another sympatric species, D. immigrans. The presence of D. affinis females was shown to significantly reduce the mating success of D. melanogaster males with conspecific females. In comparison, the presence of mated D. melanogaster females or virgin D. immigrans females had no impact on mating success in D. melanogaster males. Following exposure to D. affinis females, D. melanogaster males lessened their courtship towards both D. affinis and D. melanogaster females, suggesting that a form of interspecific courtship conditioning had occurred.

610A

Functional analyses of *fru*^{M}-expressing neurons for their role in regulating courtship initiation. David Tran[1], Ulrike Heberlein[2], Bruce Baker[1]. 1) Dept Biological Sciences, Stanford Univ, Stanford, CA; 2) Dept. of Anatomy, UCSF, San Francisco, CA.

Male courtship in *Drosophila melanogaster* is an innate and stereotypic series of contingent behaviors activated by sex-specific visual and chemosensory cues. The manifestation of male courtship behavior requires the expression of the male specific products of the *fruitless* gene, *fru*^{M}, during development. We utilize Gal4-driven, tissue-specific expression an RNAi construct inhibiting *fruitless* expression, combined with sensory deprivation to identify which specific *fru*^{M}-expressing neurons function to regulate defined sensory inputs during courtship. 119 Gal4 lines, upon UAS-*fru*^{M}IR expression affect the timing of courtship initiation, either shortening (90 fast lines) or lengthening (29 slow lines) courtship latency (measured by the time to wing extension). Courtship latency is a particularly useful behavioral parameter because it is both significantly affected by the loss of a single sensory modality and synergistically perturbed by deficits in multiple modalities. Based on that behavior, we further subdivide these lines by their response to the loss of visual cues, i.e. compounding any possible UAS-*fru*^{M}IR-dependent effects on chemosensory/mechanosensory processing. Seven lines exhibit a synergy with the loss of visual cues, where courtship latency is significantly slowed in comparison to courtship in the light. In combination with the above behavioral assays, we have developed methods to visualize the specific overlaps of the Gal4s with *fru*^{M}expression. Several patterns emerge from the expression analyses with implications for the *fru*^{M}-expressing circuitry. Our results suggest that the *fru*^{M}-expressing courtship circuitry is distributed and robust in regards to the activation of this fixed-action pattern. Conversely, there are several defined and spatially-limited neural subsets which act to inhibit courtship activation.

611B

***Catsup* Function in Dopamine Homeostasis.** Faiza Ferdousy, Hakeem Lawal, Zhe Wang, Iyare Izevbaye, Carrie Williams, Daniel Roberts, Janis M. O'Donnell. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

The neurotransmitter dopamine, the predominant form of catecholamine in the central nervous system, plays an important role in many cellular and signaling processes. Dopamine synthesis is initiated by the action of tyrosine hydroxylase (encoded by *pale* in *Drosophila*) the first and rate limiting enzyme in this pathway. Mis-regulation of dopamine homeostasis has been associated with many neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease as well as mental disorders such as schizophrenia and depression. Previously, our lab has shown that the *Drosophila* gene *Catecholamines up*, predicted to be a seven transmembrane domain protein, acts as a post-translational negative regulator of tyrosine hydroxylase, the first and rate-limiting enzyme in the dopamine biosynthesis pathway. Here we report that the *Catsup* protein plays a significant role in the synthesis of dopamine in the central nervous system. We now demonstrate that the *Catsup* protein also acts as a regulator of GTP cyclohydrolase, the first and rate-limiting enzyme in the synthesis of tetrahydrobiopterin, a necessary cofactor for tyrosine hydroxylase. Further, our data shows that *Catsup* mutant flies confer neuroprotection against oxidative insult while up-regulating BH4 and dopamine synthesis simultaneously. We also present an analysis of *Catsup* in dopamine packaging.

612C

Targeted Activation of CCAP Neurons Using the Cold-Sensitive TRPM8 Channel Reveals a Pre-eclosion Critical Period in Wing Expansion. Nathan Peabody[1], Andrew Vreede[1], Fengqiu Diao[1], Elizabeth Dewey[2], Hans-Willi Honegger[2], Benjamin White[1]. 1) Lab of Molecular Biology, NIMH/NIH, Bethesda, MD; 2) Department of Biological Sciences, Vanderbilt University, Nashville, TN.

Drosophila neurons that express Crustacean Cardioactive Peptide (CCAP) are required for wing expansion and cuticle tanning following eclosion. We have previously shown that enhancement of excitability in CCAP-expressing neurons (N_{CCAP}), using the bacterial sodium channel NaChBac, inhibits wing expansion and tanning. We used temperature-sensitive Gal80 (tub-Gal80^{ts}) to determine the developmental window during which enhancement of excitability acts to cause wing expansion deficits, and found that induction of NaChBac expression in N_{CCAP} during the last 25 percent of pupal development blocks wing expansion and cuticle tanning. To refine this time window, we made transgenic flies that express the cold-sensitive TRPM8 channel (UAS-TRPM8) and have shown that they can be used to acutely activate targeted neurons. By targeting expression of TRPM8 to N_{CCAP}, we have determined that activation of these neurons during the 9.5 h prior to eclosion leads to wing expansion deficits, with peak deficits occurring during a critical period between 1.5 and 5 h before eclosion.

In addition, we have examined the spatial requirements of enhanced excitability by restricting NaChBac expression to a subset of neurons (N_{burs}) within N_{CCAP} that expresses the hormone bursicon. To express NaChBac throughout N_{burs}, we made a burs-Gal4 driver line using the promoter region of the bursicon alpha-subunit gene. Expression of UAS-NaChBac with burs-Gal4 yielded animals with wing expansion deficits similar to CCAP-Gal4. Together, our results indicate that brief enhancement of activity prior to eclosion in bursicon-expressing neurons of N_{CCAP} interferes with a posteclosion event (i.e. wing expansion), possibly by interfering with N_{CCAP} network activity.

613A

Anatomical and Behavioral Defects in *Drosophila* Mushroom Body Mutants. Brian S. Dunkelberger, Christine N. Serway, Nicole W.C. Nolan, J. Steven de Belle. School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV.

Mushroom bodies (MBs) are paired neuronal assemblies that have been implicated as sensory integration and olfactory associative centers in the *Drosophila* brain[1]. Genes that influence MB development were initially identified in mutant screens for brain defects in the early 1980s[2,3]. Most remain poorly characterized in terms of their genetics and influences on anatomy and behavior. Here we describe the nature of MB reduction in three mutants: *mushroom body miniature B (mbmB)*[3,4], *small mushroom bodies (smu)*[4], and *mushroom bodies reduced (mbr)*[4]. Histological preparations viewed with fluorescence microscopy and planimetric measurements of the calyx verify the severe MB reductions previously reported in these mutants[4]. Mutant alleles were then combined with GAL4 enhancer elements[5,6] to target expression of green fluorescent protein in either the nuclei or the cytoplasm of MB neurons. Confocal laser scanning microscopy of whole mount adult brains revealed a reduction in cell number and aberrant patterns of axonal architecture in these mutants. Based on this analysis, it appeared that each gene affected the development of a restricted subset of lobes. We will look at the development of these flies at several key stages to determine when disruption occurs. A panel of immunohistochemical markers preferentially expressed in the mushroom bodies will be used to further characterize the mutant phenotypes. Finally, we will test these flies in a battery of behavioral assays to determine any defects caused by the mutations. 1. Heisenberg 2003 2. Heisenberg 1980 3. Heisenberg *et al.* 1985 4. de Belle & Heisenberg 1996 5. Brand and Perrimon 1993 6. Renn *et al.* 1999.

614B

Differential Induction of Short-term and Medium-term Memories by Appetitive and Aversive Reinforcements in *Drosophila* Larvae. Ken Honjo, Katsuo Furukubo-Tokunaga. Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.

The fruit fly *Drosophila melanogaster* has been utilized as a successful model to study underlying mechanisms of learning and memory. Because of its simple organization, the larva has recently attracted much interest and indeed provides us a useful system to identify neural networks involved in complex brain functions. Here, we show that appetitive and aversive memories are considerably different in their stability whereas both are localized to the mushroom bodies (MBs). We found that larval memory induced by appetitive sucrose reinforcement lasts six times longer than that induced by aversive quinine reinforcement despite comparable initial learning performances. By expressing shits1 in larval MBs, we demonstrated that disruption of neural output from MBs abolishes both appetitive and aversive memory traces. By contrast, sucrose but not quinine induces medium-term memory components that require *CREB* and *amnesiac* activities. Thus these data suggest that appetitive and aversive reinforcements stimulate different intracellular and/or intercellular signaling pathways generating different memory components in MBs.

615C

Nemy, a cytochrome B561, is required for memory formation in *Drosophila*. Konstantin Iliadi, Natalia Iliadi, Gabrielle Boulianne. The Hospital for Sick Children, Toronto, Canada.

Learning and memory represent fundamental examples of individual adaptations in higher organisms. Behavioral genetics approaches make it possible to understand these processes by revealing the specific genetic pathways that regulate the ability of animals to learn and remember. Here we report a molecular-genetics analysis of a *Drosophila* memory mutant called *nemy*. The first allele, *nemy*^{P153}, was identified in a P-element screen for mutants with 3-hour memory defects using a courtship suppression paradigm. However, similar memory defects were also revealed using the associative olfactory learning assay. Molecular characterization of *nemy* revealed that the gene encodes the *Drosophila* homolog of cytochrome b561 (Cyt651). In vertebrates, CytB561 has been shown to function as a transmembrane electron transport protein that is present in both small synaptic vesicles and large dense core vesicles where it plays a central role in the biosynthesis of several catecholamine and peptide neurotransmitters. Of note, *nemy* undergoes alternative splicing to produce 6 different transcripts, which encode 3 different proteins that exhibit tissue-specific expression. Interestingly, the largest *nemy* isoform is predominantly expressed in adult heads suggesting that this isoform may be responsible for memory formation. Within the adult brain, *nemy* is primarily expressed in mushroom bodies and antennal lobes. To unambiguously demonstrate that the memory defects were due to a loss of *nemy* function, we performed rescue experiments using the GAL4/UAS system. Specifically, transgenic flies were generated that expressed a cDNA corresponding to the longest isoform of *nemy*. These were then crossed into the *nemy*^{P153} mutant background along with an ELAV driver. We found that neuronal expression of *nemy* was sufficient to completely rescue the memory defects observed in *nemy*^{P153}. We propose that *nemy* give rise to memory loss due to defects in key neuropeptides within specific regions of the fly brain. Current studies are aimed at further elucidating the precise mechanisms by which *nemy* regulates memory formation.

616A

Heat shock drastically shortens the onset of deficits in memory and locomotion in a model for age-dependent neurodegenerative disorders,the mutants of the kynurenine pathway. Elena Savvateeva-Popova[1], Ekaterina Nikitina[1], Anna Medvedeva[1], Elena Tokmatcheva[1], Alexandr Peresleni[1], Andrei Popov[2], Peter Riederer[3]. 1) Pavlov Institute of Physiology,St Petersburg, Russia; 2) Sechenov Institute of Evolutionary Physiology and Biochemistry,St Petersburg, Russia; 3) Department of Clinical neurochemistry, Clinic and Policlinic of Psychiatry and Psychotherapy, University of Würzburg, Germany.

Neurodegenerative disorders, characterized by a late onset disturbance of memory, structural brain impairments and altered content of the intermediates of the kynurenine pathway.The ratio between kynurenate (KYNA),endogenous NMDA receptor inhibitor and the generator of oxidative stress 3-hydroxykynurenine (3-HOK)is critical for neuronal viability. The Drosophila mutants cinnabar (cn, KYNA excess) and cardinal (cd,3-HOK excess) allow to asses the specific roles of these metabolites. As shown previously, the mutant cd can serve as a model for dementia due to progressive decline in learning and memory and in the brain control of locomotion (parameters of sound production) over its life-span, accompanied by synaptic pathology and volumetric changes of the brain structures. Here, we tested the effects of HS în the main disease manifestations - impairments in 1) learning/memory and 2) locomotion. For this, we used a 30-min HS given at developmental stages, crucial for the formation of the brain structures implicated in learning and memory. Behavioral display was recorded in 5 day old flies after preceding HS given at the stage of formation of:the mushroom bodies (HS1); the central complex (HS2) or 1 hour before a test in adults (HS). Both the HS and HS2, with no effect in wild type and cn,lead to a drastic 6-fold decrease in memory retention and distortions in sound production in young cd mutants which are similar to age-dependent manifestations.Therefore,HS treatment can provide a model for the express-testing of putative drugs in experimental preclinical studies of neurodegenerative diseases.

617B

Exclusive requirement of NMDA receptors for long-term memory consolidation in *Drosophila* ellipsoid body. Shouzhen Xia[1], Chia-Lin Wu[2,3], Tsai-Feng Fu[2,3], Huaien Wang[1], Ying-Hsiu Chen[2,3], Daniel Leong[1], Ann-Shyn Chiang[2,3], Tim Tully[1]. 1) Beckman Neuroscience Center, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Sprg Harbor, NY 11724; 2) Institute of Biotechnology and Department of Life Science, National Tsing Hua University, Hsinchu 30013, Taiwan; 3) Brain Research Center, National Tsing Hua University, University System of Taiwan, Hsinchu 30013, Taiwan.

In humans and all other animals tested, memory consolidation occurs through multiple temporal phases and usually involves more than one neuroanatomical brain system. Genetic dissection of Pavlovian olfactory learning in *Drosophila* also has revealed multiple memory phases. While emerging studies suggest roles for antennal lobes and dorsal paired medial neurons, the predominant view holds that long-term memory (LTM) is consolidated in mushroom body (MB) neurons. Here, we demonstrate an acute requirement for NMDARs outside of MB during LTM consolidation. RNAi-mediated knockdowns of dNR1 or dNR2 in the cholinergic R4m subtype large-field neurons of the ellipsoid body (EB) specifically disrupt LTM. Similar knockdowns of NMDARs in MB disrupt an earlier memory phase (middle-term memory, MTM), leaving LTM intact. Our results established independent roles for NMDARs in EB during LTM consolidation and in MB during the MTM phase of early memory processing, and presented the intriguing possibility that LTM is stored outside of MB, the long-recognized anatomical locus of olfactory learning in insects. The demonstration that NMDA receptors are specifically required in EB rather than in MB for LTM consolidation shows for the first time that memory consolidation in an invertebrate model system clearly is a systems-level phenomenon, implying that the transfer of memory from one brain location to another during memory consolidation is evolutionarily conserved, in spite of huge anatomical (circuit) differences between flies and mammals.

618C

Dissection of a neuronal network required for wing expansion and tanning using the Split Gal4 System. Haojiang Luan, Fengqiu Diao, Kevin Ho Wan, Nathan Peabody, Benjamin White. Lab Molecular Biol, NIMH, Bethesda, MD.

Neurons that express crustacean cardioactive peptide (CCAP) are required at pupal ecdysis for head eversion and after eclosion for wing expansion and cuticle tanning. To determine which CCAP-expressing neurons are specifically required for each of these processes, we have used the Split Gal4 system to selectively ablate subsets of CCAP-expressing neurons. The Split Gal4 system is an extension of the classic Gal4-UAS technique that permits combinatorial restriction of gene expression (Luan et al., *Neuron*, in press). Using a hemidriver line in which the CCAP promoter drives expression of the Gal4 DNA binding domain (Gal4DBD) selectively in CCAP-expressing neurons in combination with enhancer-trap hemidriver lines that express the transcription activation domain of the VP16 transcription factor (VP16AD) in arbitrary patterns, we can selectively express UAS-transgenes in subsets of CCAP-expressing neurons. We have generated approximately 156 VP16AD enhancer-trap hemidriver lines to date and have screened 96 of them for overlapping expression within the CCAP group using UAS-EGFP and the CCAP-Gal4DBD hemidriver. Thus far, we have identified 25 lines that yield expression within subsets of the CCAP group. The numbers of CCAP-expressing neurons in these patterns range from 2 - 46 (of 50 total), and all patterns identified thus far are anatomically distinct, with some lines expressing exclusively in neurons of the abdominal ganglion, others solely in the brain, etc. We have begun to probe the functional identities of these subsets by targeted ablation using UAS-*reaper* and have identified several lines that exclusively produce deficits either at pupal ecdysis or in posteclosion processes. We are continuing to generate further VP16 AD enhancer-trap lines to compile a complete functional map of the CCAP-expressing neurons. We anticipate that this approach will be broadly useful in the dissection of neuronal network function.

619A

The proprotein convertase amontillado may function in larval growth and glucose homeostasis by processing Drosophila insulin-like peptides and adipokinetic hormone. Jeanne Rhea[1], Lowell Rayburn[1], Christian Wegener[2], Michael Bender[1]. 1) Department of Genetics, University of Georgia, Athens, Georgia; 2) Department of Biology, Phipps-University, Marburg, Germany.

Peptide hormones are potent signaling molecules that coordinate development, behavior, and physiology. Many peptide hormones are synthesized as inactive precursor molecules that must be proteolytically processed by proprotein convertases (PCs) to generate a biologically active molecule. Seven mammalian PCs have been identified, including Proprotein Convertase 2 (PC2), which is expressed in endocrine cells and acts to proteolytically cleave and activate a variety of substrates including proinsulin, proglucagon, and proopiomelanocortin. amontillado (amon), the Drosophila homolog of PC2, is expressed in neuroendocrine cells and may function in the regulation of growth and glucose homeostasis in Drosophila by proteolytically activating peptide hormones involved in these processes, including the Drosophila insulin-like peptides (dilps) and adipokinetic hormone (akh) - the analog of vertebrate glucagon. amon mutants exhibit growth defects similar to those observed in animals in which the insulin-producing cells have been ablated and amon protein co-localizes with Dilp2 in the insulin-producing cells. We have also shown that amon and components of the insulin signaling pathway genetically interact. Together, these data support the hypothesis that amon may proteolytically process one or more of the Dilps. Interestingly, trehalose levels are reduced in amon mutants, suggesting that amon may also process adipokinetic hormone. Consistent with this hypothesis, mature akh levels are greatly reduced in amon mutants as shown by mass spectrometric peptide profiling techniques. We are now determining the cell types in which amon function is required for normal larval growth and glucose homeostasis by reducing amon expression in specific subsets of cells through RNA inactivation. In a complementary approach, we are also restoring amon expression to specific cells in an amon mutant background, and assaying the effects on growth and glucose homeostasis.

620B

Evidence for Regulatory Interactions between Key Enzymes in Dopamine Synthesis. K. Bowling, C. Funderburk, D. Xu, Z. Huang, F. Ferdousy, J. O'Donnell. Biological Sciences, University of Alabama, Tuscaloosa, AL.

The cofactor tetrahydrobiopterin (BH_4) is critical in regulating the signaling molecules dopamine (DA), serotonin and nitric oxide. Cofactor deficits lead to defects in neurological processes such as learning and memory and have been linked to depression, dopa-responsive dystonia and Parkinson's disease. Recent evidence suggests that BH_4 modulates pain sensitivity and persistence. Regulation of cofactor pools resides with the first enzyme in its biosynthetic pathway, GTP cyclohydrolase (GTPCH). Because organisms must rapidly respond to environmental and developmental cues to adjust neurotransmitter output, complex regulatory mechanisms are vital for signal modulation. Interestingly, little is known about GTPCH regulation. *Drosophila* has three active GTPCH isoforms each containing a unique N-terminal extension. We have shown through kinetic analysis that the isoforms are regulated negatively via end-product feedback inhibition and this regulation is dependent upon N-terminal sequences. Feedback inhibition of mammalian GTPCH requires a protein, GTPCH feedback regulatory protein (GFRP), which is not present in the *Drosophila* genome. We suggest that sequences in the N-terminal domains of *Drosophila* GTPCH are functionally equivalent to mammalian GFRP. By conducting phosphorylation studies we have discovered that two GTPCH isoforms are positively regulated by phosphorylation as is the mammalian enzyme. These regulatory mechanisms mirror those of mammalian GTPCH and suggest that *Drosophila* GTPCH will serve as a model for studying GTPCH regulation. Protein-protein interaction methods have revealed that GTPCH isoforms physically associate with tyrosine hydroxylase (TH), the rate-limiting enzyme in DA production, and this interaction is dependent upon the phosphorylation state of each protein. We have undertaken kinetic analysis to test the consequences of the interaction between GTPCH and TH. Because these enzymes function closely in DA production, the physical association between these two proteins may affect enzyme regulation and serve to co-localize the key components of DA synthesis.

621C

DSERT mutants display altered cocaine responses, circadian rhythmicity, and startle responses. Noël C. Derecki, Erik Loken, Jay Hirsh. Biology, University of Virginia, Charlottesville, VA.

Serotonin transporters (SERTs) act to remove serotonin from the synaptic cleft. SERTs are key target sites for clinical drugs, as well as for drugs of abuse, including psychostimulants such as cocaine. Serotonin also has circadian and locomotor functions in Drosophila. To better understand the significance of SERT in Drosophila, we have targeted the dSERT sequence for mutagenesis via imprecise excision of a P[XP] element located 560 bp upstream from the transcription startsite. We identified two deletion mutants from 70 excisions, each with a ~175 bp deletion flanking the P insertion site.

Initial assays conducted using controlled exposure to volatilized free base cocaine show a significant decrease in sensitivity and sensitization in one mutant, and an increase in sensitivity and sensitization in the second as compared to wild-type. Real time RT-PCR will be performed to determine whether there is a correlation between the phenotypes and levels of dSERT mRNA expression. Locomotor and sleep assays performed with mutant flies entrained to a 12/12 L/D schedule demonstrate an increase in dark-phase locomotion, short and fragmented sleep, and a decreased amplitude of sleep-wake rhythm as compared to wild-type. Furthermore, a subsequent transition to D/D shows a total loss of rhythmicity in both dSERT mutants. Startle-response assays reveal a significantly reduced response in homozygous mutant flies to moderate and strong mechanical stimuli, and a reduced response in heterozygotes. This work will help to further clarify the role of serotonin and dSERT in both circadian and locomotor functions, and hopefully contribute to our knowledge of psychostimulant addiction.

622A

Monoamine neurotransmitter transporter expression in the Drosophila eye. Bernhard Hovemann, Guido Uhlenbrock, Anna Ziegler. Dept Chemistry, Ruhr Univ, Bochum, Bochum, DE.

We have previously shown that Ebony in vitro conjugates beta-alanine to six biogenic amines, including histamine (Richardt et al., 2003). The cysteine peptidase Tan in vitro hydrolyzes these b-alanyl conjugates. In the eye, Tan is expressed in photoreceptors and Ebony expression is restricted to epithelial glia cells. The two proteins are thought be components of a biochemical cycle, in which the neurotransmitter histamine is converted to carcinine in the glia and is subsequently hydrolyzed to histamine and b alanine after transport into the photoreceptor. In this putative cycle the interaction of Ebony in glia cells and of Tan in photoreceptors would require a transport of histamine/carcinine across cell membranes. Therefore, we are aiming at identifying transporters, which could provide the necessary function in the eye. A serotonin, a dopamine and the less specific vesicular monoamine transporter dVMAT have been described previously (Corey et al., 1994; Demchyshyn et al., 1994; Greer et al., 2005). Also, differential screenings revealed a number of computed genes with putative transporter function that are preferentially expressed in the eye (Thimgan et al., 2006). We determined by in situ hybridisation the cellular distribution of cDNA expression of these candidate transporters in the Drosophila head. The respective cDNAs were expressed in HEK cells to determine the specificity of transporter activity. Corey et al. 1994. Proc Natl Acad Sci U S A 91(3):1188-1192. Demchyshyn et al. 1994. Proc Natl Acad Sci U S A 91(11):5158-5162. Greer et al. 2005. J Neurobiol. Richardtet al. 2003. J Biol Chem 278:41160-41166. Richardt et al. 2002. J Comp Neurol 452(1):93-102. Thimgan et al. 2006. J Exp Biol 209(Pt 17):3383-3404.

623B

The 5-HT_2Dro receptor is expressed in the larva and adult CNS and modulates aspects of circadian and aggressive behaviors. Charles D. Nichols, Oralee Johnson. Department of Pharmacology and Experimental Therapeutics, LSU Health Sciences Center, New Orleans, LA.

Drosophila are known to express four serotonin receptor subtypes. These are the 5-HT_{1A}Dro and 5-HT_{1B}Dro, 5-HT_2Dro, and 5-HT_7Dro receptors. Significantly, the 5-HT_2Dro receptor is believed to be a functional ortholog of the mammalian 5-HT_2 receptor, which in humans has been most closely linked to neuropsychiatric diseases such as schizophrenia. Very little is known, however, about the expression of the 5-HT_2Dro receptor in the larva and adult, or the behaviors it mediates. Here we present data regarding the expression of the 5-HT_2Dro receptor in larva and adult CNS, and behaviors that this receptor modulates. The 5-HT_2Dro receptor is expressed in specific regions of the larva brain that dramatically change from early to late third instar. In the adult brain this receptor is expressed within the ellipsoid body, large field R-neurons, cells in the protocerebrum, glomeruli of the antennal lobe, a subset of gustatory neurons in the suboesophageal ganglia, and the lamina of the optic lobes. Behaviorally, the 5-HT_2Dro receptor modulates certain aspects of circadian rhythms, aggression, and negative geotaxis. Identifying the 5-HT_2Dro receptor circuitry and behaviors they mediate are significant steps towards understanding serotonergic function in the fly as well as conserved molecular mechanisms underlying behaviors mediated by 5-HT_2 receptors in mammalian systems.

624C

Pleiotropic behavioral phenotype of mutants of the Vesicular Monoamine Transporter. Anne F. Simon, Rafael Romero-Calderon, Anna Grygoruk, Hui-Yun Chang, Mordecai Solomon, David Shamouelian, Evelyn Salazar, David E. Krantz. Psychiat.and Biobehavior. Sci., UCLA , Brain Res Inst, Los Angeles, CA.

Aminergic signaling pathways have been implicated in a variety of neuropsychiatric illnesses and neurodegenerative disorders such as Parkinson Disease (PD). However, the mechanisms by which these pathways operate to affect behavior and neuronal degeneration remain obscure. To investigate these questions, we have cloned and characterized the Drosophila vesicular monoamine transporter (*dVMAT*). This gene has two splice variants: DVMAT-A, responsible for the vesicular storage of serotonin, dopamine and octopamine in the nervous system, and DVMAT-B, expressed in a subset of glia in the optic lobe that store histamine, a photoreceptor neurotransmitter. We have shown that over-expression of the DVMAT-A isoform in aminergic neurons has profound effects on behavior and also provides neuroprotection against toxic insults related to PD. To further determine how changes in DVMAT expression regulate behavior, we have characterized mutations in the *dVMAT* gene, affecting both isoforms. The *dVMAT* mutant phenotype includes altered locomotion and glutamatergic signaling in larvae and the conditional survival of adults. The viable adults are sterile, have a decreased longevity, and show reduced monoamine stores, including histamine. Their behavior is disrupted: they perform poorly in a geotaxis assay, but are more attracted to light, in the fast phototaxis assay. This finding suggests the possibility that mutation of *dVMAT* serves to paradoxically increase signaling in visual circuits despite the possible decrease in the histamine content of photoreceptor cells. The conditional survival, sterility and dopamine storage defects can be rescued using a DVMAT-A transgene using a ubiquitous driver. We will express DVMAT-A and DVMAT-B, as well as an RNAi against both isoforms, in particular aminergic cells and at specific times. These experiments will determine how amines regulate signaling at a glutamatergic synapse and the relationship of VMAT activity to various behaviors.

625A

Genetic and physical mapping of two spontaneous mutants that affect wing-beat frequency in *Drosophila melanogaster*. Phillip T. Barnes, Justine Miller, Slavina Georgieva. Biology Department, Connecticut College, New London, CT.

The genes for two spontaneous, recessive mutants that affect wing-beat frequency (WBF) are being mapped. Both genes, provisionally labeled *flt-2* and *wbf-2*, are on chromosome 2R. Using a tethering system and high-speed stroboscope to measure WBF, the *flt-2* mutants are unable to move their wings at all for flight (WBF = 0), while the *wbf-2* mutants have WBFs approximately 0.75-0.80 of the wild type rate (~165 vs. ~210 hz). The *flt-2* mutant maps genetically to position 106.8, and ongoing deletion mapping currently places the gene in cytological region 60A7 to 60A16. Preliminary genetic and deletion mapping places *wbf-2* at position 101.2 and tentatively in cytological region 58F3 to 59A1-3. Future work is directed at identifying the genes at the DNA level in the *D. melanogaster* genome database and, in the case of *flt-2*, examining the indirect and direct flight muscles at the ultrastructural level for any observable defects in the muscle fibers.

626B

Characterization of a fly Sensory Neuron Membrane Protein (SNMP) homologue. Harbinder Singh Dhillon[1,2], Kenny Fernandez[2], Richard Vogt[2]. 1) Biological Sciences, Delaware State University, Dover, DE; 2) Biological Sciences, University of South Carolina, Columbia, SC.

SNMPs are highly abundant proteins associated with receptor membranes of olfactory cilia in Lepidoptera. Initially identified and cloned from the antennae of Antherea polyphemus (wild silk moth), SNMP is a 69 kDa protein uniquely expressed in adult antennae and specifically localized in the receptor membranes of olfactory cilia. It has been shown earlier that SNMP expression is significantly greater in trichoid than in basiconic sensilla in male moths, and appears to express predominantly in one of the 2-3 neurons associating with trichoid sensilla. All of these findings were consistent with SNMP playing a central role in odor reception; however, SNMP is not a 7-transmembrane domain receptor and thus is not a member of the odor receptor family. Instead, SNMPs are members of a larger family of proteins characterized by the human CD36. The Drosophila genome contains at least 13 CD36/SNMP homologues, including emp, Croquemort and ninaD. The lepidopteran SNMPs are the only CD36/SNMP homologues known to associate with neurons. CG7000 is the closest Drosophila CD36/SNMP homologue to the lepodopteran SNMPs in sequence comparisons. We therefore focused our initial attention on characterizing the expression of the CG7000. Upstream fragments from CG7000 containing the presumptive regulatory region were used to drive cd8:gfp expresion in transgenic flies and characterized by fluorescent/con-focal microscopy. Our results show that CG7000 promter drives expression in subsets of chemosensory and mechanosensory neurons of adults, and chemosensory neurons of larvae, and that the CG7000 promoter is activated at around 65% of adult development in the pupa. These findings are consistent with the expression behavior of the lepidopteran SNMPs suggesting that CG7000 may be a suitable candidate for exploring SNMP function. We have directed dsRNAi against CG7000 mRNA and confirmed the knock-downs with real-time PCR, and are currently analyzing putative deficits in the knockdowns.

627C

Quantitative genomics of aggressive behavior in Drosophila melanogaster. Alexis Edwards[1], Stephanie Rollmann[1], Theodore Morgan[2], Trudy Mackay[1]. 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Division of Biology, Kansas State University, Manhattan, KS.

Aggressive behavior is important for animal survival and reproduction due to its role in the acquisition and defense of resources and mates. However, aggression can be an enormous social and economic burden for human society when it reaches pathological levels. While the role of biogenic amines in modulating aggression is well characterized, other genetic mechanisms affecting this complex behavior have not been thoroughly dissected. We developed an assay to rapidly quantify aggressive behavior in *Drosophila melanogaster*, and generated replicate selection lines with divergent levels of aggression. The realized heritability of aggressive behavior was ~ 0.10, and the phenotypic response to selection specifically affected aggression. Whole genome expression analysis identified 1,539 probe sets with different expression levels between the selection lines when pooled across replicates, at a false discovery rate of 0.001. We are quantifying aggressive behavior in co-isogenic lines that have mutations in a subset of these candidate genes, and have identified novel genes affecting aggressive behavior. Expression profiling of genetically divergent lines is an effective strategy for identifying genes affecting complex traits.

628A

MAPPING FLY COLOR-VISION CIRCUITS. Shuying Gao[1], Chun-Yuan Ting[1], Songling Huang[1], Ian A Meinertzhagen[2], Chi-Hon Lee[1]. 1) Unit on Neuronal Connectivity, NICHD, NIH, Bethesda, MD; 2) Life Sciences Centre, Dalhousie University, Halifax, Canada.

Drosophila color vision is mediated by three types of photoreceptor neurons (R1-R6, R7, and R8): R7 and R8 cells are most sensitive to UV and blue or green light, respectively, whereas R1-R6 are thought to function as a general luminance meter. Visual information gathered by all three types of R-cells converges at the medulla neuropil. All R-cells are histaminergic neurons while first-order interneurons express HisCl channels (ort). Ultrastructural analysis reveals that the first-order interneurons form synaptic connections with R7 or R8. To understand how color information is processed, we determined (a) the connection patterns of the first-order interneurons in the medulla, (b) their dendritic and axonal compartments, and (c) their expression of neurotransmitters and receptors. We identified eight types of the first-order interneurons, including one medulla intrinsic cell type, which communicates between the external and internal medulla neuropils, and seven types of transmedullary neurons (projection neurons). The projection neurons extend dendritic arbors in various medulla layers and project axons to the lobula neuropil to form a topographic map. The projection neurons, including TM5 and TM20, appear to receive input from two color-channels, suggesting that they might function as color-opponent or summation neurons. Our study suggests that fly color-vision circuits share a similar architecture to those of primates. Furthermore, these results highlight the lobula neuropil as a higher visual center for color-vision.

629B

Energy stores are genetically correlated with sleep but not altered by long-term sleep deprivation in Drosophila. Susan Harbison, Amita Sehgal. Department of Neuroscience, University of Pennsylvania Medical School, Philadelphia, PA.

Recent studies in humans indicate that an association between short sleep times and obesity exists. It is unknown, however, whether this association reflects a genetic correlation between obesity and endogenous sleep, or whether obesity may result from long-term sleep deprivation. We used *Drosophila melanogaster* as a model to distinguish between these two possibilities. To assess the connection between obesity and endogenous sleep, we measured sleep, glycogen, triglycerides, protein, and body weight in a panel of 143 *P*-element insertion lines; from this data, we calculated the genetic correlation between sleep phenotypes and energy stores. To assess the impact of long-term sleep deprivation on energy stores, we subjected four wildtype lines to chronic sleep deprivation. We measured glycogen, triglycerides, protein, and body weight in sleep-deprived flies and compared them to flies that were not sleep-deprived. We found sex-specific genetic correlations between sleep phenotypes and energy stores; however, long-term sleep deprivation per se had no impact on energy stores.

630C

Molecular and Behavioral Analyses of Transgenic α-Synuclein Flies. Ralph Hillman, Natalie Jerome, Nneka Isamah, Connie Yang, Darryl L'Heureux, Robert Pendleton. Dept Biol, Temple Univ, Philadelphia, PA.

Western blot analyses showed that flies homozygous for the human α-synuclein transgene contained α-synuclein in their central nervous systems. These transgenic flies showed a loss of both geotactic and locomotor activity in standard behavioral analyses. Phototactic activity was slowed, possibly due to a reduced locomotor response, but the final activity was not affected. Geotactic activity in these flies was restored by the addition to the medium of GABA. Geotactic activity was also restored in a dose dependent fashion by the addition of L-DOPA, a dopamine precursor, and by the addition of baclofen, a GABA-B receptor agonist. Neither of these latter compounds restored locomotor activity to the transgenic flies. A GABA-A receptor agonist, muscimol, had no effect on geotactic behavior in the transgenics. The data indicate a specific GABA receptor response for the restoration of geotactic behavior and a differential control of geotactic and locomotor activity.

631A

The CAFE assay allows precise measurement of ingestion in *Drosophila*. William W. Ja[1], Gil B. Carvalho[1], Noelle N. de la Rosa[1], Elizabeth M. Mak[1], Jonathan Liong[1], Ted Brummel[2], Seymour Benzer[1]. 1) Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125; 2) Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341-2116.

The available methods for studying feeding behavior in *Drosophila* suffer from a number of limitations. Food dyes provide low sensitivity and do not allow long-term measurements, while radioactive isotope-labeling assays cannot distinguish between ingestion and nutrient absorption. These methods also require sacrificing the flies. We describe the CAFE, a method allowing precise, real-time, unambiguous measurement of ingestion. The sensitivity of the CAFE allows the feeding behavior of single flies to be monitored over periods of time ranging from a few minutes to several days. Additionally, our method obviates the need for fly transfer or food additives such as dye or isotopes. As an illustration of the potential of the CAFE assay, we show that flies exhibit compensatory feeding in response to variations in medium concentration.

632B

A mutation in a putative Ste20 family gene eliminates ethanol-induced hyperactivity. Ian F.G. King[1], Linus Tsai[1], Ralf Pflanz[2], Herbert Jäckle[2], Ulrike Heberlein[1]. 1) Dept. of Anatomy, UCSF, San Francisco, CA; 2) Max-Planck Institute for Biophysical Chemistry, Gottingen.

We have identified EP(X)1455 as a mutant affecting sensitivity to multiple drugs of abuse. This line has a P-element insertion in the 5' UTR of the tao-1 gene, which encodes a putative serine-threonine kinase of the Ste20 family. Normal ethanol response has three phases - flies first startle in response to the smell of ethanol, then become hyperactive, then begin to sedate. EP(X)1455 flies do not become hyperactive in response to moderate doses of ethanol. However, at all doses tested they startle normally and sedate normally, implying that they are not impaired in their movement and can smell ethanol normally. They also absorb ethanol at a normal rate, indicating that they have normal ethanol metabolism. Mobilization of the P-element reverts the ethanol phenotype, restoring the hyperactivity response. The EP(X)1455 line is also resistant to the effects of cocaine and nicotine, as judged by a negative gravitaxis assay. The phenotype of EP(X)1455 is temperature sensitive - flies raised at 25?C have reduced hyperactivity, while flies raised at 18?C respond normally. Using temperature shift experiments we have determined that the critical interval for this temperature sensitivity is during pupation. This suggests that tao-1 might be required during metamorphosis for the development of structures required for ethanol-induced hyperactivity.

633C

Mutations in the α2-6 sialyltransferase gene DSiaT cause nervous system functioning defects. Kate Koles[1], Elena Repnikova[1], Yi Ren[2], Scott Selleck[2], Yi Zhou[3], Claire Haueter[3], Hugo Bellen[3]. 1) Dept Biochem & Biophysics, Texas A&M Univ, College Station, TX; 2) University of Minnesota Department of Genetics, Cell Biology, and Development, Minneapolis, MN; 3) HHMI and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

DSiaT codes for the fruit fly homologue of an a2-6 sialyltransferase, and represents the only vertebrate-like sialyltransferase gene in the Drosophila genome. We have previously characterized the in vitro enzymatic properties of DSIAT protein and in vivo expression pattern of this gene. In the larval brain, numerous motor-neurons, some cholinergic neurons and projection neurons express DSiaT. In the pupal and adult brain the optic lobes, specifically a subset of the medulla cortex and lobula plate neurons and GH146-Gal4 positive projection neurons stain positive for DSIAT protein expression, suggesting a role in visual and olfactory processing.

In order to better understand the function of sialyltransferase and to characterize the mutant phenotype of deficient flies, we generated DSiaT knock out flies. Analysis of mutant phenotypes showed that the DSiaT gene might be involved in neural transmission and functioning. Our data also suggested the possibility of neurodegeneration in DSiaT mutants. We are currently exploring the possibility of neurodegenerative phenotype in the first optic ganglion using electron microscopic and electrophysiological methods. We will describe the observed phenotypes in detail. The molecular mechanisms that cause these phenotypes have also been addressed and provide some new and interesting insights into molecular pathway that DSIAT might be involved in.

634A

Neuroanatomy of the central complex: a statistical approach. Mark H. Longair[1], Dean A. Baker[2], J. Douglas Armstrong[3]. 1) Neuroinformatics DTC, University of Edinburgh, Edinburgh, UK; 2) Department of Genetics, University of Cambridge, Cambridge, UK; 3) Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK.

We present the results of a semi-automated analysis of the neuroanatomy of the central complex of *Drosophila melanogaster*. This is based on whole-mount scans of adult brains from four P{GAL4} lines selected for interesting expression patterns in the central complex. Each brain is co-stained with the nc82 antibody, a common marker for neural architecture, although any marker in which distinctive points in the brain can be identified could be used. Using simple web-based tools for identifying landmark points in these scans, the expression patterns from multiple brains are warped into the *Drosophila* Standard Brain coordinate system. [4] Once mapped into this space we statistically analyze the connectivity between different regions of the fruit fly brain and the position of distinctive neuronal features. Using this method we can produce quantitative rather than descriptive neuroanatomical results, qualified with statistical error data. We present some novel neuroanatomical features discovered using this system. In addition, this system can be used to generate averaged images for the expression pattern of genes or reporters, or allow overlaying of arbitrary scans of the same region.

We are using the central complex as an example region of the brain to generate these results, but the method can be adapted to other regions of the brain. The tools for annotation and analysis are integrated into an online confocal scan archive which also allows simple sharing of data within a group and provides the benefit of easy centralized backup.

[4] Rein, K., Zöckler, M., Mader, M.T., Grübel, C., Heisenberg, M.: The *Drosophila* Standard Brain. *Current Biology.* 2002; 12(3):227-231.

635B

***Drosophila CG16801/NR2E3* modulates eclosion and wing expansion behaviors and fertility.** Steven Robinow, Qing Chang, Laura Wong, Gavin Ganzer, Nelson Lazaga, Elizabeth Nguyen, Michelle Varize, Carl Sung. Dept Zoology, Univ Hawaii, Honolulu, HI.

The NR2E subfamily of nuclear receptors is of particular interest to developmental neurobiologists because all characterized members of this subfamily appear to play a role in neural development or function. In *Drosophila* however, two members of this subfamily have yet to be studied, *CG10296* and *CG16801*. We have been characterizing *CG16801*, the ortholog of the vertebrate *photoreceptor specific nuclear receptor* gene, in an effort to develop a more comprehensive understanding of the role nuclear receptors play in neural development and function. *CG16801* cDNA was cloned by RT-PCR. Northern analysis demonstrates that *CG16801* generates two major transcripts of 3.3 kb and 2.2 kb. While the 3.3 kb transcript is the major species during early embryonic development, the 2.2 kb transcript becomes dominant during the mid and late stages of embryogenesis. The 2.2 kb transcript continues to be the dominant transcript during post-embryonic stages. *In situ* hybridization demonstrates that *CG16801* transcripts are localized within the central nervous system to a small number of cells in the brain and ventral nerve chord. To investigate the function of *CG16801*, a mutant allele was generated by homologous recombination. Animals lacking *CG16801* displayed one of three phenotypes. Nineteen percent of the mutant/deficiency animals failed to eclose. Twenty-four percent of the mutant animals eclosed but failed to expand their wings. The remaining 57% of the mutant animals eclosed and expanded their wings. However, all of these animals were sterile, independent of gender. We are testing the hypotheses that *CG16801* modulates the neuroendocrine control of eclosion and wing expansion, and that *CG16801* is required for the proper development or function of the neurons that innervate the testes and ovaries. A mosaic analysis should provide insights into the neuronal circuitry that modulates eclosion, wing expansion, and fertility.

636C

Neural control of respiration in *Drosophila*. Vikram Sudarsan, Helen Wiersma, Mark Krasnow. Department of Biochemistry/ HHMI, Stanford University School of Medicine, Stanford, CA.

The *Drosophila* adult tracheal system is a network of epithelial sacs and tubes that ramify throughout the body, delivering oxygen to the tissues and removing carbon dioxide. As in other insects, oxygen entry and carbon dioxide emission from the tracheal system is regulated by the spiracles, specialized valves located at the tracheal openings on the sides of adult flies. We are interested in identifying the molecular pathways and neural circuits that sense oxygen and carbon dioxide and control respiration. Towards this end, we developed an assay for direct monitoring of the status of the spiracles in living flies, and used the assay to demonstrate extremely rapid responses to hypoxia (low oxygen) and hypercapnia (high carbon dioxide) and changes in the patterns of respiration. We also characterized the anatomy of adult spiracles. A single muscle controls the activity of each spiracle. The muscle contracts to close the spiracle when internal oxygen levels are adequate, and the muscle relaxes and the valve opens to permit gas-exchange when internal oxygen levels are low or carbon dioxide builds up, as occurs for example during *Drosophila* flight (Lehmann, 2001). We have identified central and peripheral neurons that appear to innervate the spiracular closing muscle. We have also identified the first respiratory mutants using a candidate gene approach. The mutants show altered respiratory responses to acute changes in oxygen and carbon dioxide. The genes identified by the mutants are active in the nervous system, providing evidence that respiratory rhythms are neurally encoded. This establishes a tractable genetic system for the dissection of respiratory control in insects. Lehmann, F.O. Matching spiracle opening to metabolic need during flight in *Drosophila*. 2001. *Science* 294. 1926-1929*

637A

The genetic architecture of Drosophila locomotor behavior. Akihiko Yamamoto[1,3], Robert Anholt[1,2,3], Trudy Mackay[2,3]. 1) Dept Zoology, North Carolina State Univ, Raleigh, NC; 2) Genetics, North Carolina State Univ, Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State Univ, Raleigh, NC.

Locomotion is an integral component of most animal behaviors and a major component of fitness. Despite its profound importance, the genetic basis of locomotor behavior is poorly understood. To identify ensembles of genes that mediate locomotor reactivity, we screened 720 lines in which single *P[GT1]* transposons have inserted in a common isogenic background. Locomotion was measured the total period of mobility (s) in the 30s immediately following an acute mechanical stress. Statistical analysis showed that 269 lines (37%) were significantly less active than their co-isogenic control. These lines represent 156 transposon-tagged candidate genes of multiple gene categories, including genes involved in neurodevelopment and sensory organ development. To determine to what extent these genes interact as functional ensembles, we examined 15 lines with autosomal insertions in known genes. We created all possible 105 double heterozygote genotypes and 44 double homozygotes (4 second chromosome insertions x 11 third chromosome insertions) and tested their locomotor activity. We found extensive epistasis involving all of these mutations in both conditions. Whereas details of the interactions differed between double heterozygotes and homozygotes, P-element insertions *robo*, *Sema-1a*, *mir-317* and *HLHm7* show especially extensive enhancer and suppressor effects in both conditions. Thus, the genetic architecture of locomotor behavior is characterized by a highly interconnected epistatic network of pleiotropic genes. [Supported by NIH grants GM45146 and 59469].

638B

Two Genes Affecting *Drosophila* Gravitaxis. Sonia Bjorum, Kathleen M. Beckingham. Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

Using the vertical maze assay, we have isolated a collection of Drosophila P {GawB} mutants with defects in gravitaxis. Eighteen genes have thus been identified as having roles in gravitaxis, including several novel genes with unexplored functions. Many of theses genes have protein motifs that are conserved in higher organisms. Characterization of mutations affecting two of these genes will be described. Gene CG32423, also known as *alan shepard (alan)*, encodes a putative RNA binding protein similar to factors involved in mRNA processing. Gene CG16778, also known as *Tyrosine kinase-related protein (Tkr)*, has BTB-POZ and heliz-turn-helix domains, suggesting roles in ubiquitin-mediated degradation and transcription. Imprecise excision has been used to generate additional mutants of CG32423 and *Tkr* in order to understand more fully the roles of these genes. Molecular characterization of the excisions is ongoing. One of the CG32423 mutants shows even greater disorientation with respect to gravity than the original P{GawB} mutant. Expression of individual transcripts for both genes is being examined with *in situ* hybridization. Antibodies to the proteins encoded by these genes are being generated to study protein expression patterns.

639C

PHOSPHOLIPASE A2 AND ACYLTRANSFERASE ENZYMES INVOLVED IN *Drosophila melanogaster* OLFACTION AND VISION. Ismael Josafat Gimate-Baños, Juan Rafael Riesgo-Escovar. Development Biology, INB, UNAM, Querétaro, Querétaro, MX.

We are characterizing an acylglycerol-acyltransferase gene *nanahuatzin (nana)* and a *phospholipase A2* gene *(pla2)*, that have very restricted expression confined to olfactory and visual areas of *Drosophila melanogaster*. Previous work in the lab demonstrated that *nana* mutants have olfactory and visual defects in behavioral tests. Others have shown that *pla2* mutants have olfactory-memory problems. Both enzymes (Nana and PLA2) are involved in phospholipid metabolism, as these proteins catalyze opposite reactions, Nana in anabolism and PLA2 in catabolism of membranes phospholipids. We reasoned, both enzymes could be involved in the same pathway in the olfactory and visual systems. Our work focuses in the characterization of mutant allels of *nana* and of the *pla2*. Our results show that *pla2* mutant flies have similar olfactory defects to those of *nana* mutants, in behavioral tests in larvae and adults. In addition, and very surprisingly, *pla2* mutants also have visual defects in behavioral tests, again, similar to *nana* mutant. We generated a new independent *nana* allele with similar behavioral and electrophisiologal defects previously characterized in extant *nana* mutants. It is possible that *nana* mutant have synaptic problems between photoreceptor cells and lamina interneurons measured by electroretinograms, a defect that could signal more widespread synaptic problems. In this way, both *nana* and *pla2* are required for vision and olfaction.

640A

Sweet and bitter taste profiling in *Drosophila*. Beth Gordesky-Gold, Natasha Rivers, Osama Ahmed, Paul Breslin. Monell Chemical Senses Ctr, Philadelphia, PA.

The ability to perceive the taste quality of a food source is critical for the survival of an organism. Taste discrimination between nutritious and toxic substances leads to the acceptance or rejection of a potential food source, respectively. This discrimination is based on the perceived taste quality of that food source. Flies are omnivores and have very similar taste sensitivities as humans and other mammals with regard to attractants and repellents. Carbohydrates are a major food source for both mammals and flies, while many of the chemicals toxic to mammals are also avoided by flies. *Drosophila* sensitivity ranges for taste stimuli are within the perceived concentration ranges of humans. We have tested wildtype Canton-S (CS) males and females for their taste responses to a variety of compounds considered sweet or bitter by humans. Many of these compounds have never before been tested in *Drosophila*. Flies responded to and rejected many stimuli that humans find bitter. Flies reject quinine, caffeine, denatonium, and $MgSO_4$. On the other hand, flies appear insensitive to PROP, PTC, SOA, limonin, cyclo-LeuTrp, epicatichin, and naringin at the concentrations tested. CS flies were also tested for their taste response to fourteen high potency sweeteners, plus five amino acids, five sugars, two sugar alcohols, ethanol and a sweet salt ($PbCl_2$). We found that wildtype *Drosophila* responded appetitively to most high potency sweeteners preferred by humans, even those not considered sweet by rodents or new world monkeys such as; sodium cyclamate, aspartame, NHDC, Thaumatin and Monellin. We conclude that *Drosophila* taste responses are often more similar to human responses than are those of rodents and many primates.

641B

Functional roles for β1,4-N-acetlygalactosaminyltransferase-A in *Drosophila* Larval Neurons and Muscles. Nicola Haines, Bryan A. Stewart. Dept. Biology, University of Toronto, Mississauga, ON, CA.

Adult *Drosophila* mutant for the glycosyltransferase β1,4-N-acetlygalactosaminyltransferase-A (β4GalNAcTA) display an abnormal locomotion phenotype, indicating a role for this enzyme, and the glycan structures it generates, in the neuromuscular system. To investigate the functional role of this enzyme in more detail we turned to the accessible larval neuromuscular system and find that larvae mutant for β4GalNAcTA display distinct nerve and muscle phenotypes. Mutant larvae exhibit abnormal backward crawling, reductions in nerve terminal bouton number, decreased spontaneous transmitter release frequency and short, wide muscles. This muscle shape change appears to result from hypercontraction since the individual sarcomeres are shorter in mutant muscles. All of these phenotypes can be rescued by a transgene carrying the β4GalNAcTA genomic region. Tissue specific expression, using the Gal4-UAS system, reveals that neural expression rescues the mutant crawling phenotype, while muscle expression rescues the muscle defect. Tissue specific expression, did not appear to rescue the decrease in NMJ bouton number, suggesting this defect arises from co-operation between nerve and muscle. Altogether, these results suggests that β4GalNAcTA has at least three distinct functional roles.

642C

***touch insensitive larva B*, A Gene Necessary for Hearing and Male Fertility Encodes a Conserved Ciliary Protein.** Ryan G. Kavlie[1,3], Maurice J. Kernan[2], Daniel F. Eberl[1,3]. 1) Interdisciplinary Ph.D. Program in Genetics, Univ of Iowa, Iowa City, IA; 2) Department of Neurobiology and Behavior, State Univ of New York at Stony Brook, Stony Brook, NY; 3) Department of Biological Sciences, Univ of Iowa, Iowa City, IA.

Mutants in *touch insensitive larva B* (*tilB*) exhibit ciliary dysfunction in sperm flagella and the chordotonal organs that mediate adult hearing and larval touch sensitivity. Deficiency mapping placed *tilB* in cytogenetic region 20A on the X-chromosome. By sequencing candidate genes within this region, we identified two mutations in the gene CG14620 that correspond to both *tilB* alleles. *tilB*^{1} is a nonsense mutation in exon one, while *tilB*^{2} is an in-frame 6bp deletion in exon three. Rescue of adult hearing was obtained by genomic insertion of the wild-type gene. *tilB* is the *Drosophila* homolog of a gene conserved in ciliated eukaryotes; mutations of the zebrafish homolog have phenotypes associated with ciliary defects. TilB is a 395 amino acid protein with no predicted function yet has a highly conserved N-terminal leucine-rich repeat region from amino acid 16-145 and a coiled-coil domain from amino acid 171-191. The *tilB*^{2} deletion removes an alanine and a tyrosine, two non-conserved residues between two conserved C-terminal regions. RT-PCR data showed that *tilB* is transcribed in both heads and testes. A *tilB-Gal4* transgenic strain shows expression in mature sperm, chordotonal organs of the embryonic body wall, and the Johnston's organ, the auditory organ of the fly. A C-terminal TilB:GFP fusion protein shows localization to sperm flagella and to the outer dendritic segments of the Johnston's organ neurons including in the ciliary dilation. Experiments ongoing aim to determine the function of TilB conserved regions and the role of the TilB protein in axoneme construction and in auditory function.

643A

Characterization of mutants for jog, a gene with a role in gravity perception in Drosophila. Vanaja Konduri, Kathleen Beckingham. Dept Biochemistry & Cell Biol, Rice Univ, Houston, TX.

Using a vertical 8 choice-point maze as a screening assay, we have isolated a series of Drosophila P{GawB} mutants with defects in gravitaxis. This has led to the identification of 18 genes that have roles in gravity-based responses. Some of these are previously studied genes with known roles in neural processing; others are novel genes with no known roles in Drosophila. Our focus is on mutants affecting novel genes, in particular genes with conserved protein motifs suggesting particular molecular functions or genes with known homologs in higher organisms. We will present our characterization to date of gene CG11940 (named John Glenn, jog after American astronaut) a gene indicated to function in a receptor tyrosine kinase signaling pathway and gene CG7392, the Drosophila homolog of Striatin, a vertebrate WD-40 repeat-containing protein with roles in locomotor responses. Imprecise excision has been used to generate further mutants of both genes in order to gain insight into the full range of the activities of the gene. Two jog mutants have a deletion spanning 2.5 Kb and 1.6 Kb respectively that includes the translation start site for the longer isoform of the jog protein. We expect that the region of 128 amino acids specific to the longer isoform of the jog protein will not be expressed in these mutants in tissues affected by the P{GawB} insertion. An antibody against this region has been generated and immunolocalization studies are in progress. Expression of Gal4 from the jog P{GawB} mutant indicates that the brain and chordotonal organs in various tissues will be affected. Although less developed, comparable studies are in progress for CG7392.

644B

Two approaches to understanding the function of the RdgB protein. Christin M. Molnar, Kate R. Muenzer, Don W. Paetkau. Department of Biology, Saint Mary's College, Notre Dame, IN.

The *Drosophila* retinal degeneration B (RdgB) protein is the founding member of a family of membrane bound PITP proteins also found in zebrafish (pl-RdgB), mice (M-RdgB1 and M-RdgB2) and humans (Nir1-3). These proteins contain an amino-terminal phosphatidylinositol transfer domain (absent in Nir1 and pl-RdgB), a calcium binding domain, several hydrophobic domains and a conserved carboxyl terminus region required for proper protein localization. In the fly retinal cell, the RdgB protein localizes to the SRC membrane and functions in phototransduction. *Drosophila rdgB* mutants exhibit a defective electroretinogram light response followed by rapid light-enhanced retinal cell degeneration. Two screens were performed to provide insights into the function of the RdgB protein in *Drosophila* retinal cells: 1) a screen for suppressors of $rdgB^{KS222}$-mediated retinal degeneration and 2) a Clontech Matchmaker III yeast two-hybrid (Y2H) screen for proteins that directly interact with the carboxyl-terminus of the *Drosophila* RdgB protein. The *su(rdgB)69* suppressor was identified in the first screen. This mutation suppresses both the defective light response and retinal cell degeneration of the $rdgB^{KS222}$ mutation. The *su(rdgB)69* mutation was mapped to 100B5-C4 region of chromosome 3. It failed to complement the *su(rdgB)102* mutant and the $P\{SUPor\text{-}P\}CG31006^{KG09937}$ P-element, located within the *CG31006* gene. The molecular defect in the $CG31006^{su(rdgB)69}$ allele was identified as an insertion in its fifth exon of a *hobo* transposon. The molecular defect in the $CG31006^{su(rdgB)102}$ allele appears to be a deletion in the same region. Further characterization of the role of the *CG31006* gene in retinal degeneration suppression will be presented. The second screen, performed by Saint Mary's College Molecular Cell Biology students, produced a large number of candidate RdgB-interacting proteins. False positives are common in Y2H screens and so the candidates were tested for their ability to activate the Y2H system in the absent of a specific RdgB bait protein. The results of these tests will be presented.

645C

Oxygen-sensitive soluble guanylyl cyclases mediate larval hypoxia escape responses. David Morton, Anke Vermehren. Integrative Biosciences, Oregon Health & Science Univ, Portland, OR.

Conventional soluble guanylyl cyclases (sGCs) are stimulated by NO to synthesize cyclic GMP (cGMP) and mediate a wide variety of physiological processes. We have recently shown that three *Drosophila* genes, *Gyc-88E*, *Gyc-89Da* and *Gyc-89Db* code for sGC subunits that respond poorly to NO and are activated by reduced O_2 levels raising the possibility that they act as O_2 sensors signaling when an animal is in an hypoxic environment. The expression of these genes is consistent with this function as all three genes are expressed in a subset of CNS and sensory neurons. To test this hypothesis we have identified the promoter region for two of the genes, *Gyc-89Da* and *Gyc-89Db* and used the UAS-GAL4 system to disrupt the cGMP signaling cascade in these neurons. Reducing the levels of cGMP by expressing a cGMP-specific phosphodiesterase in the *Gyc-89Da* or the *Gyc-89Db* neurons significantly reduced the time taken for larvae to initiate an escape response when exposed to 5% and 10% O_2. Similarly, when levels of the PKG, dg1¸ are reduced by expressing dg1 dsRNA in these neurons we also measured significantly reduced escape responses. It is notable that there is no difference in the behavioral responses whether cGMP signaling is disrupted in either *Gyc-89Da* or *Gyc-89Db* neurons despite our findings that there is little overlap in the expression patterns of these genes. The biochemical properties of these two subunits are indistinguishable - both are inactive on their own, but require co-expression with *Gyc-88E* for activity. Chemotaxis in response to certain odors and tastants are also disrupted in these larvae (See abstract by Vermehren and Morton), but here the responses differ depending on whether the cGMP signaling is disrupted in either *Gyc-89Da* or *Gyc-89Db* neurons. This suggests that the neurons responsible for mediating hypoxia escape responses are those that express both *Gyc-89Da* and *Gyc-89Db* in addition to *Gyc-88E*. Candidate O_2 sensors are sensory neurons that innervate the caudal sensory cones and basoconical sensilla along the lateral body wall of larvae.

646A

Larval lethality in mutations of *nervana 3*, which encodes the beta subunit of Na/K ATPase. Madhuparna Roy[1], Ryan G. Kavlie[2], Daniel F. Eberl[1,2]. 1) Dept of Biological Sciences, University of Iowa, Iowa City, IA; 2) Genetics PhD Program, University of Iowa, Iowa City, IA.

The plasma membrane-localized Drosophila Na/K ATPase is dependent on the beta subunit for its transport to the plasma membrane and for regulating its activity. Two beta subunits have been characterized in detail, Nervana1 and Nervana2, which are both expressed broadly in the nervous system and epithelia. *In situ* hybridization shows that a third beta subunit gene, *nervana 3 (nrv3)*, is expressed in a subset of CNS neurons and, peripherally, in chordotonal organ neurons. This expression pattern suggested the possibility that *nrv3* could be important for hearing, mediated by the antennal chordotonal array called Johnston's organ. The *nrv3* (CG8663) gene is located at the 39D2 region of the second chromosome. We currently have three *nrv3* mutant alleles, two of which (nrv^{15} and nrv^{47}) were created by imprecise excision of the NP6215 Gal4 insertion, located near the 5'end of the *nrv3* gene. The third mutant allele, $nrv3^{f04395}$, is a piggyBac insertion just 5' of the fourth coding exon. All *nrv3* alleles result in homozygous lethality at early larval stage. Our immunohistochemical studies in late stage embryos show that the alpha subunit localizes to the plasma membrane of CNS neurons, while Nrv3 localizes to the PNS, especially the lateral pentascolopidial organ (lch5), as well as a subset of the CNS. These data agree with our *in situ* hybridization studies. Finally, the NP6215 Gal4 insertion drives reporter expression in a similar pattern. This driver also expresses in some of the adult PNS, including chordotonal organs, suggesting that *nrv3* has the potential to play a role in fly hearing. Experiments are in progress to characterize the details of the larval lethal phenotype, to rescue the lethal mutant phenotype using the UAS/Gal4 system, as well as to construct mosaic animals using the MARCM technique with a view to testing auditory function of *nrv3* in Johnston's organ.

647B

The gravitaxis-affecting protein Yuri interacts with the actin cytoskeleton. Michael J. Texada[1], Cassidy B. Johnson[1], Rebecca A. Simonette[1], Ravi P. Munjaal[1], J. Douglas Armstrong[2], Kate M. Beckingham[1]. 1) Biochemistry and Cell Biology, Rice University, Houston, TX; 2) Bioinformatics, University of Edinburgh, Edinburgh, Scotland, UK.

The force of gravity is a common stimulus affecting the behavior of most animals. We conducted a screen for mutants with aberrant behavioral responses to gravity [Armstrong et al., 2006; Genes Brain Behav. 5(3):222-39], using the maze assay developed by J. Hirsch. One of these genes we named "*yuri*," after Yuri Gagarin. The mutation at the *yuri* locus is a GAL4 enhancer-trap insertion 40 bases 5' of the *yuri* transcriptional start site. GAL4 reporter activity is widespread during pupal development and subsequently narrows to a subset of chordotonal neurons in the legs (femoral chordotonal organ) and antennae [Johnston's organ (JO), the organ of hearing in Drosophila], suggesting that the phenotype of the insertion mutant might arise from defects in these anatomical structures.

Yuri is ubiquitously expressed during development and appears to colocalize with actin-rich structures. In Yuri underexpressers, the actin-rich "investment cones" necessary for spermatid individualization are observed to disintegrate after their formation, causing a lack of mature sperm and, consequently, male sterility. Overexpression results in sluggishness and defects in the organization of the JO scolopale rods.

Tandem affinity purification ("TAP-tag") co-purification assays indicate that Yuri forms complexes containing components of the actin cytoskeleton, including actin itself, muscle myosin II (*Mhc*), the structural protein Zeelin1, the contraction-regulatory protein troponin T (*upheld*), and the filament-stabilizing protein tropomyosin (*Tm1*). Yuri also interacts genetically in synthetic-lethality assays with *zipper*, *Mhc*, and *didum*. Together, these results suggest that Yuri mutations may affect gravitaxis via effects on the actin cytoskeleton, particularly in the scolopales of chordotonal organs.

648C

Identification of the adenylyl cyclase that mediates sugar perception in *Drosophila*. Kohei Ueno[1], Yoshiaki Kidokoro[2]. 1) Department of Behavioral Sciences, Graduate School of Medicine Gunma University, Maebashi, Gunma, JP; 2) Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Gunma, JP.

In *Drosophila* and mammals, sugar perception is initiated by a specific G-protein coupled receptor in gustatory-receptor neurons. We previously identified the gene for the sugar receptor in *Drosophila* as *Gr5a*. By genetic and physiological analyses, we found that the G-protein coupled to Gr5a is DGs, a homolog of mammalian Gs. In mammals, it is known that the Gs family is coupled to adenylyl cyclase that generates cAMP. Hence, it is likely that adenylyl cyclase and cAMP mediate the transduction pathway for sugar perception in *Drosophila*. In *Drosophila* genome, there are 13 adenylyl cyclase genes. We then asked which adenylyl cyclase is required for sugar perception and found two adenylyl cyclase genes, *AC78C* and *ACXE*, are expressed in gustatory organ by the RT-PCR analysis. The expression level of *AC78C* was decreased in transgenic flies lacking sugar-sensitive gustatory-receptor neurons. We generated transgenic flies expressing double-stranded RNA against these adenylyl cyclase genes in sugar-sensitive gustatory-receptor neurons by the GAL4/UAS system. The electrophysiological responses to sugar in sugar-sensitive gustatory-receptor neurons were impaired in the transgenic flies carrying UAS-RNAi against *AC78C*. Finally, we generated mutant flies of *AC78C* by imprecise P-element excision. Behavioral and electrophysiological responses to sugar were also impaired in the mutant flies. Taken together, we conclude that this adenylyl cyclase gene is required for sugar perception, and suggest that cAMP conveys sugar signaling initiated by Gr5a in the sugar-sensitive gustatory-receptor neurons.

649A

Atypical soluble guanylyl cyclases in Drosophila may be involved in feeding preference behaviors. Anke Vermehren, David Morton. Integrative Biosciences, OHSU, Portland, OR.

The intracellular second messenger cGMP regulates many different physiological processes including neuronal development and sensory physiology. The Drosophila genome contains five genes that code for soluble guanylyl cyclase (sGC) subunits. Two of these are the conventional Drosophila α and β subunits which form obligate NO-sensitive α/β heterodimers. The remaining genes code for the atypical sGC subunits and include Gyc-88E, Gyc-89Da and Gyc-89Db, which are relatively NO-insensitive. Recent studies of members of the atypical sGCs, have identified them as likely molecular O_2 sensors in invertebrates (see abstract by Morton and Vermehren). Using promoter-GFP transformed flies, Gyc-89Da and Gyc-89Db expression has been localized to the CNS and peripheral sensory neurons; and no co-expression was observed with the exception of a very small subset of neurons. Gyc-89Da and Gyc-89Db are expressed in some neurons of the dorsal and terminal organs (perception of volatile and contact chemo-receptors respectively) in larvae. To determine the role played by these two atypical sGC subunits in feeding preference behaviors we used the GAL4/UAS system to express a cGMP-specific phosphodiesterase (PDE5) to break down the natural production of cGMP in these cells, as well as double-stranded RNA complementary to a cGMP-dependent protein kinase (dg2), all under the UAS promoter. We tested several odorants (propionic acid, 1-butanol, cyclohexanone, ethyl acetate and n-octyl acetate) and tastants (sucrose, fructose, glucose, maltose, trehalose, quinine and NaCl) using standard larval feeding preference assays. Reduction of cGMP and dg2 levels in Gyc-89Da neurons inhibited attraction to ethyl acetate but had no effect on the response to any tastant whereas reduction of cGMP and dg2 levels in Gyc-89Db neurons inhibited responses to n-octyl acetate and sugars. Activation of the atypical sGCs with reduced oxygen levels restored the ability of these animals to respond to sugars. *Support contributed by: NS29740*.

650B

Molecular and Neural Regulation of Social Response to Aversive Stimuli in Drosophila melanogaster. Jie Xu, Ping Shen. Department of Cellular Biology, University of Georgia, Athens, GA.

Diverse animals including mammals and insects display cooperative behavior in response to stressful stimuli. However, little is known about how such complex social behaviors are initiated, maintained and terminated. We previously showed that developmental downregulation of a neuropeptide Y-like activity in maturing D. melanogaster larvae triggers food-averse migration (also known as wandering, Wu et al, 2003; Neuron 39:147-61); On hard food media, newly-emerged wandering larvae instinctively take on a group burrowing behavior, providing a rare opportunity to elucidate the neuronal network and underlying molecular processes that regulate social response to aversive cues.

In this report, we show that larval group burrowing involves sensation of chemical and mechanical cues. The fly nociceptive gene (pain), which encodes a transient receptor potential (TRP) ion channel protein, is essential for sensing noxious food stimuli that elicit the group burrowing. We also show that the neural activity of cAMP-dependent protein kinase (PKA) regulates food-conditioned group burrowing behavior. PKA overexpression directed by a pain promoter led to food-independent group burrowing. We also identified and characterized a genetic mutation that renders animals to burrow solitarily but in nearby sites. Our data suggest that PAIN/PKA sensory neurons modulate at least three downstream neuronal pathways that differentially control converging, grouping and burrowing behaviors.

651C

A Potential Role for the Cytoskeletal Linker Protein, Moesin, in *Drosophila* Neuromuscular Junction Morphology. Sara Seabrooke, Bryan A. Stewart. Department of Biology, University of Toronto, Mississauga, Ontario, CA.

Although it is known that actin plays a role in synaptic development, the precise role of actin and actin-binding proteins remains unknown. Moesin is a member of the ERM family, which link F-actin to the plasma membrane. Moesin is the only ERM homolog present in *Drosophila* providing a unique opportunity to gain an understanding of the role of ERM proteins in actin dynamics. In the neuromuscular junction (NMJ), overexpression of Moesin was able to rescue the morphology of NSF2-induced NMJ overgrowth. This NSF mutant is known to have reduced synaptic strength and reduced F-actin in the nerve terminal. To further investigate the potential role of Moesin in NMJ development, immunocytochemical quantification of NMJ branch length was completed for gain-of-function and loss-of-function *moesin* in conjunction with the overgrown phenotype. Loss-of-function *moesin* enhanced the NMJ overgrowth indicating a likely role for Moesin in normal NMJ development. However, using electrophysiology it was determined that while overexpression of Moesin rescues the morphology, there was not a corresponding rescue of synaptic strength in the NSF2 mutant. Together this implies a role for *moesin* at the NMJ as well as implying the possibility that there are distinct mechanisms involved with the morphology and physiology of the *Drosophila* NMJ.

652A

A conserved muscle differentiation complex in *Apis mellifera* and *Tribolium castaneum*. Jessica Cande, Michael Levine. Department of Molecular & Cell Biology, UC Berekely, Berkeley CA.

There are only a handful of developmental control complexes, such as the Hox complex, the *Enhancer of split* complex or the *achaete-scute* complex conserved across the sequenced insect genomes. One notable example is a 200 kb cluster of homeobox transcription factors (*tinman*, *bagpipe*, *ladybird late*, *ladybird early*, *C15* and *slouch*) that control muscle differentiation. This complex is present in both the flour beetle *Tribolium castaneum* and the honeybee *Apis mellifera*, with the inclusion of an additional member *msh*. I have used a combination of bioinformatics methods, microarray tiling data and *in situ* hybridization to look at functional versus phylogenetic homology of these genes in the honeybee and the flour beetle. I propose a model for the evolution of an ancestral muscle differentiation complex. This model focuses on the duplication and divergence of *msh* expression in beetles and in honeybees. Understanding the evolution of this complex and the regulatory linkages within it may help explain why the physical linkage of these genes has been maintained across 300 million years of evolutionary time.

653B

Analysis of Netrin Expression in *Artemia franciscana* provides Evidence for Conserved Roles of Netrins during Arthropod Development. Molly Duman-Scheel, Stephanie Clark, Eric Grunow, Andrew Hasley, Brandon Hill, Wendy Simanton. Dept. of Biology, Albion College, Albion, MI.

Although many similarities in arthropod CNS development exist, differences in midline cell formation and ventral nerve cord axonogenesis have been noted in arthropods. It is possible that changes in the expression of axon guidance molecules such as Netrin, which is expressed by a subset of *Drosophila* midline cells and functions to regulate commissural axon guidance, may parallel these differences. In this investigation, we investigate this hypothesis by examining Netrin expression during development of the brine shrimp *Artemia franciscana*, a branchiopod crustacean. An *Artemia franciscana netrin (afrnet)* orthologue was cloned. An antibody to the afrNet protein was generated and used to examine the pattern of afrNet expression during brine shrimp development. Despite differences between fly and brine shrimp nerve cord development, examination of afrNet expression suggests that this protein regulates axon guidance during *Artemia* CNS development. However, midline expression of afrNet and commissural axon guidance occurs at a relatively later time point in *Artemia* as compared to *Drosophila*. Expression of afrNet in a subset of midline cells that closely resemble Netrin-expressing cells at the *Drosophila* midline provides evidence for homology of midline cells in arthropods. Expression of Netrins in many other tissues is comparable, suggesting that Netrin proteins may play many conserved roles during arthropod development. The discovery that the afrNet antibody recognizes Netrin proteins in many arthropods will enable examination of Netrin expression in additional species.

654C

Molecular Population Genetics of a cis-regulatory network in *Drosophila melanogaster*. Ian Dworkin[1,2], Greg Gibson[1]. 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Department of Biology and Carolina Center for the Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

In *Drosophila* cis regulatory modules have been functionally characterized for a large number of genes which span multiple developmental functions. In particular, the molecular characterization of these regions focuses on minimal enhancer elements which can recapitulate native transcript expression. Computational analysis and discovery of cis regulatory elements in the genome often rely on the information from these minimal enhancers as “training sets”. While particular transcription factor binding sites are often degenerate, it is unclear if this impacts the accumulation of polymorphism in natural populations. In particular whether the transcription factor binding sites will show relatively high levels of polymorphism in comparison to nearby sites that are not functionally constrained. To address this question, we have surveyed large samples of alleles from two North American populations of *D. melanogaster* for a number of minimal enhancer elements that all participate in the genetic network involved with wing patterning. We present the results of our tests of the hypothesis of increased functional constraint for the transcription factor binding sites, and the minimal enhancers as a whole. In addition, preliminary data pertaining to the association of polymorphism with wing shape for these lines will be presented.

655A

Rapid evolution at some, but not all, proteins involved in the control of Drosophila germline stem cell differentiation. Heather A. Flores[1], Vanessa L. Bauer DuMont[1], Aalya Fatoo[1], Mohammed A. Hijji[2], Diana Hubbard[3], Danial A. Barbash[1], Charles F. Aquadro[1]. 1) Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY; 2) Cornell Medical College in Qatar, Doha, Qatar; 3) University of Chicago, Chicago, IL.

Drosophila germline stem cell (GSC) differentiation is controlled at several levels by numerous genes. GSCs are present in a niche environment, and close proximity to the niche allows for multiple, short-range mechanisms to repress stem cell differentiation. In addition, genes expressed within the GSCs also help these cells to maintain their identity as stem cells. GSCs undergo asymmetric division and the daughter cell that is moved out of the niche begins to differentiate. Given the intricate control exhibited in this pathway, one might have expected that the proteins involved would be under extreme functional constraint. However, recent work has shown that two of these proteins, Bag of marbles (Bam) and Benign gonial cell neoplasm (Bgcn), are rapidly evolving in *Drosophila melanogaster* and *D. simulans*. With the goal of better understanding the selective forces acting on this pathway, we have surveyed DNA sequence variation at several other genes involved in the control of GSC differentiation. Analyses of polymorphism and/or divergence have revealed evidence of recurrent and recent adaptive fixations in some but not all genes in this pathway. We hope to use a "selection map" coupled with putative protein/gene interactions to test alternative hypotheses as to what intrinsic or extrinsic factors are shaping the molecular evolution of this critical differentiation pathway.

656B

Genotype x environment interaction in response to novel stress combinations. Clayton Hallman, James Thompson. Zoology Dept, Univ Oklahoma, Norman, OK.

Physical stresses can affect the stability of development, as measured by deviations from phenotypic symmetry (fluctuating asymmetry, FA), but both stresses and traits differ in their effect. Our interests in these relationships were initiated by questions of adaptation to novel or marginal habitat conditions, like those that could be experienced by organisms adapting to a space environment. Physical stresses there can include vibration, periodic hypergravity, microgravity, increased radiation, and temperature fluctuations. We currently focus on vibration, hypergravity, and their interaction. The hypothesis is that exposure to one stress, such as hypergravity, can induce a protective stress response that will reduce the impact of subsequent exposure to a different stress, such as vibration. In addition to treating virgin females with hypergravity (5g for 4 hours) and vibration (modeled after Shuttle launch profiles), a group was given the sequential stress of hypergravity followed within one hour by the vibration exposure to assess the hypothesized protective benefit of a single condition activating a generalized stress response (cf., hormesis effect). Females were then mated and their offspring were collected in three 3-day broods. Resulting trends from Drosophila wing shape morphometrics in wild type, stress resistant, and stress sensitive strains support a model of differential stress reaction among brood periods and reduced FA in response to sequential physical stress exposures. (Supported by NASA grant NCC 2-1355).

657C

Understanding the evolution of cis-regulatory sequences determining patterns of gene expression in *Drosophilids*. Karolina M. Jastrzebowska, Pavel Tomancak. MPI-CBG, Dresden, DE.

The development of a multi-cellular organism from one cell is achieved through finely regulated gene transcription. It leads to formation of cells that express certain sets of genes and in turn acquire identity and ability to build tissues and organs that will fulfil specialized tasks. The fine-tuning of spatiotemporal gene expression is provided by *cis*-regulatory regions of DNA, which contain multiple binding sites for transcription factors (TFs). Depending on availability of a unique combination of TFs the expression of a certain gene is switched on or off.

Drosophilids can provide an insight into how this process evolved and how it differs from one species to another. Availability of 12 fully sequenced fruit fly genomes allows comparing sibling species as well as those that diverged from each other as long ago as 40 million years, yet have similar development and remain closely related. We aim to identify differences in gene expression regulation between them and directly link these changes to the divergence in genome sequence.

In a pilot experiment we used atlas of patterns of gene expression in *D. melanogaster* embryos to select ~150 genes with specific expression patterns. We compared expression of these genes in *D. melanogaster* and *D. pseudoobscura* by RNA *in situ* hybridization. Apart from highlighting the technical difficulties associated with such approach, this study also revealed significant differences in expression patterns of at least 6 genes. We are currently performing a detailed study of one gene in order to develop a toolkit necessary to work with different *Drosophilids*.

Having the methods optimized we will expand the number of genes examined. The genes of our particular interest are TFs that represent the core of the transcriptional regulatory network. Additional non-TF candidates will be genes with divergent regulatory sequences identified by multiple genome alignments, especially genes located at the borders of synteny breaks.

658A

Evolution of Ubx Transcription Activation Domains. Ying Liu, Kathleen Matthews, Sarah Bondos. Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005.

Hox proteins define regional identities along the anterior-posterior (A-P) axis and are critical throughout animal development. The ability of Hox proteins to rearrange body plans when mutated or misexepressed suggests these factors are also key to the morphological evolution of animals. Given the high conservation of Hox DNA-binding homeodomains, transcription activation and repression domains are likely to be customized during evolution to provide the diversity across species. To reveal the underlying molecular basis, we are focusing on the identification and comparison of the transcription activation domains in Ultrabithorax (Ubx) orthologues, including Ubx from the beetle Tribolium castaneeum (Tc Ubx), the butterfly Junonia coenia (Jc Ubx), the shrimp Artima franciscana (Af Ubx), and the Onychophoran velvet worm Akanthokara kaputensis (Ak Ubx) using a yeast one-hybrid system. This system allows us to isolate domains with intrinsic activity of transcription activation from in vivo assays, independent of DNA binding, transcription repression, or protein interactions. We further compared the identified Ubx activation domains with the previously dissected Ubx activation domain from Drosophila Melanogaster (Dm Ubx). Our studies demonstrate fly and shrimp Ubx proteins possess stronger activation domains than those of beetle, butterfly and worm. An ancestral activation domain was located that contains both evolutionarily conserved motifs and unique sequences. Unique sequences enriched in different amino acids are required by different Ubx proteins suggesting the possibility of distinct mechanisms of transcription activation. We also localized transcription repression regions and demonstrate the core activation domain in Dm Ubx maps to a homologous protein region that represses transcription in other species. Thus, whereas some aspects are conserved across species, transcription regulation appears to be a rapidly evolving function.

659B

Genetic analysis of segmentation patterns in Drosophila: gap and pair-rule gene expression in relation to embryo size within and between species. Susan E Lott[1], Michael Z Ludwig[2], Arnar Palsson[2], Martin Kreitman[1,2]. 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

Egg size is an adaptive trait in Drosophila, varying clinally within species and differing between species. Because the A-P axis in Drosophila is established by diffusion of maternally supplied morphogens, egg length can be viewed as an external challenge to mechanisms establishing precision in the spatial localization of segmentation landmarks. Here we carry out genetic analysis of gap and pair-rule gene expression patterns in two strains of *D. melanogaster*, India (In) and France (Fr), which produce eggs (and embryos) differing by approximately 25% in egg length when reared under identical conditions. We also analyze crosses between *D. simulans* and *D. sechellia* isolines, which also differ by approximately the same magnitude. Previous analysis showed that the establishment of the gap genes Kruppel and giant and the pair-rule gene even-skipped, is robust to genetic variation in embryo size in four *D. melanogaster* strains but differs between two closely related species, *D. simulans* and *D. sechellia*. Reciprocal crosses of Fr and In isolines to F_3's provided additional evidence for robust positioning of expression patterns within *D. melanogaster*. Regardless of differences in embryo size, which is maternally inherited, and the number of novel genotypes these crosses created, localization of expression domains relative to embryo length remained constant. These results confirm our previous finding showing robust stripe positioning in *D. melanogaster*.Pattern formation in hybrids between *D. melanogaster* w1118 and *D. simulans* and between *D. simulans* and *D. sechellia* are both consistent with maternal inheritance of the stripe positioning phenotype. F2 backcrosses between *D. simulans* and *D. sechellia* produce stripe patterns at intermediate positions along the embryo compared to the parental strains, indicating additivity of this trait. This shift in patterning in the backcross embryos provides genetic evidence for the evolution of stripe "setpoints" between species.

660C

Do host plant toxins protect Drosophila larvae from wasp parasitism? Neil Milan, Todd Schlenke. Department of Biology, Emory University, Atlanta, GA.

Drosophila species utilize a wide variety of host plants, many of which contain toxins. Certain fly species have evolved resistance to the toxins, and some have even evolved a preference for these toxins. Shifting to a normally toxic host plant may allow these species access to an underutilized food resource or may be a means of escaping parasitism. One possible example of the latter is the rarity with which cactophilic and fungivorous fly species are attacked by parasitic wasps. We set out to test 1) the level of resistance of various wasp parasitoids of Drosophila to toxins naturally found in Drosophila host plants, 2) whether parasitic wasps prefer to attack host larvae grown on standard Drosophila media as opposed to standard media supplemented with various natural plant toxins, 3) and whether wasp eggs are less likely to survive in Drosophila larval hosts grown on toxic media. The following combinations of flies and toxins are being tested: D. melanogaster grown on food containing ethanol, D. sechellia grown on food containing octanoic acid, and D. tripunctata grown on food containing alpha-amanitin. Among our preliminary results, we find that L. boulardi, a specialist parasitoid of D. melanogaster, is more resistant to ethanol knockdown than more generalist parasitoids. Furthermore, when given a choice between D. sechellia larvae growing on standard media versus media containing octanoic acid, L. boulardi females show strong avoidance of larvae grown on media containing octanoic acid.

661A

Do innate behaviors change with inbreeding? Steven Nilsen, Camayd Cristina, Nelson Dylan, Kravitz Edward. Dept Neurobiology, Harvard Medical Sch, Boston, MA.

Wild type flies that have been housed in laboratories for thousands of generations, may have undergone genetic drift in behavioral traits that have no selective advantage in crowded conditions. One behavior likely to undergo such changes is territorial behavior, both because it is highly complex and because it either is not displayed or is difficult to induce in *Drosophila* that have been socialized in crowded conditions. To test this, we made a detailed comparison of the territorial behavior of a recently isolated wild stock (D26 — Tucson stock # 14021-0231.26) with two lines inbred for over 80 years: Oregon-R (O-R) and Canton-S (C-S). Using our standard behavioral paradigm for territoriality, pairs of socially naive males were tested after 5 days of isolation. As in most species, *Drosophila* territorial fights, involve brief meetings during which flies display behavioral patterns that we previously characterized in C-S (Proc Nat Acad Sci 101:12342, 2004). In these studies, we asked whether any new or unusual behavioral patterns were seen, measured the frequency of use of the various patterns, their transitional order, and whether hierarchical relationships were established. We measured between 2400-3900 behavioral transitions per line and found no behavioral patterns unique to O-R or C-S not also displayed by D26. Certain elaborate wing movements shown by D26 were rarely seen in C-S or O-R. O-R showed large reductions in the amount of usage of the commonly seen “chasing” behavioral pattern, and less consistent use of wing movements. The transition matrixes of D26 were different from C-S or O-R, which were similar to each other. All three stocks were capable of forming hierarchical relationships, with varying frequency. Therefore, while the effects are not dramatic, differences are seen between the recently isolated line and the two established lines. It will be necessary to re-test D26 after several hundred more generations of inbreeding to obtain more definitive proof that inbreeding has caused these changes in innate behavioral patterning (supported by NIGMS).

662B

Shifting sands of heterochromatin? A rapidly evolving heterochromatin protein, Su(var)3-7. Joshua J. Bayes[1,2], Harmit S. Malik[2]. 1) Molecular & Cellular Biology Program, University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Heterochromatin is a major constituent of our genomes. Because of the highly repetitive nature of heterochromatic DNA sequences, our understanding of the true functionality and evolutionary pressures acting on these regions lags behind. As an alternative to studying heterochromatin sequences directly, we have taken an evolutionary approach to the study of proteins that bind heterochromatin. One such protein is Su(var)3-7 which is found exclusively in *Drosophila*. Su(var)3-7 is an essential, heterochromatin binding protein shown to modify position-effect variegation (PEV) in the appropriate genetic backgrounds. It contains seven C2H2 type Zinc finger motifs and a BESS domain, which facilitates homodimerization. Pairwise nucleotide sequence comparisons between *D. melanogaster* and its sibling species, *D. simulans*, show elevated non-synonymous (Ka; nucleotide changes affecting the encoded amino acid) to synonymous (Ks; silent nucleotide changes not affecting the encoded amino acid) rates in windows throughout the 5’ and Zinc finger encoding regions of the gene, indicative of positive selection in these regions. Using divergent and polymorphism information from a population data set, McDonald-Kreitman analysis confirms that *Su(var)3-7* has been subject to evolutionary pressures consistent with positive selection. When all fixed changes were assigned to either *D. melanogaster* or *D. simulans* by comparing sequences to an outgroup species, the McDonald-Kreitman test shows that positive selection has occurred only in the *D. simulans* lineage. In contrast, a gene duplication of *Su(var)3-7*, *Ravus*, shows no signs of positive selection. This data implicates the involvement of *Su(var)3-7* in a genomic conflict with rapidly evolving heterochromatic DNA sequences consistent with a ‘centromere-drive’ model that we have previously suggested.

663C

Molecular Evolution of a Sperm Specific Gene Family. Bruce Bryan[1], Timothy Karr[2], David Rand[1]. 1) Dept of Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Dept of Biology and Biochemistry, University of Bath, Bath, UK.

Novel, duplicate and fast evolving genes are often highly represented in reproductive functions. We have identified a novel family of 12 testis expressed genes, seven of which encode proteins known to be present in mature sperm. All are unique to Drosophila, and have no known function, predicted structure, or known functional protein motifs. Eight of these proteins are predicted to be targeted to the mitochondria, as predicted by the mitochondrial targeting sequence prediction program MitoProt. Proteins in this family share two main regions of conserved amino acid sequence. In addition, preliminary sliding window analyses of dN/dS ratios show that a subset of these genes contain two regions of putative positive selection that map to the same regions of the aligned proteins. These regions are around amino acid AA 70 and around AA 220. All proteins showing increased dN/dS values around AA 220 also show increased values around AA 70, and interestingly, an indel from amino acid 60 to 68 in the aligned sequence distinguishes the proteins that shown no evidence of positive selection from those that do. Here, we test these genes for deviations from neutrality. These findings have important implications for the evolutionary pressures placed on gene families and novel reproductive genes.

664A

Genome decay during the evolution of host specialization in *Drosophila sechellia*. Ian Dworkin[1,2], Corbin Jones[2]. 1) Dept Genetics, North Carolina State Univ, Raleigh, NC; 2) Department of Biology and Carolina Center for the Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Changes in host specialization have driven the incredible diversification of phytophagous insects. When shifting to a new host, specialists often evolve a suite of new physiological, morphological, and behavioral adaptations. Understanding the genetics underlying these adaptations gives insight into the frequency with which they involve gains or losses of function and could indicate the tradeoffs associated with these adaptations. Previously we have shown that *Drosphila sechellia*—a recently evolved specialist on the fruit of Morinda citrifolia—has rapidly accumulated loss-of-function alleles and reduced gene expression at genes affecting olfaction, detoxification, and metabolism relative to *D. simulans*. To further address this question, we have expanded our analysis to include the use of both the DGRC and Affymetrix arrays, to provide increased coverage of the relevant gene families. We contrast and compare the results of our heterospecific hybridizations on two common array platforms, and demonstrate a high degree of overlap in the results.

665B

Genome-wide patterns of evolution of the metabolic network in the *Drosophila* clade. Anthony Greenberg, Andrew Clark. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

We used information from the 12 *Drosophila* genomes project to study patterns of evolution of genes involved in metabolism. We have identified 675 sets of orthologous genes that code for enzymes acting in a variety of pathways. Approximately a third of these underwent lineage-specific changes in copy number. We are using this gene set to study patterns of gene duplication in the clade. A second collection (372 genes) comprises genes with exactly one ortholog in each of the 12 species. This set will let us assess patterns of synonymous and non-synonymous nucleotide substitutions, leading to inferences of levels of constraint and prevalence of positive selection. The overall goal is to test the idea that global properties of the metabolic network influence evolutionary rates of enzyme-coding genes.

666C

Rapid divergence and genome rearrangements during *swallow* gene evolution. Mary Ann Knox, Edwin Stephenson. Dept of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Localization of *bicoid* mRNA at the anterior tip of the oocyte and egg is essential for the establishment of the embryonic anterior-posterior axis. Despite the importance of the bicoid morphogen for *Drosophila* development, lower Diptera and other insects lack *bicoid* and probably specify the A-P axis by different mechanisms. We are interested in understanding the origins and evolution of the oocyte anterior mRNA localization machinery. Three genes have relatively specific roles in *bicoid* mRNA localization, *exuperantia, staufen and swallow*. The first two genes are ancient, with orthologs in other insects (*exuperantia*) or in all metazoans (*staufen*), and other functions in *Drosophila. Swallow* is a novel gene with several features that suggest that it is a recently added component of the localization machinery, and thus represents an opportunity to understand the origin of new cellular functions. *Swallow* has no identifiable orthologs in mosquitoes, bees or non insects, no obvious paralogs in *Drosophila*, and no known function in *Drosophila* other than anterior mRNA localization. We show that most of the *swallow* gene and protein have undergone rapid sequence divergence, with some conserved motifs that probably represent functional constraints. Two features of genome evolution are of interest with respect to *swallow*: First, pseudogenes have been generated by independent tandem duplication events in two lineages of the *melanogaster* species group, suggesting that this may be a hotspot for such duplications. Second, the *swallow* gene has undergone a transposition event within the *melanogaster* species group, landing in a novel genomic position in *melanogaster* and sibling species. These results are discussed with respect to the mechanism of gene duplication and transposition, and the evolution of novel genetic functions.

667A

Evolution of the Y-chromosome: changes in gene content in 8 Drosophila species. Leonardo Koerich[1], Andrew Clark[2], A. Bernardo Carvalho[1]. 1) Department of Genetics, Federal Univ. of Rio de Janeiro, Brazil; 2) Molecular Biology and Genetics, Cornell University, USA.

Y-chromosomes evolved independently many times and are a major biological phenomenon. However, they are well studied in very few species. The recent sequencing of 12 Drosophila genomes allows a detailed study of their origin and evolution. We previously showed that the *D. melanogaster* Y-linked genes were acquired from the autosomes. Here we investigate the fate of Y-linked genes and their autosomal parents, by identifying the orthologs of ten Y-linked genes of *D. melanogaster* (*kl-2, kl-3, kl-5, ORY, CCY, PRY, PPr-Y, ARY, FDY* and *WDY*) in 7 Drosophila species (*D. yakuba, D. ananassae, D. pseudoobscura, D. willistoni, D. virilis, D. mojavensis* and *D. grimshawii*). The Y-linkage of the genes was tested with PCR. We found that the composition of the Y-chromosome is quite fluid: *FDY* is present only in the *D. melanogaster* Y, making it less than 3.5 Myr old. On the other hand, the arrival of *kl-2, kl-3, ORY, PRY* and *PPr-Y* onto the Y predates the split of the Drosophila and Sophophora subgenus (63 Myr ago). We also found intermediate cases: *WDY* is restricted to the Y of the melanogaster group, and *CCY* is Sophophora-specific. Once a duplication occurs, there are three potential outcomes: (i) both genes continue to exist (perhaps with functional divergence); (ii) loss of the autosomal copy; (iii) loss of the Y-linked copy. Phylogenetic comparisons showed that the three occurred. In *FDY, PPr-Y* and *ORY* both genes were retained (the autosomal parents are *CG11844, CG13125* and *CG6059*, respectively), whereas *kl-3, kl-5, CCY,* and *WDY* exemplify the loss of the autosomal parent. Finally, the *PPr-Y* gene was lost in the *D. grimshawii* lineage. Our results identify a total of 5 gene gains and 1 gene loss from the Y on this phylogeny, and showed that the Drosophila Y is at least 63 Myr old, predating the split of all sequenced species. The surprising degree of fluidity of the Y motivates additional research to understand the evolutionary determinants of its gene composition.

668B

Structure of the Dras1 gene control region in a set of Drosophila species. Alex Kulikov[1], Anna Chekunova[1], Oleg Lazebny[1], Irina Lazebnaya[2], Vladimir Mitrofanov[1]. 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow; 2) Dept Animal Genetics, Vavilov Inst General Genetics, Moscow.

Comparing of full-length sequences of the Dras1 gene has been conducted in seven Drosophila species: D. virilis, D. mojavensis, D. melanogaster, D. simulans, D. yakuba, D. ananassae, D. pseudoobscura. The gene sequences were aligned by the ClustalW method in MegAlign. To identify the promoter region, the interval (-60 to +30) around the TSS was analyzed using the JDSA program (Boulay et al., 2004). Four known promoter regions (TATA, DRE, INR & DPE) and six new determined with the help of the Ohler's algorithm (Ohler et al., 2002) were used for the promoter searching. The consensus sequence KNNCAKCNCTRNY is located in the interval 1 to 10 bp upstream from the TSS in the species studied. The DREF binding sequence TATCGATA is located 7 to 19 bp upstream from the consensus sequence. The lengths of noncoding sequences located upstream of the Dras1 promoter were limited with the Rlb1 gene and varied from 75 to 440 bp in six species studied. Negative correlation was observed in these species between the length of the noncoding sequence at the promoter region and the length of the first intron. Inversion has occurred in D. virilis that moved away the Rlb1 gene in 12 kb distance. A highly homologous conservative region has been revealed while aligning sequences spreading from the promoter to the first intron. Its length was 61 bp and it was located at the second part of the first intron. There were no other homologous sequences of comparable lengths at this region. Hypervariability of the regulatory region of the highly conservative Dras1 gene in seven drosophila species studied suggests that regulation of its expression occurs with the help of either different enhancers arbitrarily located within the noncoding region or the conservative sequence revealed within the first intron. Supported by the Russian Foundation for Basic Research (RFBR) grant 05-04-49450 and Russian State contract "Biodiversity and Dynamics of Gene Pools of Plants, Animals, and Humans".

669C

Wolbachia influences mating preferences in Drosophila melanogaster. Alex Kulikov[1], Alexander Markov[2], Irina Goryacheva[3], Oleg Lazebny[1], Maxim Antipin[3]. 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow; 2) Institute of Paleontology, Moscow; 3) Vavilov Inst General Genetics, Moscow.

Investigation of the effect of Wolbachia infection on mating preferences in Drosophila melanogaster is being conducted. Wild type strains along with strains carrying white mutation are used. Part of them is contaminated with Wolbachia. Preliminary result was obtained in a series of competitive mating tests. Wolbachia infection leads to assortative matings in "white" strains. Apparently, this effect lacks in wild type strains. Supported by the Russian Foundation for Basic Research (RFBR) grant 05-04-49702.

670A

Rapid evolution of the *mu2* gene. James Mason, Raghuvar Dronamraju. Lab Molec Genetics, NIH/NIEHS, Res Triangle Pk, NC.

Telomeres are required at chromosome ends for stability. After ionizing radiation of *mu2* mutant females, however, fertility is reduced, and the frequency of progeny with one-break deficiencies is increased dramatically. The deficient chromosomes arise from loss of chromosomal material distal to the radiation-induced break, including the telomere. We proposed earlier that the MU2 protein mediates DNA repair by controlling chromatin structure. Consistent with this, *mu2* mutations are recessive suppressors of position-effect variegation, and two-hybrid data suggest that the MU2 protein interacts with HP1, a highly conserved chromosomal protein. Thus, *mu2* appears to play an important role in DNA repair. Surprisingly, DNA sequence data suggest 2% amino acid divergence between our Oregon R control and the sequence published by the Drosophila Genome Project, and 20% amino acid divergence between *D. melanogaster* and *D. yakuba*. While BLAST does not identify a *mu2* homologous sequence in *D. virilis*, short conserved amino acid motifs are shared between *D. melanogaster* MU2 and the conceptual translation of the predicted transcribed *mu2* region of *D. virilis*, indicating that *mu2* is present, but changing rapidly among Drosophila species. The predicted *mu2* coding sequence in several species indicate a lack of codon usage bias, but ka/ks ratios less than one, consistent with weak purifying selection. We will test these expectations by sequencing cDNAs from several *D. melanogaster* wild type strains and from an array of Drosophila species and address the question of why a seemingly important gene is subject to rapid drift.

671B

Phylogenetic incongruence arising from chromosomal inversions. Bryant McAllister, Amy Evans, Paulina Mena. Biological Sci, Univ Iowa, Iowa City, IA.

Discrepancies among resolved phylogenies are common when evolutionary relationships are inferred using different loci. For example, the inferred evolutionary history of human, chimp and gorilla depends on which gene sequence is analyzed. Many species relationships in the genus Drosophila demonstrate a similar pattern of phylogenetic incongruence. Two mechanisms generally considered to be responsible for this incongruence are: incomplete lineage sorting and introgression. We propose that chromosomal rearrangements provide a separate mechanism of generating pervasive phylogenetic incongruence and we demonstrate this effect by examining sequence relationships within and around a chromosomal inversion in *Drosophila americana* and *D. novamexicana*. These sibling species have allopatric distributions, and for many loci in the nuclear genome and for mitochondrial DNA, sequences of *D. novamexicana* form a monophyletic group embedded within the highly variable sequences of *D. americana*. A paracentric inversion [*In(4)a*] arose in the common ancestor of these species. This inversion is fixed among existing lines of *D. novamexicana*, but it is segregating in natural populations of *D. americana*. Within the inverted region, a sister-group relationship is present between *D. novamexicana* and inverted chromosomes of *D. americana*. Therefore, loci in this large inversion encompassing an approximately 13-Mb region of Chromosome 4 exhibit incongruence with loci in other regions of the genome. Non-neutral forces influencing the inversion have generated a lasting phylogenetic imprint. Incongruence is not a consequence of introgression, but due to meiotic segregation and recombination within *D. americana* which redistributes loci having distinct phylogenetic histories. The current pattern of incongruence may persist through future speciation events. Reinterpretation of incongruent phylogenetic relationships within other Drosophila species groups suggests that segregating chromosomal inversions may have impacted their genomes similarly.

672C

Microarray analysis of interspecific interactions affecting gene expression. Colin Meiklejohn[1], Yasuhiro Go[2], David Rand[1], Daniel Hartl[2]. 1) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Interspecific hybrids allow the study of the phenotypic consequences of interactions between divergent alleles. A set of interspecific introgression genotypes, whose genomes consist of a small, precisely defined segment on the 3rd chromosome from *Drosophila mauritiana* in an otherwise *D. simulans* background, was used to study the effects of these interactions on gene expression. Microarray analyses of RNA profiles from adult males and females of these introgression lines were used to screen for genes with significantly different expression in these lines relative to the parental *D. simulans* strain. The results indicate the extent to which these genetic interactions involve cis-regulatory divergence versus trans-acting affects and how these effects are partitioned between the sexes.

673A

On the origin and evolution of segmentally duplicated genes in the *Drosophila pseudoobscura* genome. Richard P. Meisel, Nadia Hasan, Ben B. Hilldorfer, Robin P. Le Gros, Rachel L. Zindren, Stephen W. Schaeffer. The Pennsylvania State University, University Park, PA.

We identified duplicated genes that arose along the *Drosophila pseudoobscura* lineage via segmental duplication after the divergence with *D. melanogaster*. Intrachromosomal duplications are longer than interchromosomal duplications, and tandem duplications are longer than non-tandem duplications. Intergenic sequences flanking the duplications are enriched for a common *D. pseudoobscura* repeat sequence relative to all intergenic regions in the genome. We propose that the segmental duplications arise when double-strand breaks are repaired using a non-allelic sequence as the template. The resolution of the repair process determines whether a duplication or other genome rearrangement occurs, and the size of the duplication depends on the relative location of the template used. Partially duplicated coding sequences have a greater likelihood of accumulating substitutions that interrupt their open reading frame (i.e., frameshift, nonsense, and intron splice site mutations) than completely duplicated coding sequences. Surprisingly, the length of a duplicated region is a better predictor of whether a duplicated gene will have an interrupted reading frame than the nucleotide divergence between paralogs. This trend holds true for both partial and complete duplications of coding sequences. There are multiple duplications of complete coding sequences in which the amino acid sequence of the two paralogs are evolving at unequal rates. This may be evidence for neofunctionalization, but more data are required to reach a definitive conclusion. Many of the duplications have diverged less than 5% in their nucleotide sequences, suggesting that they may be segregating as copy number polymorphisms in natural populations. We present a preliminary analysis of the copy number frequency of duplications that have yet to fix in the species.

674B

The base composition evolution of Drosophila genome. Yu-Ping Poh[1], Chau-Ti Ting[1,2], Charles H. Langley[3]. 1) Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan; 2) Department of Life Science, National Taiwan University, Taipei, Taiwan; 3) The Center for Population Biology & The Section of Evolution and Ecology, UC Davis, Davis, CA.

Unveiling the patterns of selection pressure and mutational bias work on the dynamics of base composition among taxa are major issues in molecular evolution studies. Evidence showed that *Drosophila melanogaster* genome is under relaxation of codon bias, which might be due to inefficient selection and the bottleneck effect caused by reduced population size. As most of the preferred codons are ended with GC, it is likely that *D. melanogaster* had undergone a shift in mutational bias towards AT component. Thus, it would be of great interest to clarify the underlying mechanism of base composition evolution in *D. melanogaster* and compare with its sibling, *D. simulans*. According to the whole genome comparison, *D. melanogaster* and *D. simulans* genomes harbor more AT than GC substitutions during evolution with more biased occurred in *D. melanogaster*. The AT over-representation in *D. melanogaster* genome is found across all sequence categories, and the bias is more salient in coding regions. On the contrary, no such heterogeneity between coding and noncoding sequences has been observed in *D. simulans* genome. Comparing the frequency spectra for AT and GC polymorphism in *D. simulans* genome, the AT derived alleles are at lower frequency than GC ones. Accordingly, we conclude that *D. melanogaster* and *D. simulans* genomes both experienced a shift in mutational bias towards AT. The level of AT richness is not correspondingly similar is due to either *D. simulans* is more efficient to remove excess AT mutations from the population or a mutation shift is more recent comparing to *D. melanogaster*. Furthermore, the homogeneity of the substitution pattern in coding and noncoding regions of *D. simulans* genome suggests a mutation shift is the more reasonable mechanism contributing to the base composition evolution. These results provide significant implications in understanding the patterns of base composition evolution in *Drosophila* siblings.

675C

Evolutionary analysis of the D. melanogaster betaNACtes gene family. Lev Usakin, Oxana Olenkina, Vladimir Gvozdev. Animal Molecular Genetics, Institute Molecular Genetics, Moscow, RU.

Previously we have elaborated a scenario for the origin of the D. melanogaster repeated Stellate genes. They emerged as a result of the acquisition of a preformed alien testis-specific promoter of the betaNACtes genes (Usakin, et.al, MBE, 22(7):1555). We proposed that the surviving of the newly emerged Stellate genes was connected with the new transcription status. Here we address the question if this event of sharing the same promoter between two non-homologous gene families (Stellate and betaNACtes) influenced the evolutionary history of these genes. Stellate genes are characteristic only for D. melanogaster, whereas betaNACtes are present in several close species. In D. melanogaster genome there are more betaNACtes genes than in the genomes of other species (five genes vs. two or one). We detected a distinct expression pattern of betaNACtes genes in different species. In D. melanogaster betaNACtes are transcribed practically only in testes, while in D. simulans and D. yakuba - in many tissues. We performed McDonald-Kreitman test for positive selection for betaNACtes genes. The results show the involvement of positive selection and high rate of the evolution of betaNACtes genes. Thus, in D. melanogaster species betaNACtes genes amplified, acquired specificity of transcription, underwent the positive selection and these data indicate that Stellate genes influenced betaNACtes genes evolution in D. melanogaster. The impair of proper Stellate functioning leads by unknown way to the formation of stellate-like protein crystals in testes and male sterility. The putative function of betaNACtes is an involvement in cytosolic protein folding as a component of NAC (nascent peptide associated complex). We proposed that the crystal formation is the result of dysfunction of betaNACtes genes under the influence of Stellate repeats sharing the same promoter. We constructed transgenic lines carrying a hairpin for the knockdown of betaNACtes genes. If the hypothesis is true the knockdown of betaNACtes genes will lead to the crystal formation in testes. Now we are awaiting the results.

676A

Transposable element estimate on selection constraint in the Drosophila genome. Jun Wang[1], Hsin-Chien Cheng[2], Pei-San Li[2], Daniel Barbash[1], Hsiao-Pei Yang[1,2]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853, USA; 2) Faculty of Life Sciences and Institute of Genomic Sciences, National Yang-Ming University,Taipei, Taiwan, R. O. C.

Knowing the proportion of the genome which evolves under selective constraint is of great importance for understanding many fundamental problems in evolutionary biology. For example, an accurate estimation of selective constraint provides the key parameter for testing the hypothesis that efficient removal of deleterious mutations explains the origin of sexual reproduction. To estimate genomic constraints, one needs to know the rate of evolution under neutrality. Negative selection quickly removes most transposable elements (TEs) from the genome. Ancient TEs, which presumably remain in the genome because they do not experience negative selection, may therefore evolve neutrally. Here we analyze the molecular evolution of the ancient and highly abundant TE, Drosophila interspersed element-1 (*DINE-1*). Previous work showed that *DINE-1*s have been inactivated in the lineage leading to the common ancestor of *D. melanogaster* and *D. simulans*. Based on the analysis of 66 orthologous *DINE-1*s (including polymorphism for ~20 sites) between *D. melanogaster* and *D. simulans*, we estimate that the average divergence is 14.3% (with s.e. 0.4 %). Based on comparisons to substitution rates observed for different regions of the genome we suggest that at least 70% of the Drosophila genome is under purifying selection, and up to 80% of the genome may be under functional constraint. We also provide information on the mutational pattern for neutrally evolved sequences, including for base composition and for insertions and deletions. Our results indicate that the rate of deleterious mutation for Drosophila is at least 1 mutation per diploid individual per generation, supporting the hypothesis of deleterious mutation being an important force driving the evolution of sexual reproduction.

677B

Rapid increase in viability due to new beneficial mutations in Drosophila melanogaster. Ronny Woodruff[1], Priti Azad[1,2]. 1) Dept Biol Sci, Bowling Green State Univ, Bowling Green, OH; 2) Dept of Neurology, College of Medicine, UTHSC, Memphis, TN.

It is usually assumed that new beneficial mutations are extremely rare. Yet, few experiments have been performed in multicellular organisms that measure the effect of new beneficial mutations on viability and other measures of fitness. In most experiments, it is difficult to clearly distinguish whether adaptations have occurred due to new beneficial mutations or by selection on preexisting genetic variation. Using a modification of a Dobzhansky and Spassky (1947) assay to study change in viability over generations, we have observed an increase in viability in lines homozygous for the second and third chromosomes of Drosophila melanogaster in 6 to 26 generations, due to the occurrence of new beneficial mutations in population sizes of 20, 100 and 1000. These results show that new beneficial mutations, along with selection, can quickly increase viability and fitness even in small populations. Hence, new advantageous mutations may play an important role in adaptive evolution in higher organisms.

678C

Searching for the autonomous transposable elements responsible for the transpositional burst of DINE-1s in Drosophila genomes. Hsiao-Pei Yang, Sherry Lin. Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

We have previously presented evidence that the dispersed repeat *DINE-1*, originally discovered in *D. melanogaster*, is part of a highly abundant family of MITEs (Miniature Inverted-repeat Transposable Elements) found in all 12 sequenced genomes of Drosophila. While these elements share overall structure, our phylogenetic analysis of *DINE-1*s and their host species suggest that each *DINE-1* is species-specific and in general transmitted vertically. We also found that the transpositional activity of *DINE-1* is extremely dynamic, with some lineages showing evidence for recent transpositional bursts and other lineages appearing to have silenced their *DINE-1*s for long periods of time. However, no known *DINE-1*s appear to code for any transposase. They are therefore likely to be non-autonomous and use enzymes from other transposable elements for their transposition. We have developed a bioinformatic approach to search for potentially autonomous *DINE-1*s in five Drosophila species which experienced recent transpositional bursts. Our preliminary data suggest that a few autonomous TEs may serve as the autonomous elements which catalyze transposition of *DINE-1*s. Identification of these autonomous elements will provide important clues for understanding the highly dynamic and species-specific distribution of *DINE-1*s.

679A

The rate of unequal crossing over in the PIGSFEAST repeat array in the Drosophila *dumpy* gene. Amber Carmon, Matthew Larson, Ross MacIntyre. Dept Molec Biol & Genetics, Cornell Univ, Ithaca, NY.

The product of the complex *dumpy* gene includes some 28-45 nearly identical repeats of 102 amino acids which contain the "words" PIGS and FEAST in the single letter amino acid code. We have shown that different geographic strains of *D. melanogaster* have different numbers of repeats in their arrays. Furthermore, when species of the *melanogaster* subgroup are compared, intraspecific variation is consistently less than interspecific variation when the repeats are analyzed, and several in frame indels have spread through the entire arrays of certain species. These observations strongly indicate that the "PIGSFEAST (PF)" repeats are evolving concertedly, presumably by unequal crossing over (UCO). In order to obtain an estimate of the rate of unequal crossing over, we are screening the mutation accumulation lines established and maintained by L. Higgins and M. Wayne. The lines were set up using a full sib approach with single pair matings from each of two nearly isogenic genotypes, one selected for high, the second for low ovariole numbers. 182 lines are being maintained with 21-25 generations of mutation accumulation. We use long range PCR with the SpeedSTAR polymerase (Takara) to amplify the PF array from these lines and have identified the sizes of the two different "ancestral" arrays from the high (45 repeats) and low (41 repeats) lines respectively. New variants generated by UCO are present in both sets of lines, and their numbers in the lines analyzed so far (38) lead to a preliminary estimate of UCO in the PF array of $3X10^{-3}$.

680B

Asymmetrical reproductive isolation between *Drosophila albomicans* and *D. nasuta*. Hwei-yu Chang, Yu-ta Tai. Dept. Entomology, National Taiwan University, Taipei, TW.

The species status of a pair of taxa, *Drosophila albomicans* and *D. nasuta*, has been an issue for no obvious reproductive isolation has been reported. For either *D. albomicans* or *D. nasuta* females, the successful mating percentage of intra-specific crosses is the same as that of inter-specific ones in a single-choice mating test. However, by comparing "multiple-choice", "dual-choice" and "single-choice" cage experiments, there is evidence for intra-specific mating preference in *D. albomicans* females. Another sexual selection component, male-male interaction, was also observed in *D. albomicans.* The implication of this asymmetric premating sexual behavior in *D. albomicans* on the speciation history of this species is discussed.

681C

Genetic changes on a non-recombining chromosome of *Drosophila*. Ting-yi Gong, Hwei-yu Chang. Dep.of Entomology, National Taiwan University, Taipei, TW.

Recombination taking place during meiosis is an important source of genetic variability that organisms need to survive changing environment. A hybrid strain named H10 with fixed karyotypes (*i.e.*, 2n = 6 in females but 2n = 7 in males) has been established in our laboratory from a cross between *D. albomicans* female (2n = 6) and *D. nasuta* male (2n = 8). The separate 3rd chromosome in H10 can only exists in males together with the Y chromosome and can be regarded as a Y-like chromosome. This Y-like chromosome is not physically attached to the real Y chromosome, therefore can be transmitted to females or form homozygotes through proper cross and backcross experimental design. Using PCR-RFLP markers, it is possible to reveal whether sexually antagonistic alleles and recessive deleterious alleles are accumulated on it as expected according to the general assumption of no recombination in *Drosophila* males.

682A

An experimental test of the X-inactivation hypothesis. Winfried K Hense, John F Baines, John Parsch. Department of Biology II, Section of Evolutionary Biology, University of Munich (LMU), Munich, DE.

Genes with male- and testis-enriched expression are underrepresented on the *D. melanogaster* X chromosome. There is also an excess of retrotransposed genes, many of which are expressed in testes, that have "escaped" the X chromosome and moved to the autosomes. It has been proposed that early inactivation of the X chromosome during spermatogenesis contributes to these patterns: genes with a beneficial function late in spermatogenesis should be selectively favored to be autosomal. To test this, we used the promoter sequence of the autosomal, sperm-specific *ocnus* gene to drive expression of a *lacZ* reporter gene in a transgenic vector. Autosomal inserts of this vector showed the expected pattern of male- and testis-specific expression. However, X-linked inserts showed no detectable reporter gene expression. Thus, we find that X-linkage does inhibit the activity of a testis-specific promoter. We obtained the same result using a vector in which the transgene was flanked by chromosomal insulator sequences. This is consistent with global inactivation of the X chromosome and suggests a selective explanation for X chromosome avoidance of genes with beneficial effects late in spermatogenesis.

683B

The evolution of and divergent expression in closely linked members of a gene family. Diana L.E. Johnson, Paaqua Grant. Dept Biological Sci, George Washington Univ, Washington, DC.

Gene families are ubiquitous and are assumed to contain genes with divergent functions. We have been investigating the four genes in the Glutathione S-Transferase (GST) omega family in *Drosophila melanogaster*. GST omega family genes have been implicated in a wide variety of processes in humans. The GST omega-1 gene affects age of onset in Alzheimer and Parkinson diseases and has been implicated in differential sensitivity to arsenic. We are examining the pattern of gene expression of transcripts in the family and are characterizing the evolution of GST omega genes in *D. melanogaster*. The four genes in the family produce five transcripts. We have found divergence in temporal expression among the transcripts at different life stages. We plan to compare quantitative expression of the transcripts also. In addition, we have assigned a specific function to one member of the family. Other members do not show functional redundancy for this character. We have constructed deletions of the whole family and will characterize their effects. Finally, a gene tree will be presented demonstrating a model of the evolution of the family.

684C

Adaptive radiation of digestive proteases in Drosophila female reproductive tracts. Erin Kelleher[1], Willie Swanson[2], Therese Markow[1]. 1) Deparment of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Department of Genome Science, University of Washington, Seattle, WA.

Extensive examination of the protein composition and molecular evolution of the *Drosophila* male ejaculate indicates this trait evolves rapidly: frequently as a result of directional selection. Additionally, extensive studies of biochemical and physiological functions of these proteins in *D. melanogaster* reveal they interact directly with molecules in the lumen of female reproductive tract, mediating essential post-copulatory processes such as sperm storage, ovulation and oogenesis. Although, these interactions create the potential for molecular coevolution between the sexes, little is known about the biochemistry and evolution of female proteins involved.

We have performed a comparative EST screen of female reproductive tracts from *D. arizonae*. The screen identified 673 unique proteins, ~57 of which show evidence of adaptive evolution in pairwise comparisons with the genome sequence of *D. mojavensis*, its close relative. Biochemical functions of secreted and cell membrane female reproductive proteins were enriched in proteolysis, hydrolysis of glycoconjugates, and transport. Additionally, three families of secreted digestive serine proteases were discovered, all of which exhibit strong signatures of adaptive evolution and high amino acid diversity between paralogs. Curiously, these gene families have arisen recently, and indicate that *D. arizonae* female reproductive tracts exhibit digestive function that is subject to strong directional selection. These proteases likely have important implications for the fate of the male ejaculate, and the evolution of the proteins contained therein.

685A

The evolution of mitochondrial physiology and intergenomic epistases across the Drosophila phylogeny. Kristi Montooth, Colin Meiklejohn, Dawn Abt, David Rand. Dept Ecol & Evol, Brown Univ, Providence, RI.

Mitochondrial and cytoplasmic energetics have evolved tightly coordinated function, and efficient performance demands that nuclear genes be regulated in response to mitochondrial demands. Coupled with the dual nuclear and mtDNA encoding of the respiratory complexes, this produces a co-evolutionary dynamic between nuclear and mitochondrial genomes. We have recently assembled mitochondrial genomes from the twelve Drosophila species for which we have nuclear genomes. These mitochondrial genomes allow progress on two complementary fronts: the molecular evolution of inter-genomic relationships and the physiological consequences of divergence within the oxidative phosphorylation complexes. Here we describe how mitochondrial genomic divergence relates to divergence in mitochondrial traits, such as NADH dehydrogenase (ND) and cytochrome oxidase (COX) activity, ATP synthesis, and metabolic rates, across the phylogeny. While purifying selection is the predominant force shaping mitochondrial divergence across the phylogeny, complex-specific rate variation indicates differential selection across the oxidative phosphorylation complexes. In particular, the genes encoding ND have accumulated the greatest amount of amino acid change (median pairwise dn/ds = 0.09), while those encoding COX are most conserved (median pairwise dn/ds = 0.03). We discuss whether this pattern of genomic divergence is reflected in patterns of phenotypic divergence in ND and COX activity or whether the accumulated amino acid change in ND is essentially neutral with respect to ND activity. These data also inform a project that reveals intergenomic epistases underlying mitochondrial physiology and fitness by introgression of *D. mauritiana*, *D. simulans* and divergent *D. melanogaster* mitochondrial haplotypes into a controlled *D. melanogaster* nuclear background.

686B

Horizontal transmission of male-killing *Wolbachia* in *Drosophila*. Sara Sheeley, Bryant McAllister. University of Iowa, Iowa City, IA.

Reproductive parasitism by *Wolbachia* is common among arthropods, with phenotypes including cytoplasmic incompatibility (CI), feminization, parthenogenesis, and/or male-killing. CI is the most commonly observed phenotype, and CI *Wolbachia* have undergone frequent horizontal transmission, evidenced by incongruent parasite and host phylogenies. Male-killing strains have evolved repeatedly from ancestral CI strains, but evidence of horizontal transmission of male-killing strains is rare. To date, only two strains of male-killing *Wolbachia* have been identified infecting species within the genus *Drosophila*, and phylogenetic evidence indicates that these cases originated independently. Here we report the characterization of a strain of male-killing *Wolbachia* infecting *Drosophila borealis*, as well as evidence for horizontal transmission of the male-killer between two distantly related *Drosophila* species with non-overlapping geographical ranges. A recent collection of *D. borealis* yielded an iso-female line that produced 100% female offspring in laboratory culture. A survey of ovarian bacterial flora of the line revealed *Wolbachia*, while no bacterial DNA was detected in ovaries of a *D. borealis* line collected at the same locality that produces a 1:1 sex ratio. Elimination of *Wolbachia* with antibiotics restored a 1:1 sex ratio. The *Wolbachia*-infected line exhibits a reduction in egg hatch of approximately half the rate observed for cured and control lines. Furthermore, attempts to amplify a Y-linked gene from phenotypically female flies from all-female broods were unsuccessful. These results indicate that the all-female line of *D. borealis* is infected with an embryonic male-killing *Wolbachia* strain. Phylogenetic analysis of this strain in *D. borealis* indicates that it is nearly identical to a previously identified male-killing strain infecting *Drosophila innubila*. Identification of male-killing *Wolbachia* in these two distantly related *Drosophila* species will allow investigation of factors that make host species susceptible to invasion and capable of maintenance of an embryonic male-killer.

687C

The projectin protein and the evolution of asynchronous physiology in insect flight muscles. Richard Southgate, Catherine Kramp, Agnes Ayme-Southgate. Dept Biol, Col Charleston, Charleston, SC.

Insect flight is powered by two types of muscles known as either synchronous or asynchronous. The success of insects as a major animal group has been partly attributed to the acquisition of asynchronous physiology, a critical adaptation that is believed to have independently evolved on multiple occasions during insect evolution. The asynchronous mode relies on the physiological mechanism of stretch-activation, a property leading to multiple contractions for each nerve impulse. Stretch activation is made possible, at least in part, by the presence of connecting filaments linking Z-bands and myosin filaments. In Drosophila melanogaster, these structures are composed of two proteins, projectin and kettin. One step towards the acquisition of asynchronous physiology may, therefore, be the mechanical specialization of these two protein sequences to the stretch-activation process. Drosophila projectin is a large modular protein composed of two repeated motifs and a unique sequence, the PEVK domain that is known to work as an elastic region in other proteins. The sequence of the PEVK domain is different between the synchronous and asynchronous isoforms of Drosophila projectin. This structural difference is proposed to be an adaptation to the PEVK domain's elastic function within the asynchronous muscles. We propose that the fundamental physiological mechanism of asynchronous muscles in insects has evolved partly through sequence modifications in the projectin PEVK domains. To address this hypothesis, we have started a functional analysis of the projectin protein in various insects. Projectin genes have been characterized from several insect orders, including coleoptera, hymenoptera and diptera. The splicing pattern and isoform within the PEVK domains will be analyzed by an RT-PCR methodology, including the alternative splicing patterns in different muscle types. We will present the phylogenetic analysis of the completed amino acid sequences using standard alignment and phylogenetic programs such as CLUSTALW, PHYLIP and MACCLADE.

688A

Phylogenetic analysis suggests a functional relationship between *kayak* the Drosophila Fos homolog and *fig* a predicted PP2C phosphatase rested within a *kayak* intron. Stephanie Hudson, Elliott Goldstein, Stuart Newfeld. Sch Life Sci, Arizona State Univ, Tempe, AZ.

We have generated a new model for the gene structure of the gene, kayak (kay) and its nested gene, fos-intronic gene (fig). kay is the D. melanogaster homolog of the human oncogene c-fos, which codes for part of the AP-1 transcription factor. fig, codes for a predicted PP2C phosphatase. It is a large gene region of 27Kb and has multiple promoters for both kay and fig giving 6 different transcripts. The most distal promoter of kay gives rise to the kay-α transcript of 3.8Kb with two introns and three exons. The second promoter leads to the kay-β transcript of 3.4Kb with 4 exons and 3 introns. The most proximal promoter produces the kay-γ transcript, which is 3.1Kb with 3 exons and 2 introns. All 3 isoforms splice into the kay mainbody, which contains the bZIP and DNA binding domain. The fig gene has two alternate promoters leading to 2 forms of the transcript. The fig-α transcript is 1.4Kb and codes for a putative PP2C phosphatase. The fig-β transcript is 629bp and produces a predicted protein which is truncated. Now that multiple species of Drosophila have been sequenced, it is informative to ask what the structure of this complex region is in the multiple species and if this region of ~27Kb has a conserved gene structure or is unique to D. melanogaster. We have completed a structural analysis of the region for the 12 Drosophila species. We have constructed alignments of the predicted protein sequences with phylogenetic trees. We examined the sequences and annotations for the twelve species of Drosophila with fully sequenced genomes to identify the regions containing kay and fig. We found an extensive diversity of gene structures for kay as shown by the presence of multiple chromosomal inverstios and the repeated loss of individual kay 5' exons. Nevertheless, fig is divergently transcrivbed and nested in a kay intron in all twelve species - a level of conservation suggesting a funcitonal relationship between them D. melanogaster.

689B

Phylogenetic analysis of *Drosophila virilis* species group by two different mtDNA approaches. Svetlana Sorokina. Dept Genetics, Koltsov Inst Dev Biology, Moscow, RU.

Mitochondrial DNA is useful marker to study molecular history of species. Two different approaches based on mtDNA polymorphism were used to infer phylogenetic relationships between twelve species of *Drosophila virilis* species group. Nucleotide sequences of 630 kb fragment of mitochondrial gene COX1 was determined and *Hinf I* RFLP analysis of total molecule of mtDNA was made for 92 isofemale strains of *D. virilis* species group. Phylogenetic tree was constructed. Levels of inter and intraspecific polymorphism were estimated for this group. Possible trends of events in species history are discussed. The study was supported by Russian Foundation for Basic Research (RFBR) grant 06-04-49369-a.

690C

Functional evolution of *X*-linked odorant binding protein genes in *Drosophila melanogaster*. Gunjan Arya[1,3], Ping Wang[2,3], Richard Lyman[2,3], Trudy Mackay[2,3], Robert Anholt[1,2,3]. 1) Department of Zoology, North Carolina State Univ., Raleigh, NC; 2) Department of Genetics, North Carolina State Univ., Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State Univ., Raleigh, NC.

The ability to respond to chemical signals from the environment is essential for the survival and reproduction of most organisms. Odorant binding proteins (Obps) are encoded by a diverse family of about 50 rapidly evolving genes and are the first components of the insect olfactory system to interact with odorants. Whereas their precise functions in olfaction are largely unknown, altered transcriptional regulation of some Obps has been observed in lines selected for sensitivity to alcohol exposure, copulation latency, and aggression, indicating pleiotropic effects on behavior. We have re-sequenced all 6 *Obp* genes on the *X*-chromosome in 50 wild-derived inbred lines of *D. melanogaster* and analyzed sequence variation. The number of polymorphic sites ranged from 8 in *Obp18a* to 36 in *Obp19b* and comprised both SNPs and insertion/deletions. LD analysis shows evidence of extensive historical recombination. Population genetic analyses indicate no departures from neutrality for *Obp18a* and the *Obp19a-d* cluster. However, values for Fu and Li's D* and F* suggest departure from neutral evolution for *Obp8a*. Preliminary association analyses did not detect statistically significant associations for starvation resistance, longevity, and copulation latency for any of the *Obp* genes. However, a polymorphic region in *Obp19b* shows association with olfactory behavior and an insertion/deletion that causes a frame shift in the coding region of *Obp8a* shows association with trehalose preference that exceeds the Bonferroni-corrected threshold for statistical significance. The segregation of this low frequency (q=0.06) null mutant in the population suggests functional redundancy among members of this multigene family. Further studies with increased sample sizes are needed to confirm associations with olfaction and gustation, respectively, in these two *Obp* genes.

691A

Identification of loci contributing to mating success and multicomponent sexual signals in female *Drosophila melanogaster*. Brad R Foley[1], Steve F Chenoweth[2], Sergey V Nuzhdin[1], Mark W Blows[2]. 1) Dept of Evolution and Ecology, UC Davis, Davis, CA; 2) Dept of Integrative Biology, the university of Queensland, Brisbane AU.

The genetic basis of sexually selected traits within natural populations has received considerable attention in quantitative genetic studies, but the molecular genetic basis of such variation is generally unknown. We previously conducted a QTL mapping experiment to characterize the genetic basis of a large suite of sexually selected traits within a set of Recombinant Inbred Lines (RILs) derived from a natural population of *Drosophila melanogaster*. Male and female choice mating assays were also performed on these lines to obtain a measure of attractiveness. While no QTL pleiotropic for both CHC expression and mating success were mapped, evidence was found for genetic variation in female, but not male, mating success mediated by CHC expression. Multiple regression and model selection were used to identify 6 CHC QTL which together explained 18.1% of the variation in female mating success.

692B

First evidence for natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. Brad R Foley[1], Steve F Chenoweth[2], Mark W Blows[2], Sergey V Nuzhdin[1]. 1) Dept of Evolution and Ecology, UC Davis, Davis California, CA; 2) Department of Integrative Biology, the University of Queensland, Brisbane AU.

Cuticular Hydrocarbons (CHCs) act as contact pheromones in *Drosophila melanogaster* and are an important component of several ecological traits. Segregating genetic variation in the expression of CHCs at the population level in *D. melanogaster* is likely to be important for mate choice and climactic adaptation; however this variation has never been characterized. Using a panel of Recombinant Inbred lines (RILs) derived from a natural population we found significant between-line variation for nearly all CHCs in both sexes. We identified 25 QTL in females and 15 QTL in males that pleiotropically influence CHC expression. There was no evidence of colocalisation of QTL for homologous traits across the sexes, indicating that sexual dimorphism and low intersex genetic correlations between homologous CHCs are a consequence of largely independent genetic control. This is consistent with a pattern of divergent sexual and natural selection between the sexes.

693C

Evolution of water balance and gene expression in desiccation-selected Drosophila melanogaster. Allen G. Gibbs, Cheryl H. Vanier. School of Life Sciences, University of Nevada, Las Vegas, NV.

Because of their small size and large surface-area-to-volume ratio, insects face significant problems in maintaining positive water balance. These problems are particularly severe in arid environments such as deserts. To investigate the mechanistic basis of differences in water balance, we subjected replicated populations of Drosophila melanogaster to selection for resistance to desiccation for 30 generations. The major physiological responses were a 30% increase in body size and water storage, a doubling in carbohydrate stores, and a reduction in water-loss rates mediated by reduced activity and respiratory water loss. Over 90% of the variation in desiccation resistance could be explained by differences in carbohydrate storage and water-loss rate. We also used oligonucleotide microarrays to identify evolved changes in patterns of gene expression. Relatively few genes (~60) evolved different expression patterns, whereas >500 genes were differentially expressed when flies were exposed to desiccation or starvation stress. The latter category included many genes involved in energy metabolism, consistent with our physiological results.

694A

Divergence population genetics of the *Drosophila simulans* species complex. Richard Kliman, Shannon McDermott. Dept Biological Sci, Cedar Crest Col, Allentown, PA.

The *Drosophila simulans* species complex serves as an important model for the study of new species formation. *Drosophila sechellia* and *Drosophila mauritiana* are island endemics. The three species are partially reproductively isolated; hybrid males are infertile. To better understand the natural historical context in which reproductive isolation has arisen, we have measured polymorphism and divergence at ~30 loci, applying the divergence population genetics approach to estimate divergence times, to estimate ancestral population sizes and to infer possible gene flow subsequent to initial isolation of the incipient island species. A particularly striking finding is very low divergence among all three species at "hitchhiking" genes, despite typical divergence at these genes from *Drosophila melanogaster*. This indicates either a very large ancestral effective population size for the specie complex or gene flow in hitchhiking regions.

695B

Linkage disequilibrium analyses of synonymous and replacement polymorphisms in *Drosophila* chemoreceptor genes. Rumi Kondo[1], Miki Oshima[1], Yukako Yoshifuji[1], Nobuyuki Inomata[2], Masanobu Itoh[3], Toshiyuki Takano-Shimizu[4]. 1) Dept Biology, Ochanomizu University, Tokyo, JP; 2) Dept Biology, Graduate School of Sci, Kyushu University, Fukuoka, JP; 3) Dept Applied Biology, Kyoto Institute of Technology, Kyoto, JP; 4) Dept Population Genetics, National Institute of Genetics, Shizuoka, JP.

To assess the degrees of natural selection acting on *Drosophila* chemoreceptor genes, we examined the linkage disequilibrium (LD) patterns of synonymous and replacement polymorphisms. Fly samples were collected from Kyoto, Japan in the fall of 2001 (Ky01au) and spring of 2003 (Ky03sp). We examined 73 polymorphisms (40 replacement SNPs from chemosensory receptor genes and 33 synonymous SNPs from chemosensory receptor or other genes) on the second chromosome of Ky01au (282 chromosomes) and Ky03sp (361 chromosomes), respectively. LD was examined for 2,628 SNP pairs. The amount of LD (r^2) was much larger in the spring sample than in the autumn one. In the spring samples, the distribution pattern of LD (D') differed between replacement and synonymous SNPs. Associations between one frequent allele and one less common alleles were significantly high for the replacement SNPs ($D'<0$), but significantly low for synonymous SNPs ($D'>0$). Together with our previous observation from the spring of 2002 samples (Ky02sp), these results suggest the effects of seasonal bottleneck and natural selection due to synergistic interaction among rare replacement polymorphisms in shaping the pattern of LD in *Drosophila* chemoreceptor genes.

696C

Evidence of spatially varying selection acting on the chromatin remodeling gene, chameau. Mia Levine, David Begun. Section of Evolution & Ecology, Univ California, Davis, Davis, CA.

Chromatin remodeling factors regulate gene expression by modifying chromatin into permissive and restrictive transcriptional states. Temperature may also influence chromatin state, with warmer temperatures associated with a relatively decondensed, permissive state. Natural populations of Drosophila melanogaster occur along wide latitudinal gradients over which temperature varies dramatically, raising the possibility of thermal adaptation of chromatin remodeling proteins. To identify such candidate proteins, we took advantage of a whole-genome tiling array study of genetic differentiation between tropical and temperate populations from both Australia and the United States. The two continents share several differentiated regions, one of which contains the histone acetyltransferase, chameau, which modulates two chromatin-associated phenotypes—position effect variegation and aberrant sex comb expression. We sequenced over 30 alleles of both the putative region of differentiation and several 1 kb fragments that span 10 kb up- and down- stream of chameau in both continental samples. We find a strikingly similar distribution of Fst values that peak at the fourth exon of chameau and decay rapidly with distance. These data are consistent with spatially varying selection acting on a chromatin remodeling gene.

697A

Evolution at two levels revisited: The role of transcriptional and functional variation in host adaptation. Luciano Matzkin, Therese Markow. Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ.

More than 30 years ago King & Wilson (1975) proposed that changes in gene regulation plays a role in the diversification of species. Since then, and especially within the last few years extensive levels of transcriptional variation in many taxa have been discovered. Furthermore, studies suggest that a significant portion of the observed interspecific transcriptional variation has been shaped by natural selection. The question then becomes, what are the relative contributions of transcriptional and coding sequence variation in evolution? Unfortunately, most models focus on developmental genes. We have developed a model for metabolic enzymes in which standing *cis* binding domain variation and/or coding sequence variation can play a significant role in evolution. We have tested this model by examining the pattern of transcriptional and coding variation in metabolic and detoxification genes of cactophilic *Drosophila.*

D. mojavensis contains four genetically isolated host races each specializing in the necrotic tissues of different cactus species. The necrosis of each cactus species provides the resident populations with a distinct chemical environment. We previously investigated the role of transcriptional variation in the adaptation of *D. mojavensis* to its hosts and have produced a set of candidate loci associated with host shifts, among them was Glutathione S-transferase-D1 (*GstD1*), a known detoxification gene. We have examined the pattern of sequence variation at *GstD1* in *D. mojavensis*, *D. arizonae* and *D. navojoa*. The data suggest that in two populations of *D. mojavensis GstD1* has gone through a period of positive selection. Of the seven amino acid fixations that occurred in the *D. mojavensis* lineage, two of them occur in the active site pocket, potentially having a significant affect on substrate specificity and possibly in the adaptation to alternative cactus hosts. Thus in the case of *GstD1* both transcriptional and functional variation have been shaped by adaptation of *D. mojavensis* to their cactus hosts.

698B

Chromosomal Polymorphisms and Associated DNA variation in *D. americana*. Paulina Mena, Bryant McAllister. University of Iowa, Iowa City, IA.

Geographically distributed genetic polymorphisms suggest the presence of natural selection in response to variable climate. Chromosomal inversions are a common polymorphism associated with climatic gradients. Their hypothesized role is to protect co-adapted gene complexes. *D. americana* is an excellent model for examining the involvement of genome organization in facilitating a correlated response to environmental conditions. A derived chromosomal rearrangement consisting of a centromeric fusion between the autosomal 4th and the X is present in *D. americana*. Frequency of the X-4 fusion is positively correlated with latitude. Neutral loci show no detectable population structure, suggesting substantial gene flow among populations. Thus, the maintenance of the X-4 fusion cline suggests a balance between gene flow and natural selection. Several polymorphic inversions are also present in *D. americana*. Here we report the geographic distribution of these inversions by examining samples representing the latitudinal extent of the species' range. Clinally distributed inversions were observed on chromosomes X, 4 and 5. Xc is strongly associated with the centromere arrangement (found only on chromosomes with the fusion). Analyses of DNA variation on the X were used to examine the history and effects of these rearrangements. Sequences were obtained for 5 regions along the X chromosome for 25 inbred lines with known chromosomal arrangements. Within and around Xc, sequence differentiation is high between fused and unfused chromosomes, and near the Xc proximal breakpoint the X-4 fusion exhibits less nucleotide variation than the unfused X. No sequence divergence between alternative arrangements is observed closest to the centromere although the level of nucleotide variation on the ancestral unfused X is extremely low and no variation is observed on the X-4 arrangement. The data suggests that the Xc is older than the fusion and previously existed in a cline similar to the current X-4 fusion cline. The fusion occurred later on an inverted X, followed by a rapid increase in frequency.

699C

Abundant genetic variation in transcript level during early Drosophila development. Sergey Nuzhdin[1], Danielle Tufts[1], Mathew Hahn[2]. 1) Dept Evolution & Ecology, Univ California, Davis, Davis, CA; 2) Department of Biology and School of Informatics, Indiana University, Bloomington, IN 47405.

Variation in gene expression may underlie many important evolutionary traits. However, it is not known at what stage in organismal development changes in gene expression are most likely to result in changes in phenotype. One widely held belief is that changes in early development are more likely to results in changes in downstream phenotypes. In order to discover how much genetic variation for transcript level is present in natural populations, we studied zygotic gene expression in nine inbred lines of Drosophila melanogaster at two time points in development. We find abundant variation for transcript level both between lines and over time: close to half of all expressed genes show a significant line effect at either time point. We examine the contribution of maternally-loaded genes to this variation, as well as the contribution of variation in upstream genes to variation in their downstream targets in two well-studied gene regulatory networks. Finally, we estimate the dimensionality of gene expression in these two networks and find that—despite large numbers of varying genes—there only appear to be two factors controlling this variation.

700A

Maintenance of a gene arrangement polymorphism in natural populations of *Drosophila pseudoobscura*. Stephen Schaeffer. Dept Biol, Pennsylvania State Univ, University Park, PA.

The 30 different gene arrangements observed in natural populations of *Drosophila pseudoobscura* were generated through a series of overlapping inversions. The gene arrangements have been thought to be the target of strong selection because they form classical clines across diverse geographic habitats, they cycle in frequency over seasons, and they form stable equilibria in population cages. Clustering populations based on chromosomal frequencies show that populations within the cline occur in one of six major climatic zones. Molecular sequence data show that migration rates are sufficient within *D. pseudoobscura* to homogenize inversion frequencies among these different climatic zones suggesting that strong selection in heterogeneous environments is responsible for the gene arrangement clines. A numerical approach was used to estimate the fitness sets for 15 gene arrangement karyotypes in the six climatic zones across the southwestern United States based on a model of selection-migration balance. The fitness estimates were used in standard selection recursion equations that allow migration among the six populations. Fitness sets obtained with different migration rates were highly correlated, the only difference being the magnitude of selection parameters. The migration rates chosen were sufficient to homogenize inversion frequencies without selection. The gene arrangement frequencies arrived at a stable equilibrium in all climatic zones with frequencies near the observed values in nature. The model was extremely robust to the initial gene arrangement frequencies including cases where chromosomes were introduced one at a time based on their estimated time of origin from molecular phylogenies. The model shows the complex dynamics of how selection can act on standing variation in populations. This analysis shows that nucleotide sequences within the gene arrangements are not likely to conform to current simple models of selection. The approach described here provides a method to estimate fitness values from static gene frequency data in organisms that migrate extensively.

701B

Biparental inheritage of mtDNA in *D. melanogaster*. Christian Schloetterer, Daniela Nunes. Inst Tierzucht, VMU Wien, Wien, AT.

DNA sequence analysis of the cytochrome c oxidase subunit I in a world-wide collection of *D. melanogaster* populations revealed the presence of heteroplasmic individuals. Further analyses using Realtime PCR showed a high proportion of flies with two distinct mtDNA haplotypes. Interestingly, the transmission of the two distinct haplotypes was not stable, showing a large heterogeneity among offspring from the same female. We propose that biparental inheritage of mtDNA is common in natural *D. melanogaster* populations and may result in a low association between mtDNA haplotypes and different Wolbachia genotypes.

702C

Variations in the chromosomal locations of the rRNA genes and pairing ability during male meiosis in the *D. ananassae* complex. Mami Shibusawa[1], Yoshiko Tobari[2], Muneo Matsuda[1]. 1) Biology, Kyorin University, Mitaka, Tokyo, JP; 2) Institute of Evolutionary Biology, Setagaya, Tokyo, JP.

In *D. ananassae*, it has been shown that there are two NO loci, one on the distal part of the long arm of the 4th chromosome and the other on the short arm of the Y chromosome. Pairing among the 4th, X, and Y chromosomes during male meiosis has been observed despite the fact that the X chromosome lacks NO. We investigated the chromosomal locations of the rRNA genes in 37 strains of 10 species belonging to the *D. ananassae* complex using fluorescence *in situ* hybridization (FISH). For these FISH analyses, we used the pDmr-a51#1 probe, which contains 18S, 5.8S, and 28S rDNAs and an intergenic spacer region including 240-bp repeats. 1) We found no signals of rRNA genes on the X chromosomes of any the strains examined in this study. 2) On the tips of the long arms of the 4th chromosomes of all the strains examined, we found strong signals of the rRNA genes. 3) Two types of the Y chromosome, one of which shows a strong signal for rRNA genes and the other shows no rRNA gene signal, are found in the Tonga population of *D. ananassae*. 4) All the strains examined exhibit the tetravalent configuration among 4th, X, and Y chromosomes during male meiosis, and the paired regions are hybridized with the pDmr-a51#1 probe. These results suggest that the entire sequence of the rRNA genes is not necessary for pairing and that the X chromosome does carry a necessary sequence for pairing. 5) One of the three strains of Taxon K collected in Okinawa has two sites of rRNA genes on the 4th chromosome, one on the long arm and the other on the short arm. The loop configuration of the 4th chromosomes in the tetravalent is found in the male meiosis of this strain. This result indicates that the two rRNA gene clusters on the 4th chromosome have the ability to pair with each other.

703A

Associations of SNPs in Odorant Binding Protein Genes with Olfactory Behavior in *Drosophila melanogaster*. Ping Wang[1,2], Richard F. Lyman[2], Svetlana Shabalina[3], Theodore J. Morgan[1,2], Trudy F. C. Mackay[1,2], Robert R. H. Anholt[1,2,4]. 1) W. M. Keck Center for Behavioral Biology, NC State Univ, Raleigh, NC; 2) Dept. Genetics, NC State Univ, Raleigh, NC; 3) NCBI, NIH, Bethesda, MD; 4) Dept. Zoology, NC State Univ, Raleigh, NC.

Odorant binding proteins are the first components of the insect olfactory system to encounter odorants and are encoded by a family of 50 genes. Despite their abundant expression, their functions remain poorly characterized. We designed a population genetics strategy to uncover historical patterns of natural selection acting on these genes, while at the same time providing information about their binding specificities. We analyzed sequence variation in two clusters of *Obp* genes, *Obp56a-i*, and *Obp99a-d* in a population of wild-derived inbred lines, using *D. simulans* as outgroup. We identified 152 SNPs in the *Obp56* genes and 154 SNPs in the *Obp99* genes with SNP densities ranging from a single SNP in *Obp56f* to 77 SNPs in *Obp99c*. Molecular evolutionary analyses show that most *Obp* genes evolve according to neutral expectations, but we found signatures of balancing selection (*Obp56g*, *Obp56h*, *Obp99b*, *Obp99c* and *Obp99d*) and positive selection (*Obp56a* and *Obp56c*), indicating different evolutionary histories. Association analyses with olfactory responsiveness to benzaldehyde measured in 193 of the lines revealed statistically significant associations that exceeded the permutation threshold for multiple testing for 4 SNPs in the *Obp99* cluster. Two SNPs are in introns of *Obp99a* and *Obp99c*, and two are in the coding region of *Obp99d*, comprising a nonsynonymous substitution (G67A) and a synonymous substitution (T78G). We found significant differences in olfactory responsiveness between the GT and AG haplotypes. mRNA structure predictions for these haplotypes show changes in the stem-loop structure of the pre-mRNA. Our results show that *Obp99a*, *Obp99c* and *Obp99d* contribute to recognition of benzaldehyde. Thus, this population genetic approach will allow us to elucidate binding specificities of members of the *Obp* gene family.

704B

Quantitative trait loci affecting plasticity and allometry of ovariole number and body size. Alan Bergland[1], Anne Genissel[2], Sergey Nuzhdin[2], Marc Tatar[1]. 1) Dept Ecology & Evolution, Brown Univ, Providence, RI; 2) Section of Evolution and Ecology, University of California Davis, Davis, CA.

Environmental factors during larval growth such as temperature and nutrition have major effects on adult morphology and life-history traits. In Drosophila, ovary size, measured as ovariole number, and body size, measured as thorax length, are developmentally plastic traits with respect to larval nutrition. The genetic basis for plasticity of ovariole number and body size, as well the genetic basis for their allometric relationship was investigated using recombinant inbred lines (RILs). We reared 200 RILs under four yeast concentrations and measured ovariole number and body size. There is considerable genetic variation for ovariole number and body size within environments, and for plasticity across environments. Further, there is genetic and environmental variation in the allometric relationship between ovariole number and body size. Main effect and epistatic QTL along all the 2nd and 3rd chromosomes were identified that show consistent effects across environments for ovariole number and body size. Despite the high amounts of genetic variation in plasticity, we identify no main effect or epistatic QTL-environment interactions for ovariole number and only one main effect QTL-environment interaction for thorax length. We identify epistatic QTL and QTL-environment interactions for the allometric relationship between ovariole number and thorax length. These data suggest that ovariole number and body size plasticity is a function of genetically determined allometric growth.

705C

A microarray approach to understanding the genetic basis of variation in age-specific immune response in Drosophila melanogaster. T. M. Felix[1], J. M. Drnevich[2], K. A. Hughes[3], J. W. Leips[1]. 1) Dept. of Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD; 2) W.M. Keck Center for Comparative and Functional Genomics, University of Illinois, Urbana IL; 3) School of Integrative Biology and Institute for Genomic Biology, University of Illinois, Urbana IL.

Senescence is the age-related deterioration in physiological function reflected in the decline of a number of traits. Age-related deterioration in immune response is one of the hallmarks of aging in vertebrates; however, microarray studies on aging flies indicate that many of the innate immune response genes are up-regulated with age. Moreover, a recent study using chromosome II substitution lines derived from a natural population of *Drosophila melanogaster* found genetic variation in the influence of age on the immune response, with some lines actually showing an improvement in immune response with age. The mechanism responsible for variation in age-specific immune function or the genetic basis of such changes is not known. We tested the hypothesis that variation in age-specific immune response is due to transcriptional changes and used a microarray approach to identify candidate genes responsible for age-related changes in this trait. Virgin females of twenty inbred lines derived from the population in Raleigh, NC were screened for their ability to clear an artificially induced bacterial infection at one and four weeks of age. Six lines that showed deterioration in immune response with age and six lines that improved with age were analyzed using microarrays. These data will identify candidate genes for which age-specific expression is associated with age-specific immune function and so elucidate the influence of transcriptional variation in producing age-related changes in immune response.

706A

A novel method for measuring total fitness of outbred genotypes. James D. Fry. Dept Biol, Univ Rochester, Rochester, NY.

I describe a novel, balancer-based method for measuring total fitness of chromosome heterozygotes. In contrast to a previous method, the method is not restricted to chromosomes with pre-existing lethals, and does not require two balancers, making the results somewhat easier to interpret. In the first of two experiments to test the method, a third chromosome from an ethanol-adapted population increased fitness on ethanol-supplemented medium, but decreased fitness on regular medium, relative to a chromosome from a control population. A second experiment using an independent pair of chromosomes gave more ambiguous results, possibly due to smaller sample size. Nonetheless, the method gives reproducible fitness estimates, and could be used for a variety of applications, including measuring the mutational variance for total fitness, and mapping QTL causing fitness trade-offs between environments.

707B

Are the genes which contribute to species differences in sex comb tooth number also associated with tooth number variation in natural populations of *D. simulans*? Rita M. Graze[1], Elena Naderi[2], Sergey V. Nuzhdin[2]. 1) Genetics Graduate Group, University of California, Davis, CA 95616; 2) Center for Population Biology, Section of Evolution and Ecology, University of California, Davis, CA 95616.

The sex comb is a male secondary sexual character which forms on the prothoracic legs of *Drosophila* males. Previously, a large effect QTL for divergence between *D. simulans* and *D. mauritiana* in sex comb tooth number was mapped to the third chromosome, in the 73A-84AB region. We hypothesized that genes located in 73A-84AB also make a similar contribution to variation in sex comb tooth number within species. To test this hypothesis, we introgressed this region from a set of natural population derived *D. simulans* isogenic lines into a common isogenic background using the flanking visible markers *scarlet* and *ebony* (*st e*). After > 10 generations of backcrossing, we scored sex comb tooth number for two replicate introgression lines per wild-derived chromosomal region. Mean sex comb tooth number was analyzed by ANOVA with model $Y_{ijk} = Line_i + Replicate_j(Line_i) + error$. The wild-derived st^+ e^+ regions were not significantly associated with variation in sex comb tooth number. We conclude that, in our system, the genes primarily accounting for interspecific differences in sex comb tooth number have minor, or no effect, on intraspecific variation.

708C

Genetic variation in the plastic response of life history traits, energy metabolism, and age-specific immunity to different diets. Mary F. Kaminski[1], Michelle Moses[2], Maria DeLuca[2], Jeff Leips[1]. 1) Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD; 2) Department of Nutrition Sciences, University of Alabama at Birmingham.

Caloric and dietary restriction have been shown to affect longevity, reproduction and immune response in a wide range of organisms. Variation in resource quality and quantity is common in natural populations, and adjustment of energy allocation to different traits in response to such variation is likely to have important effects on fitness. In this study we used *chromosome III* substitution lines derived from the natural population in Raleigh, NC to examine the effect of variation in diet on several life history traits and to characterize genetically based variation in the plastic response of these traits to dietary restriction. We crossed females from each of six *CIII* substitution lines to males of an unrelated *CIII* line and measured the following traits on the offspring: development time, body size at eclosion, age-specific immune response, and age-specific energy storage and metabolic rate under two food conditions: regular cornmeal based fly food and the same food with a 60% reduction in yeast. Our results will shed light on the genetic interrelationships among these traits under different diets and explore the possibility of trade-offs among traits. We will also use these data to begin to explore the genetic basis of variation in the plastic response of these traits to dietary restriction.

709A

Quantitative Genetics of Antibacterial Immunity in *Drosophila*. Brian Lazzaro. Dept Entomology, Cornell Univ, Ithaca, NY.

Quality of immune response can reasonably be considered an important fitness trait, and therefore potentially subject to the actions of natural selection. We have previously documented substantial genetic variation for antibacterial defense capability in natural populations of *D. melanogaster*, which could provide the substrate for selection. The efficiency of selection on this trait, however, will depend strongly on its genetic architecture and ecological context. We know that flies in nature are subject to infection by a diversity of pathogens, so we have measured correlations among natural genotypes in resistance to infection by various bacteria. These correlations are surprisingly weak, suggesting that resistance to different bacteria may be independently selectable. We have also intercrossed parental lines with extreme phenotypes in a diallel structure to estimate the heritability of resistance to infection. Genotypic information is known for all of the phenotyped *Drosophila*, making it possible to associate phenotypic with genetic variation in all of these studies. In general, we find that individual loci make small contributions to phenotypic variance and suggest that these effects are partially determined by genotype-by-environment interactions. Importantly, this series of experiments has an element of repetition that makes it possible to evaluate the replicability of our quantitative genetic results.

710B

Quantitative trait loci analysis of cryptic female choice between *Drosophila mauritiana* and *D. simulans*. Cheng-Lin Li[1], Chen-Hung Kao[2], Chau-Ti Ting[1,3]. 1) Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan, ROC; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan, ROC; 3) Department of Life Science, National Taiwan University, Taipei, Taiwan, ROC.

Multiple mating results in coexistence of sperm from different males in female reproductive track and sperm competition. In addition to sperm competition within species, con-specific sperm precedence has also been well demonstrated. Genetic studies suggested that both within species sperm competition and con-specific sperm precedence not only depend on the genetic variation of males but also are mediated by the genetic background of females. In males, accessory gland proteins play important roles in sperm competition. However, little is known about the genes involved in female mediated sperm competition, namely cryptic female choice. To address this question, we generated the backcross generation one females of *Drosophila simulans* and *D. mauritiana* to analyze the overall genetic architecture of cryptic female choice. In control over loci causing reduction in fecundity of hybrid females, adjusted quantitative trait loci mapping has identified four QTLs, two on each major autosome, associated with cryptic female choice. More interestingly, two of the QTLs show negative effects on the usage of *D. simulans* sperm, *i.e.*, individuals with homozygous *D. mauritiana* alleles favor the sperm of *D. simulans* males. These results suggest although only a few chromosomal regions are involved in cryptic female choice, yet the underlying mechanism is far more complex.

711C

Naturally segregating polymorphisms responsible for quantitative variation in gene expression and wing vein position in *Drosophila melanogaster*. James Lorigan, Fangfei Ye, Jason Mezey. Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

The position of the longitudinal wing veins in *Drosophila* shows considerable variation in both wild and artificially-selected populations. The Hedgehog and Decapentaplegic signal transduction pathways have been found to play a role in generating quantitative variation in the distances between wing veins L3-L4 and L2-L5, respectively. Differential expression of genes in these highly-conserved pathways is therefore of interest for understanding the production of quantitative variation in the position of the longitudinal veins. Here we identify naturally occurring polymorphisms in coding and regulatory regions of candidate genes. We use association mapping to identify those polymorphisms responsible for variation in the expression of Hedgehog and Decapentaplegic pathway genes in the wing discs of third-instar larvae. In addition, we test for associations of these polymorphisms with quantitative variation in wing vein position. These results are being used to construct a quantitative developmental model that can explain quantitative variation in wing venation.

712A

Genetic Analysis of Intraspecific and Interspecific Sexually Dimorphic Trait Differences in the *Drosophila bipectinata* Species Complex. Chen Siang Ng, Andrew Hamilton, Artyom Kopp. Section of Evolution and Ecology, University of California, Davis, CA 95616, USA.

Sexually dimorphic traits evolve rapidly among closely related species, presenting good opportunities to study sexual selection as well as genetic basis of morphological changes. Here we investigate the genetic basis of sexually dimorphic trait differences in the *bipectinata* species complex. The six closely related species and subspecies of the *bipectinata* species complex belong to the *ananassae* subgroup and are widespread from eastern Africa to Australia and South Pacific islands. They show striking intraspecific and interspecific morphological variation in two sexually dimorphic traits - pigmentation and the sex-comb. *D. parabipectinata*, *D. malerkotliana malerkotliana* and *D. pseudoananassae nigrens* show a distinct sexually dimorphic pattern where the last three abdominal segments are melanized in males. Males of *D. bipectinata*, *D. p. pseudoananassae* and *D. m. pallens* almost entirely lack dark abdominal pigmentation. *D. bipectinata* and *D. parabipectinata* also have unique rotated sex-combs that are distinct from all other members of the *ananassae* subgroup. Possible candidate loci in the pigmentation and sex-comb development pathway, as well as neutral molecular markers, were used to map QTLs responsible for these differences. QTLs are mapped in the F_2 hybrids in the crosses between *D. m. malerkotliana* and *D. m. pallens* for intraspecific pigmentation differences and between *D. bipectinata* and *D. m. markotliana* for interspecific pigmentation and the sex-comb differences. We will determine if the regions delimited by the QTL include any known candidate genes. Spatial or temporal differences of expression levels of candidate genes will be investigated using molecular and developmental genetic approaches.

713B

The genetic basis of eye size and shape differences between two closely related species of Drosophila. Maria Margarita Ramos[1], David Stern[1], Peter Grant[1], Andrew I. Hanna[2], Enrico Coen[3]. 1) Ecology & Evolutionary Biol, Princeton Univ, Princeton, NJ; 2) Signal and Image Processing Research Group, Royal Society Wolfson Bioinformatics Laboratory, University of East Anglia, Norwich,UK; 3) Cell and Developmental Biology Department, John Innes Centre, Norwich,UK.

What are the mechanisms and laws underlying morphological evolution? To generate a comprehensive understanding of how evolutionary change proceeds we need to characterize both proximate (genetic basis, development) and ultimate causes (selective forces in nature) generating phenotypic variation. A combination of both laboratory and field studies of closely related species allows us to identify genetic changes responsible for phenotypic differences and the selective forces that brought them forth. The eye is a complex morphological structure that has diversified into a varied array of types to fit the lifestyle of its bearer. Within the melanogaster species subgroup, Drosophila mauritiana has larger eyes (about 30% more ommatidia) than its sibling species Drosophila simulans. In this ongoing project we seek an integrative approach to understand the evolution of eye size and shape differences in Drosophila mauritiana and Drosophila simulans by examining both its proximate and ultimate causes. Here I present an approach to quantifying the differences in eye size and shape between these two species and to mapping the genetic basis of this morphological trait. Using MatLab based software, we decomposed eye size and shape variation in a population of backcrosses using principal component analysis. The resulting PC values were used as phenotypes in QTL mapping. A total of five PCs map (in total explaining 71.9% of the total biological variation in the PCA and 63.2% of the difference between the species) between vermillion and forked on the X chromosome suggesting that at least one major QTL is located in this region. Currently we are expanding the mapping to the rest of the genome and finely mapping the QTL on chromosome X. Identifying the genetic basis of morphological differences between closely related taxa might help us to better understand patterns of morphological evolution. Such studies are likely to pinpoint important mechanisms generating variation in morphological characters from a conserved set of genes.

714C

Transcriptional profiles of high and low selection lines for the temperature knock down phenotype in Drosophila melanogaster. David Rand[1], Colin Meiklejohn[1], Donna Folk[2], George Gilchrist[2]. 1) Ecology & Evolutionary Biol, Brown Univ, Providence, RI; 2) Dept. of Biology, College of William & Mary, Williamsburg, VA.

We have carried out an artificial selection experiment on thermal tolerance in Drosophila melanogaster with the aim of dissecting the genetic architecture of thermal traits. Replicate populations of flies have been subjected to high and low selection for temperature knock-down (Tkd) in a 'Weber column'. Natural populations of D. melanogaster show a clear bimodality in the distribution of Tkd suggesting some major-effect genes underlying this trait. The high and low selection lines appear to have pulled the two modes apart, further implicating major-effect genes. A microsatellite scan uncovered a highly significant QTL in band 3, which is definitively not the period locus, and may be the shaggy locus. Shaggy protein levels are significantly different between the high and low lines, and the SGG allele that has been increased in the high selection lines is more common in Florida than in Maine, suggesting that laboratory artificial selection and latitudinal selection may have acted on the same variants. To gain a more complete understanding of the genomic response to this thermal selection regime, we determined the transcriptional profiles of replicate high and low selected populations using microarrays. A factorial design was used where high and low populations were each subjected to a heat shock or no heat shock before freezing the flies. This allows us to identify the effect that thermal selection has had on the constitutive vs. the inducible response to temperature stress. The analyses show that the high selection treatment had a more significant effect on elevating the constitutive levels of transcripts involved in the heat shock response, as compared to inducible levels of these genes. Shaggy transcripts were also affected by the selection process, and further show differences between the heat shock and non heat shock treatments. This study reveals how selection can act on standing vs. plastic variation for complex phenotypes.

715A

Genetics of alcohol sensitivity in *Drosophila melanogaster*. Reba M. Royster[1,3], Robert R. H. Anholt[1,2,3], Trudy F. C. Mackay[1,3]. 1) Genetics, NC State University, Raleigh, NC; 2) Zoology, NC State University, Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, NC State University, Raleigh, NC.

Drosophila melanogaster provides an attractive model for studies on the genetic architecture of alcohol sensitivity. Alcohol sensitivity in *Drosophila* can be measured as the elution time from an "inebriometer" upon exposure of flies to ethanol vapors. The inebriometer is a 4 foot long glass tube with mesh partitions to which ethanol-intoxicated flies can adhere. We measured inebriometer elution times following exposure to saturated ethanol vapors for 21 recombinant inbred introgression lines of *Drosophila melanogaster*, derived from parental Oregon and 2b inbred strains, to identify quantitative trait loci (QTL) for alcohol sensitivity. Analysis of variance showed significant variation among the introgression lines with phenotypic values of the introgression lines exceeding those of the parental strains providing evidence for extensive epistasis between genes in the introgressed 2b regions and the Oregon host genome. We identified 11 QTL regions, which harbor genes that contribute variation in alcohol sensitivity. Diallel crosses between 10 introgression lines with significant effects were made to quantify epistasis. Because genotypic variation among the Oregon and 2b lines is limited, we also assessed naturally occurring variation for alcohol sensitivity in 341 wild-derived inbred lines from a natural Raleigh population. Statistical analysis revealed significant variation among lines and between sexes, with greater sensitivity among males than females. To account for previously observed effects of alcohol dehydrogenase (ADH) alleles on alcohol sensitivity, we genotyped these lines for fast and slow ADH alleles and selected 10 sensitive and 10 resistant lines with half of each group harboring the fast ADH allele and half the slow ADH allele. These lines will be used in future studies for further QTL mapping and transcriptional profiling aimed at identifying quantitative trait genes responsible for variation in alcohol sensitivity.

716B

Dynamic Genetic Interactions Determine Odor-Guided Behavior in *Drosophila melanogaster*. Deepa Sambandan[1,2], Trudy F.C. Mackay[1,2], Robert R.H. Anholt[1,2,3]. 1) Department of Genetics; 2) W.M. Keck Center for Behavioral Biology; 3) Department of Zoology, North Carolina State University, Raleigh, NC.

Understanding the genetic architecture of complex traits requires identification of the underlying genes and characterization of gene-by-gene and genotype by environment interactions. Behaviors that mediate interactions between organisms and their environment are complex traits expected to be especially sensitive to environmental conditions. Previous studies on the olfactory avoidance response of *Drosophila melanogaster* showed that the genetic architecture of this model behavior depends on epistatic networks of pleiotropic genes. Here we characterized the expression profiles of ten *p[GT1]*-element insertion lines and showed that the effects of the transposon insertions are often dependent on developmental stage. Hypomorphic mutations in developmental genes can elicit profound adult behavioral deficits. We assessed epistasis among these genes by constructing all possible double heterozygotes and measuring avoidance responses under two stimulus conditions. We observed enhancer and suppressor effects among subsets of these genes, and surprisingly, epistatic interactions shifted with changes in the concentration of the olfactory stimulus. Our results show that the manifestation of epistatic networks dynamically changes with alterations in the environment.

717C

Natural genetic variation on the 3rd chromosome influencing a host of quantitative traits and their interactions in *Drosophila melanogaster*. Adrienne Starks, Jeff Leips. Dept Biological Sciences, UMBC, Baltimore, MD.

Senescence, the age related decline in physiological performance, is reflected in a number of traits including fecundity, susceptibility to disease, agility, and mobility. When senescence is observed in an organism, it is not clear if there is a general decline in function across all traits or if certain traits decline at different rates. In this study, we investigated the effects of age on a host of quantitative traits including: immune response, fecundity (reproduction), triglyceride level (energy storage), and lifespan in the fruit fly, *Drosophila melanogaster*. We used a panel of 3rd chromosome extraction lines derived from a natural population in Raleigh, NC to construct a partial reciprocal diallel cross to measure each of these traits. A partial reciprocal diallel cross provides insight to understand inheritance through multiple shared parental crosses and also provides the ability to identify genetic interrelationships between each of the traits. These traits were chosen because of possible positive/ negative interactions due to competing fitness strategies for the allocation of energy. In addition, each trait (except lifespan) was measures at an early age (1 week) and a late age (6 weeks) to identify age related changes for each trait. Analysis of this data through phenotypic trait values for each individual and through group comparisons would identify: (1) genetic variation within each trait, (2) genetically based age related differences within each trait, and (3) genetic correlations for each trait along with any age related differences. Results from this study will be presented.

718A

Investigating interactions between HP1 and the hybrid incompatibility protein LHR. Nicholas J. Brideau, Xu Wang, Daniel A. Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Interspecific crosses between *D. melanogaster* females and *D. simulans* males produce inviable F1 males and sterile, semi-viable F1 females. The gene *Lethal hybrid rescue* (*Lhr*) in *D. simulans* contributes to this hybrid incompatibility and has recently been cloned in our lab. A YFP::LHR fusion protein predominantly localizes to heterochromatic regions of polytene chromosomes. In congruence with this localization pattern, *D. melanogaster* LHR has been shown to interact with Heterochromatin Protein 1 (HP1) in a yeast two-hybrid screen. *Lhr* orthologs are highly divergent among *Drosophila* species. Despite this divergence we have found that *D. melanogaster* HP1 also interacts with LHR from the following six species: *D. simulans*, *D. yakuba*, *D. erecta*, *D. ananassae*, *D. pseudoobscura*, and *D. virilis*. In addition, we have found that the chromo-shadow domain of HP1 is required for this interaction, which is consistent with data from other HP1-interacting proteins. We are currently attempting to identify the region within LHR necessary and sufficient for interacting with HP1 using yeast two-hybrid analysis and other approaches.

719B

Adaptive evolution underlies genetic divergence contributing to hybrid incompatibilities in *Drosophila*. Corbin Jones[1], Alisha Holloway[2]. 1) Department of Biology & Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC; 2) Section of Evolution and Ecology & Center for Population Biology, UC-Davis, CA.

We do not currently know how important adaptive evolution is to speciation, the process by which one species splits into two. In *Drosophila* and other organisms, recent work has identified a few genes affecting a key aspect of speciation, hybrid incompatibility. These data suggest that incompatibility between species is frequently the byproduct of adaptive protein divergence within species. Evolutionary theory suggests that both adaptive evolution and genetic drift can produce the genetic divergence that leads to hybrid incompatibilities. We, however, have little data indicating which force predominates. To address this question, we have combined a genome-wide survey of genes experiencing selection, interspecific gene expression data, and genetic data on hybrid incompatibilities from the autosomes of *Drosophila melanogaster* and *D. simulans*. Our analysis shows that chromosomal regions affecting hybrid inviability between *D. melanogaster* and *D. simulans* are enriched for genes that have undergone recurrent directional selection relative to the rest of the genome. We also show that these same regions harbor more genes that have significant expression level divergence since the recent common ancestor. Interestingly, the genes in these intervals represent a non-random subset of biological and molecular functions. Genes involved with the nuclear pore are over-represented, which is congruent with Presgraves *et al* (2003) data showing that an adaptively evolving Nucleoporin gene (*Nup96/Nup98*) in one of these intervals affects hybrid inviability. Genes important to chromosome structure are also over-represented, including the gene *cid* that has been suggested as contributing to hybrid incompatibilities (Malik and Henikoff, 2001). Interestingly, these regions are also enriched for Myb complex genes (the X linked hybrid male rescue gene, *Hmr*, is Myb-like [Barbash et al 2003]). In total, our analysis suggests a critical role for adaptive evolution in speciation.

720C

Population genetics of a recently divergent group of Hawaiian *Drosophila*. Richard Lapoint, Dr. Patrick O'Grady. ESPM, UC, Berkeley, Berkeley, CA.

The Hawaiian Islands are the most isolated archipelago on Earth and contain a remarkable diversity of endemic species. Their isolation from the mainland, complex geological history, and dynamic landscape make these islands the perfect system to study evolutionary processes. The Hawaiian *Drosophila*, a hyperdiverse lineage of close to 1000 species, is an excellent model for understanding the formation of biodiversity. These species have diverged both morphologically and ecologically, further increasing their utility as an evolutionary model. The population genetics of this system are similarly complex. The Hawaiian *Drosophila* have repeatedly undergone bottlenecks as they colonize new islands or as populations are divided by lava flows, elevational and rainfall gradients, or erosional processes. This consequent reduction in effective population sizes, known as founder effect, changes allele frequencies via drift and may encourage diversification of the resultant populations. Hybridization has also been implicated as an agent of diversification in Hawaiian *Drosophila*. Divergence population genetics (DPG), an approach combining population genetic and phylogenetic analyses, was used to examine the processes connecting micro and macroevolution during the formation of new species. We used DPG to study several genetic markers in the spoon tarsus species group of Hawaiian *Drosophila*. This clade consists of 8 closely related species, all of which are found on the island of Hawaii and have diverged within the past ~400,000 years. We employed tests of neutrality and examined levels of polymorphism to search for evidence of bottlenecks in the populations. Phylogenetic analysis was used to examine the relationships between species and uncover any potential instances of hybridization between species. A molecular clock was calibrated using the known ages of the Hawaiian Islands to place the divergence of these taxa within a temporal context.

721A

An investigation into the molecular function of the hybrid incompatibility gene, *Lhr*. Shamoni Maheshwari, Daniel A. Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Hybrid sons produced from a cross between *D. melanogaster* mothers and *D. simulans* fathers die as third instar larvae. Removal of the *Hmr* gene from *D. melanogaster* can suppress this incompatibility and thus produce viable F1 sons. The evolution of such loci that reduce hybrid fitness has been explained by Dobzhansky and Muller as resulting from a two-locus interaction. In this model loci accumulate species-specific mutations in each parental lineage, the byproduct of which is an incompatible interaction in the hybrid background. Our lab recently cloned a second rescue mutation *Lhr* from *D. simulans*, which together with *Hmr* forms a candidate DM pair of interacting loci. The *Lhr* gene has functionally diverged between the two species; only removal of the *D. simulans* allele of *Lhr*, but not the *D. melanogaster* allele, suppresses hybrid lethality. The *D. simulans* ortholog has a derived 16 aa insertion relative to *D. melanogaster*, and Lhr^2, a new rescue allele, is missing this insertion. We will construct synthetic transgenes to test if this indel is necessay/sufficient to complement the Lhr^1 rescue mutation. We are also identifying LHR-interacting proteins in order to learn more about *Lhr* function within species and in hybrid lethality. LHR contains a BESS domain, which mediates protein-protein interactions between a number of DNA binding proteins and transcription factors. Moreover, LHR interacts with Heterochromatin Protein 1 (HP1) in yeast two-hybrid assays. This is suggestive of a chromatin-associated function and identification of other interaction partners will help to place *Lhr* in the context of defined molecular networks, which will facilitate an understanding of wild type function. We are using tandem affinity purification (TAP tagging), and have identified several candidate interaction partners from *Drosophila* embryonic cell lines.

722B

The genetic basis of segregation distortion and male sterility in the F_1 hybrids between *Drosophila pseudoobscura* USA and Bogotá sub-species. Nitin Phadnis, H. Allen Orr. Department of Biology, University of Rochester, Rochester, NY.

The idea that selfish genetic elements, such as segregation distorters, may play a role in speciation is intuitively appealing and has a controversial history. Recent work has shown that "sterile" F_1 hybrid males between the USA and Bogotá subspecies of *Drosophila pseudoobscura* - taxa considered paradigmatic of the early stages of speciation - become weakly fertile when aged. These weakly fertile males produce almost all daughters, apparently reflecting sex chromosome segregation distortion. Mapping studies have shown that the same regions on the Bogotá X-chromosome underlie both hybrid male sterility and hybrid segregation distortion. The critical question is whether the *same* genes cause both hybrid male sterility and hybrid segregation distortion. We are attempting to answer this question by fine-mapping the genes that cause hybrid male sterility and hybrid segregation distortion through a large introgression experiment. In particular, we focused on a region linked to the visible mutation sepia on the *D. pseudoobscura XR*, which is known to play a large - and essential - role in both hybrid male sterility and segregation distortion. We have introgressed approximately 200 independent copies of the *sepia* region from USA into an otherwise Bogotá background, and performed backcrosses to Bogotá for 28 generations (14 recombinational). So far, we have been unable to meiotically separate the genes causing hybrid male sterility from those causing hybrid segregation distortion. Phenotypic characterization and genotyping of these lines using molecular markers has allowed us to fine-map the genes responsible for both hybrid sterility and segregation distortion to an interval containing five genes. It seems likely that the same gene(s) cause both hybrid problems in the F_1 hybrids between these young taxa.

723C

A new type of apoptosis-induced compensatory proliferation requires activity of effector caspases. Yun Fan, Andreas Bergmann. Department of Biochemistry & Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Cell death and cell proliferation are coordinated during development of multicellular organisms. Recently, apoptotic cells have been found to be able to induce compensatory proliferation in *Drosophila* wing discs when they are kept alive by blocking cell death using the caspase inhibitor P35. Under these conditions, *dpp* and *wg* signaling cascades are activated in the undead cell to induce compensatory proliferation. This requires the function of the Dronc caspase and the *dp53* gene. Here, we show that eye-specific expression of the proapoptotic gene *hid* under GMR control (GMR-*hid*) in the absence of P35 reveals a different type of apoptosis-induced compensatory proliferation in *Drosophila*. GMR-*hid* induces cell death in both differentiating cells and differentiated neurons in the 3rd instar eye discs. Interestingly, a wave of compensatory proliferation behind the second mitotic wave is also induced in *hid*-expressing differentiating cells. This suggests that cells under stress have the capacity to induce proliferation. Strikingly, unlike wing discs, this *hid*-induced compensatory proliferation in the eye disc can be inhibited by simultaneous expression of P35. Moreover, induction of the *dpp* and *wg* signaling and a requirement of *dp53* were not observed in this process. Altogether these data suggest that the compensatory proliferation in eye discs is triggered and regulated in a different way compared to wing discs. Further genetic analysis using multiple alleles of cell death regulators revealed that, in addition of the apical caspase Dronc, the effector caspases DrICE and Dcp-1 also play a key role to coordinate cell death and proliferation in eye discs. The possible signaling pathways that are triggered by DrICE and Dcp-1 are currently under investigation. In summary, these studies illustrate that different types of compensatory proliferation in response to apoptotic activity exist.

724A

The caspases Strica and Dronc function redundantly during programmed cell death in oogenesis. Kim McCall[1], Jason S. Baum[1], B. Paige Bass[1], Jeanne S. Peterson[1], Antony Rodriguez[2], John M. Abrams[2]. 1) Department of Biology, Boston University, Boston, MA; 2) Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Programmed cell death (PCD) in the Drosophila ovary occurs either during mid-oogenesis, resulting in degeneration of the entire egg chamber, or during late oogenesis, to facilitate the development of the oocyte. PCD during oogenesis is regulated by mechanisms distinct from those that control cell death in other tissues. We have analyzed the role of caspases in PCD of the female germline by examining caspase mutants and overexpressing caspase inhibitors. Surprisingly, mutants of the initiator caspases Dronc or Dredd did not affect either cell death pathway. Imprecise P element excision was used to generate mutants of the third initiator caspase strica. While null mutants of strica displayed no significant phenotype, we have determined that strica exhibits redundancy with dronc during both mid- and late oogenesis. Ovaries of double mutants contain defective mid-stage egg chambers similar to those seen in dcp-1 mutants, and mature egg chambers with persisting nurse cell nuclei. In addition, the effector caspases drice and dcp-1 also display redundant functions during late oogenesis, resulting in persisting nurse cell nuclei. Mutants of dark showed a similar late oogenesis phenotype but surprisingly did not show any defects in mid-oogenesis. These findings indicate that caspases are required for germline cell death in mid-oogenesis, and participate in developmental nurse cell death during late oogenesis. Both pathways involve redundancy between the initiator caspases strica and dronc.

725B

Expression of a caspase-resistant nuclear lamin disrupts cell death and morphogenesis in oogenesis. Elizabeth A Tanner, Margaret Barkett, Kimberly McCall. Department of Biology, Boston University, Boston, MA.

During Drosophila oogenesis, cell death plays a role in the maturation of egg chambers. Each oocyte develops in an egg chamber composed of the oocyte and fifteen nurse cells, surrounded by somatically derived follicle cells. During normal development, the nurse cells produce factors essential for early development of the embryo and then deposit their cytoplasmic contents into the oocyte. What remains of the nurse cells then dies through programmed cell death (PCD). To determine the role of a known caspase substrate in developmental nurse cell death, a mutant form of Lamin Dm_0 was generated which is resistant to caspase cleavage. Overexpression of the mutant lamin Dm_0 in the germline using the UASp/nanos GAL4 system resulted in abnormal stage 14 egg chambers, which commonly displayed persisting nurse cell nuclei and cup-shaped phenotypes, while lines overexpressing wild-type lamin looked mostly normal. The cup shape is distinguished by the lack of dorsal appendages. To determine if dorsal/ventral polarity was disrupted we analyzed Gurken (Grk) signaling. Grk localized properly from early stages to stage 10 and downstream targets of Grk signaling were not altered. This suggests that the disruption of dorsal appendage morphogenesis in egg chambers expressing a caspase-resistant nuclear lamin is not due to an alteration in dorsal-ventral patterning. To determine whether the abnormal phenotypes seen in the caspase-resistant lamin lines are due to increased levels of lamin or the absence of lamin cleavage events, we are performing expression analysis of mRNA and protein levels in the transgenics. Our findings indicate that caspase cleavage of nuclear lamins may play a role in nurse cell death as well as dorsal appendage morphogenesis.

726C

Ionizing radiation induces caspase-dependent but Chk2- and p53-independent cell death. Anita Wichmann[1], Burnley Jaklevic[2], Tin Tin Su[1]. 1) Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO; 2) Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, CA.

Ionizing radiation (IR) can induce apoptosis via p53, which is the most commonly mutated gene in human cancers. Loss of p53, however, can render cancer cells refractory to therapeutic effects of IR. Alternate, p53-independent pathways exist but are not as well understood as p53-dependent apoptosis. Studies of how IR induces p53-independent cell death could benefit from the existence of a genetically tractable model. In *Drosophila melanogaster*, IR induces apoptosis in the imaginal discs of larvae, typically assayed at 4-6 hours after exposure to a LD_{50} dose. In mutants of *Drosophila* Chk2 or p53 homologs, apoptosis is severely diminished in these assays, leading to the widely held belief that IR-induced apoptosis is dependent on these genes in *Drosophila*. Here we show that IR-induced apoptosis still occurs in the imaginal discs of *chk2* and *p53* mutant larvae, albeit with a delay. We demonstrate that this is a true apoptotic response, as it requires caspase activity and the chromosomal locus that encodes the pro-apoptotic genes *reaper*, *hid* and *grim*. Quantitative RT-PCR experiments show the increase in transcript levels of these pro-apoptotic genes is delayed and reduced, relative to wild-type, in *chk2* and *p53* mutants. These data are consistent with a threshold model in which it takes longer for pro-apoptotic gene products to accumulate to an apoptosis-stimulating level in *chk2* and *p53* mutants. We also show that Chk2- and p53-independent apoptosis is IR-dose dependent and is therefore likely triggered by a DNA damage signal. We conclude that *Drosophila* has Chk2- and p53-independent pathways to activate caspases and induce apoptosis in response to IR. This work establishes *Drosophila* as a model for p53-independent apoptosis, which is of potential therapeutic importance for inducing cell death in p53-deficient cancer cells.

727A

C-type lectin, Furrowed, aborts development of malaria parasite, *Plasmodium berghei* in tropical vector, *Anopheles gambiae*. Hiroka Aonuma[1,2], Stephanie Brandt[3], Shinya Fukumoto[1], Tokiyasu Teramoto[1], Masayuki Miura[4], Takeshi Yagi[2], Hirotaka Kanuka[1], David Schneider[3]. 1) NRCPD, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan; 2) Graduate School of Frontier Bioscience, Osaka University, Suita, Osaka, Japan; 3) Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA; 4) Graduate School of Pharmaceutical Sciences, University of Tokyo, Bunkyo, Tokyo, Japan.

Despite Malaria being a debilitating disease causing at least 1 million of deaths a year, the exact interaction between the mosquito vector and malaria parasite, *Plasmodium*, remains obscure. Understanding how mosquito resists invading parasites and how parasites fight back against the mosquito immune system may be key to clear the whole system of malaria. Unfortunately, not much is known due to the difficulty of genetic analysis using mosquitoes. Therefore, in order to understand the mosquito's overall immune mechanisms against *Plasmodium*, we used *Drosophila* as a substitute model for mosquitoes. *Drosophila* were screened for mutant that affected *Plasmodium* growth in their body. From these mutants, we have determined 1 gene responsible for hindering *Plasmodium* growth in *Drosophila*. Here we show that the gene, *furrowed*, is responsible for hindering *Plasmodium* growth not only in *Drosophila* but also in the natural mosquito vector, *Anopheles gambiae*. Reduction of Furrowed function in *Anopheles gambiae* using RNAi resulted in increased number of *Plasmodium* oocysts in the mosquito midgut, indicating that Furrowed is involved in hindering *Plasmodium* growth in anopheline mosquitoes. Since knockdown of *furrowed* resulted in increased *Plasmodium* oocysts, Furrowed likely functions around the mosquito midgut cells, where ookinetes cross through and develop into oocysts. We will show where and how Furrowed functions against *Plasmodium* in *Anopheles gambiae* and it's relation to the mosquito's ability to fight and abort invading parasites.

728B

Functional analysis of a major phagocytic pattern recognition receptor in *Drosophila*. Ju Hyun Cho, Christine Kocks. Department of Pediatrics, Harvard Medical School, Developmental Immunology, Massachusetts General Hospital, Boston, MA.

Phagocytosis is a complex, evolutionarily conserved process that plays a central role in host defense against infection. Recently, we have identified a predicted transmembrane protein, termed Eater, which plays a critical role in the survival of bacterial infections in *Drosophila* (Kocks et al., 2005, Cell 123: 335-46). Our results suggest that Eater is a novel, scavenger-like pattern recognition receptor that mediates the phagocytosis and inactivation of a broad range of microbial pathogens. Eater mediates pathogen defenses independently of the nuclear factor kappa B-like immunity signaling pathways Toll and IMD. In order to address the mechanisms of how Eater mediates microbe internalization and destruction, we have generated epitope-tagged variants of the Eater molecule. By analyzing the role of these variant molecules in transfected cell lines, we hope to be able to shed light on the molecular events that underlie Eater function.

729C

Undertaker, a new Drosophila mutant with defects in phagocytosis of apoptotic cells. Nathalie C. Franc, Leigh Cuttell, Emeline Van Goethem, Claire Escaron, Christina Bakatselou, Mark Lavine, Magali Quirin. MRC LMCB & CBU, University College London, London, GB.

Apoptosis allows for the maintenance of homeostasis and tissue remodelling during development of all multicellular organisms. Professional phagocytes, such as macrophages, swiftly recognize, engulf and digest apoptotic cells. In *Drosophila* embryos, macrophages are efficient phagocytes and our lab focuses on the study of these cells with respect to clearance of apoptotic cells. A deficiency screen identified a large number of mutants with defects in all aspects of embryonic macrophage biology, from their formation, differentiation or maturation, to their proliferation or survival, migration, and phagocytic capacity. We characterized a new phagocytosis-defective mutant, *undertaker*, and identified the gene responsible for this phenotype. It encodes a MORN repeat-containing protein and is autonomously required for efficient engulfment of apoptotic cells by embryonic macrophages. In mammals, MORN-repeat containing proteins of the junctophilin family have been shown to participate in calcium influx regulation, controlling intracellular calcium concentration. Other proteins with MORN repeats have also been proposed to participate in cytoskeleton rearrangement during plant chloroplast and parasite division. We are currently investigating the possible role of *undertaker* in regulating such cellular events and addressing their potential relationships with our mutant's phagocytosis defect. Progress made will be presented.

730A

Rel signaling guides immune homeostasis in *Drosophila*. Nina Matova, Kathryn V. Anderson. Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Previous investigation of double mutants for two Rel genes, *Dif* and *dorsal*, showed a central function for blood cells in the *Drosophila* immune response that is dependent on Rel signaling and is essential for the survival of the animal. Cell-autonomous activities of Dif and Dorsal are required for survival of hemocytes and effective phagocytosis of microbes. Here, we performed tissue-specific rescue experiments of *Dif dorsal* mutants and uncovered a network of interactions among the immune-responsive organs—blood cells, the lymph gland, the epidermis and the fat body—that are governed by Rel proteins in *Drosophila*. Expression of *dorsal* in the hematopoietic organ, the lymph gland, rescued blood-cell numbers in circulation and cleared infection. Surprisingly, expression of either *Dif* or *dorsal* exclusively in the fat body also rescued blood-cell numbers, revealing a non-autonomous effect of Rel signaling on blood cells. Epidermal expression of *dorsal* was sufficient to protect *Dif dorsal* animals against infection without affecting blood-cell numbers or morphology. Thus, Dorsal has an immune function that is independent of blood cells. Together these experiments showed that Rel-dependent transcription leads to the production of molecules that ensure communication among immune tissues and maintenance of immune homeostasis in *Drosophila*.

731B

Study of a new phagocytosis of apoptotic cells-defective mutant in Drosophila. EMELINE VAN GOETHEM, NATHALIE C. FRANC. MRC-LMCB/CBU, UNIVERSITY COLLEGE LONDON, LONDON, GB.

Apoptosis, a form of programmed cell death, is an important part of the development of all multicellular organisms as it controls homeostasis and tissue remodelling. We study phagocytosis, the removal of apoptotic cells by macrophages. In a genetic screen of the deficiency collection using acridine orange that stains all apoptotic cells in the fly embryo, we found 13 new deletions with phagocytosis defects based on their lack of clustering of apoptotic corpses in a macrophage distribution pattern. Among those, two overlapped and deleted the *Drosophila ced-12* gene (*dced-12*). These two mutants have increased programmed cell that is likely the result of their segmentation defect as they are also deleted for the *paired* gene. Because *ced-12* was shown to be involved in the engulfment of apoptotic corpses in *C. elegans* and mammals (*elmo1*), we decided to study these deletions further. However, our genetic analysis of new deficiencies in the region appear to dismiss *dced-12* as being the gene responsible for the phagocytosis defect seen in the deficiency lines, and instead delineate a new region of interest covering 26 genes. As the defect could be masked by a maternal contribution of *dced-12*, we continue to assess whether it may play a role in phagocytosis of apoptotic corpses using RNA interference. In parallel, genetics and molecular studies are combined to identify the candidate gene responsible for the phenotypes observed within our deficiency lines. Progress made will be reported.

732C

Control of larval hematopoiesis by the Posterior Signaling Center. Alain Vincent[1], Joanna Krzemien[1], Rami Makki[1], Laurence Dubois[1], Marie Meister[2], Michele Crozatier[1]. 1) Developmental Biology, UMR 5547 CNRS/UPS, Toulouse, France; 2) UPR 9022 CNRS, Strasbourg, France.

Drosophila hematopoiesis occurs in two phases during development. A first population of hemocyte precursors, specified during embryogenesis gives rise to an invariant number of plasmatocytes and crystal cells. A second population of hemocytes are specified during larval development from a specialised hematopoietic organ, the lymph gland, which disrupts at metamorphosis and releases its prohemocytes and hemocytes into the hemolymph Larval hematopoietic progenitors (prohemocytes) give rise to three types of circulating hemocytes : plasmatocytes, crystal cells and lamellocytes. Lamellocytes, which are devoted to encapsulation of large foreign bodies are not found in healthy larvae but only differentiate in response to specific immune threats such as parasitization by wasps. Here we show that a small cluster of signaling cells, termed the PSC (Posterior Signaling Center) controls the balance between multipotent prohemocytes and differentiating hemocytes and is necessary for the massive differentiation of lamellocytes that follows parasitization. Communication between the PSC and hematopoietic progenitors strictly depends on the PSC-restricted expression of Collier, the Drosophila ortholog of mammalian Early-B Cell factor. PSC cells act, in a non cell autonomous manner, to maintain JAK/STAT signalling activity in prohemocytes, preventing their premature differentiation. The key role of the PSC in controlling blood cell homeostasis is reminiscent of interactions between hematopoietic stem cells and their micro-environment (niche) described in vertebrates, thus further highlighting the interest of Drosophila as a model system for studying the evolution of hematopoiesis and cellular innate immunity.

733A

An isoform specific requirement for lola during programmed cell death in oogenesis. B. Paige Bass, Kim McCall. Dept Biol, Boston Univ, Boston, MA.

Programmed cell death (PCD) occurs during early, mid-stage, and late-stage *Drosophila* oogenesis. In response to nutrient deprivation, chemical induction, or abnormal development, germline cyst cells may undergo PCD in the germarium or egg chambers may undergo PCD around stage 7/8, serving as a developmental checkpoint. During late *Drosophila* oogenesis, as a part of normal egg chamber development, the nurse cells undergo PCD after dumping their cytoplasmic contents into the developing oocyte. The checkpoint and developmental PCD involve distinct pathways. We have performed a screen to identify genes involved in these pathways. From this screen we recovered an allele of *lola*, a gene encoding several different zinc-finger transcription factors previously characterized as being involved in axon guidance. A time-course study has revealed that *lola* germline clones exhibit abnormal nuclear organization which becomes increasingly severe with age. Specifically, chromatin appears diffuse and fails to condense properly in dying nurse cells. Large masses of chromatin accumulate in the ovaries of older flies containing germline clones. In *lola* germline clones, DNA fragmentation does not occur although caspase activation is seen. This results in a disruption of both mid-stage and late stage PCD. We propose that *lola* is required for proper nuclear condensation which is necessary for DNA fragmentation during PCD in the ovary. The mutation in our screen line, $lola^{629}$, was mapped to an isoform K specific exon. We have analyzed the ovarian phenotype of several other *lola* alleles, including other isoform K mutants. Our phenotypic analysis suggests that isoform K plays an essential role in the ovary. Interestingly, previous studies have shown that isoform K mutants do not exhibit the severe defects in the nervous system seen in other *lola* alleles suggesting a differential requirement for individual *lola* splice forms in the ovary and in the embryonic nervous system. Studies aimed at identifying a potential mechanism of action for *lola* in the ovary are underway and will be presented.

734B

The RING-finger protein elfless: at the crossroads of spermatogenesis and apoptosis. Jason Caldwell, Daniel Eberl. Dept Biological Sci, Univ Iowa, Iowa City, IA.

In overlapping deficiencies *Df(2L)TW119/Df(2L)TW201* three phenotypes are evident: deafness, reduced ocelli and male sterility. The male sterile phenotype results from the failure of spermatids to individualize. Recently, it has been shown that apoptosis is an essential process during individualization. *elfless* was a candidate for the male sterile phenotype in region 36D as a full-length *elfless* cDNA had previously been isolated from an adult testis EST library. *elfless* is predicted to encode a a 187 amino acid zinc finger Cys3-His-Cys4 RING protein. RING domains are known to be involved in protein-protein interactions and have been shown to mediate E2 ubiquitin conjugase-dependent ubiquitination and may also function as essential regulators of apoptosis. Indeed, *elfless* is predicted to encode an E3 ubiquitin ligase and has recently been shown, in a yeast two-hybrid assay, to interact with the E2 ubiquitin conjugating enzyme, *ubcD1*. Several experimental approaches were used to characterize *elfless* and attribute defects in this gene to the male sterile phenotype in 36DE. rtPCR analysis indicated that *elfless*expression is greatly enhanced in the testes and the expression pattern of a UAS-*elfless*-EGFP driven by *elfless*-Gal4 is likewise restricted to the testes specifically in the tail cyst cell nuclei. Two genetic techniques were used to generate mutations of elfless; 1) imprecise excisions with an upstream P-element, KG02815, and 2) FLP-FRT deletion of *elfless* with two neighboring piggyBac elements, yet disruption of *elfless* fails to induce male sterility although fertility may be slightly reduced. We addressed the in vivo role of *elfless* in a gain-of-function experiment with *GMR*-Gal4. Our present model is that *elfless* promotes ubiquitination of *DIAP1* (*Drosophila inhibitor of apoptosis*) and this, in turn, induces apoptosis through the downstream activator caspase *Dronc*. The overall conclusion from these analyses is that *elfless* is not absolutely essential for male fertility, but may reduce fertility, and *elfless* may function redundantly in the apoptotic pathway in the testes.

735C

Identification of recessive suppressors and enhancers of Hid-induced cell death. Hans-Martin Herz[1,2], Zhihong Chen[1], Andreas Bergmann[1]. 1) Dept Biochem & Molecular Biol, MD Anderson Cancer Ctr, 1515 Holcombe Blvd, Houston, TX 77030, USA; 2) Center for Molecular Biology (ZMBH), University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

Apoptosis plays an important role in regulating the development of organisms as well as maintaining proper tissue homeostasis. Interference with the integrity of the cell death pathway has been shown in manifold ways to result in developmental abnormalities as well as different diseases including cancer. In order to gain a deeper understanding of the regulation of the apoptosis pathway in *Drosophila*, we performed an EMS screen to identify new components on chromosome arm 2L. Overexpression of the proapoptotic gene *hid* under the eye specific *GMR* promoter (*GMR-hid*) results in an eye ablation phenotype. This phenotype was used to screen for recessive suppressors and enhancers of *GMR-hid* by creating mutant clones via the *FLP/FRT* technique in the fly eye. Mutants were grouped according to their strength of suppressing the *GMR-hid* eye. Furthermore two classes of mutants could be distinguished by their ability to suppress Hid-induced cell death either autonomously or non-autonomously. The characterization of these mutants will be presented.

736A

The antiapoptotic effect of overexpressing the Drosophila homolog of the putative Phosphatidylserine receptor is mediated through modulation of the N-JNK pathway. Ronald Krieser, Douglas Dresnek, Brett Pellock, Kristin White. Cutaneous Biology Research Center, Massachusetts General Hospital 149 13th Street Charlestown, MA 02129.

The externalization of membrane phophatidylserine (PS) during apoptosis is thought to be a major cue directing engulfment by a phagocytic cell. A putative receptor for PS (PSR) was identified in mammals, but subsequent genetic analysis of its function as a PS receptor directing engulfment remains controversial. We have found that the Drosophila PSR does not function in apoptotic cell engulfment by hemocytes in the developing embryo. Our observations are consistent with this molecule functioning to inhibit apoptosis in Drosophila. Loss of dPSR enhances cell death in the eye. Overexpression of this protein produces phenotypes that overlap with phenotypes observed in flies that lack the proapoptotic genes hid or dronc. One of these hid phenotypes is rotated terminalia of the adult male fly. This phenotype has also been reported in flies that lack PVR and flies that overexpress Ras, Puc, or p35. Interestingly, activating the N-JNK pathway by reducing Puc was shown to suppress the rotated terminalia phenotype observed by loss of PVR. We have found that reducing Puc also suppresses the rotated terminalia phenotype observed in flies that overexpress PSR. These data support a role for PSR in inhibiting JNK activation. JNK appears to regulate apoptosis in the developing genital disc, possibly by modulating Hid or Dronc.

737B

Genetic analysis of steroid-triggered cell death during Drosophila metamorphosis. Lei Wang, Arash Bashirullah, Carl Thummel. Department of Human Genetics, University of Utah School of Medicine, 15 N 2030 E, Room 2100, Salt Lake City, UT 84112-5330 USA.

The steroid hormone ecdysone triggers the massive destruction of obsolete larval tissues during *Drosophila* metamorphosis. Earlier studies in our lab identified several genes that are required for larval salivary gland destruction, but the mechanisms that regulate steroid-triggered cell death remain unclear. To unravel this complex biological process, we conducted a large-scale open-ended EMS screen on the third chromosome, looking for defects in salivary gland cell death. We generated 8,636 lethal mutations and recovered 566 highly penetrant pupal lethal mutations. These mutants were screened for persistent “glowing glands” using a GFP reporter transgene. Forty-six mutants were identified that displayed persistent salivary glands in an otherwise normal pupa. These mutations fell into 37 complementation groups, seven of which are represented by multiple alleles. Persistent salivary glands from each of the seven multi-allelic complementation groups fail to stain with antibodies against activated Caspase-3, indicating a block in cell death. Consistent with this, northern blot analysis shows that some mutants display reduced levels of *rpr* and *hid* in persistent salivary glands although, interestingly, others are normal. This suggests that the mutations we identified function both upstream and downstream of the transcriptional induction of *rpr* and *hid* during salivary gland cell death. We will present our preliminary results on mapping and characterizing these mutations. Our goal in this study is to provide a better understanding of the hormonal regulation of programmed cell death as well as insights into how a systemic hormonal signal is refined into precise stage- and tissue-specific biological responses during development.

738C

Identification and characterization of Cbl function in cell death and cell differentiation in the *Drosophila* eye. Yuan Wang, Zhihong Chen, Dongbin Xu, Andreas Bergmann. Biochemistry & Molecular Biol, M D Anderson Cancer Ctr, Houston, TX.

The *Drosophila* compound eye has long served as an outstanding model system to study essential cellular processes, since exactly the same number and types of cells in each ommatidia are controlled by strict regulatory mechanisms. Although many specific signals have been demonstrated to regulate several cellular processes in the *Drosophila* compound eye, still much remains to be elucidated about the regulatory mechanisms required for proliferation, differentiation and cell death. Recently, we isolated five alleles of Casitas B-lineage lymphoma (Cbl), that recessively suppress the eye ablation phenotype caused by eye-specific over-expression of the pro-apoptotic gene *head involution defective (hid)*. The Cbl family of ubiquitin ligase plays a major role in ligand-dependent ubiquitination of many receptor tyrosine kinases (RTKs). This ubiquitination triggers the internalization of activated RTKs and their trafficking to the lysosome for degradation. Using *eyFLP*-Minute to induce mitotic recombination, *cbl* mutants show overgrowth phenotype with enlarged adult heads and eyes. There was a considerable increase in spacing between photoreceptor R8 in homozygous *cbl* mutant clones as compared with wild-type regions. Moreover, mutant ommatidia had extra numbers of photoreceptors, cone and pigment cells. The sizes of all cell types were normal, although the morphology was often abnormal. Over-expression of dominant-negative form of the *Drosophila* epidermal growth factor receptor (DER) in fly eye shows that this RTK can partially suppress the over recruitment of photoreceptor cells. Here we will present our most recent results and discuss their implications for the role of Cbl in cell death and differentiation during *Drosophila* eye development.

739A

Isolation of Mutations of Apoptotic Genes in 3rd Chromosome in *Drosophila*. Dongbin Xu, Andreas Bergmann. Dept Biochemistry & Molec Biol, Univ Texas MD Anderson CA Ctr, GSBS, Houston, TX.

In order to isolate mutations in some essential apoptosis regulatory genes and further investigate the regulatory mechanisms of apoptosis, we performed *GMR-hid ey-FLP* (*GheF*) screens for the 3rd chromosome in *Drosophila*. In *GheF* screens, we used *ey-Flp* to generate clone cells containing homozygous mutations in eyes and recovered mutations which recessively suppress the *GMR-hid* induced eye ablation phenotype.

Besides 4 *dronc* alleles, 1 *drICE* allele and 6 *cbl* alleles, we recently established 6 additional complementation groups of the mutants from the screens. All mutants suppress the *GMR-hid* induced eye ablation phenotype. Some of them also show outgrowth of cuticle tissue around adult eyes when *ey-FLP* is used to generate clones. In addition, we also isolated dozens of single mutants from the screens. The ongoing work on mapping and molecular analysis of these mutants will be presented.

740B

Analysis of *Drosophila* TAB2 mutants reveals that IKK, but not JNK pathway activation, is essential in the host defense against Escherichia coli infectionsAnalysis of Drosophila TAB2 mutants reveals that IKK, but not JNK pathway activation, is essential in the host defense against *Escherichia coli* infections. Dominique Ferrandon[1], Alain Jung[1], Vanessa Gobert[1], Rui Zhou[2], Nicholas Paquette[3], Sophie Rutschmann[1], Marie-Claire Criqui[1], Marie-Céline Lafarge[1], Matthew Singer[4], David Ruddy[4], Tom Maniatis[2], Jules Hoffmann[1], Neal Silverman[3]. 1) IBMC, CNRS UPR 9022, Strasbourg, FR; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA; 3) Division of Infectious Disease, Department of Medicine, University of Massachusetts Medical School,Worcester MA; 4) Exelixis, Inc. South San Francisco, CA.

Gram-negative bacterial infections trigger the NF-κB-like Immune deficiency (IMD) pathway, which controls the expression of antibacterial peptide genes as well as the expression of dozens of other genes. The IMD pathway controls on the one hand the phosphorylation and cleavage of the Relish transcription factor, and thus its subsequent nuclear uptake. On the other hand, it also triggers the JNK pathway with immediate early kinetics. The TAK1 kinase plays a crucial role in controlling both the activation of the I-κB kinase complex and that of the JNK pathway. It has been recently proposed that the JNK pathway also controls the expression of antibacterial genes. Here, we report the phenotypic analysis of *galere* mutants, which affect the *TAK1-associated Binding Protein2* (*TAB2*) gene. We demonstrate that TAB2 acts as a regulatory subunit of the TAK1 kinase complex. Like *TAK1*, *TAB2* null mutants are not as sensitive to *E. coli* infection as *imd* or *kenny* null mutants, which act respectively upstream and downstream of *TAB2* in the IMD pathway. Thus, an alternate branch may function downstream of IMD and parallel to TAK1 for full IκB Kinase complex activation. Strikingly, our data further demonstrate that IKK, but not JNK pathway, activation is essential to the host defense against *E. coli* infections. Thus, the physiological role of the short-lived JNK pathway activation during the immune response remains mysterious.

741C

Infection of *Drosophila melanogaster* with *Providencia* species, natural bacterial pathogens. Madeline R. Galac[1], Brian P. Lazzaro[1,2]. 1) Field of Genetics and Development, Cornell University, Ithaca, NY; 2) Department of Entomology, Cornell University, Ithaca, NY.

Much of what we know about innate immunity comes from studies in *Drosophila melanogaster*. These studies are often conducted with bacteria that can kill the fly or illicit an immune response but these bacteria are not necessarily ecological pathogens of *D. melanogaster*. In order to better understand the interaction between the flies and bacteria, it is important to look at natural pathogens of *D. melanogaster* instead of just the idealized fly response. Bacteria that naturally infect flies are likely to have evolutionary context with their host and may respond to the host's immune system in a specific way. We isolated several strains of bacteria from the genus *Providencia* from wild caught *D. melanogaster*. These include the previously described species *P. rettgeri* as well as two novel species. One novel species in particular causes substantial mortality within three days of pinprick infections. It also causes an apparently depressed pattern of antimicrobial peptide (AMP) induction over the forty-eight hours following infection. This could either result from by active suppression of the immune response or evasion of the host detection system, possibilities we have explored experimentally. Other aspects of *Providencia* infections in flies have also been examined.

742A

A Comparative Dissection of Innate Immune Pathways in *Drosophila melanogaster* using RNA Interference. David Kuttenkeuler, Michael Boutros. Functional Genomics, German Cancer Research Center, Heidelberg, Heidelberg, DE.

Innate immunity is the most ancient defense strategy of multi-cellular organisms to fight microbial infections. Most metazoans rely solely on the rapidly initiated innate defense, demonstrating its importance for survival. Two signaling pathways govern the response to invading pathogens in *Drosophila*, commonly referred to as IMD-(immune deficiency) and Toll-pathway. Although both pathway signal through the activation of NF-κB transcription factors, they induce the expression of discrete sets of Toll or IMD effector genes after infection. The current working model states that infection of Gram-positive bacteria and fungi results in the activation of Toll signaling and subsequent to the expression of Toll dependent target genes. Gram-negative bacteria induce IMD signaling, which in turn yields in an IMD specific change of gene expression. The understanding of how these pathways signal to the activation of NF-κB, how they are separated or what they have in common is of principal importance to decode the innate immune response with an eye on potential therapeutic interventions. To dissect the Toll and IMD signaling pathways we performed comparative genome scale RNA interference screens to identify specific and shared components of both pathways. Luciferase based assay systems established in our lab were used to detect dsRNA induced phenotypes in *Drosophila* cultured hemocytes. In total, we screened a genomic dsRNA-probe library in duplicates independently for each immune pathway and retested dsRNA-probes which induced significant phenotypes. Out of 127 candidates which we could independently confirm in secondary assays, we grouped 43 to regulate both pathways, 19 to be specific positive regulators and 53 to be specific negative regulators of IMD signaling. 13 candidates scored only as Toll positive regulators. Currently, we further analyze these phenotypic groups in order to understand principal mechanisms of signaling specificity in NF-κB pathways.

743B

Identification of Novel Genes Affecting the *Drosophila melanogaster* Immune Response to *Drosophila* X Virus. Anne M Macgregor, Louisa P Wu. Center for Biosystems Research, UMBI, College Park, MD.

The innate immune system is an important component in an organism's overall immune response to various pathogens. Much of the primary research in the field of innate immunity was accomplished using *Drosophila melanogaster*, as *Drosophila* are only equipped with an innate immune response. The focus of viral research in our lab lies in understanding the innate immune response to viral infection. To this end, I have employed a forward genetic screen to identify mutant *Drosophila* lines, which are more susceptible to viral infection. *Drosophila* X Virus (DXV), a double-stranded RNA virus belonging to the Birnaviridae family, is known to induce anoxia sensitivity and death in *Drosophila*. Experiments show that this heightened sensitivity occurs between seven and ten days post-injection and correlates with amplified DXV titer loads. To date, over 160 lines have been screened and 12 have been identified as more susceptible to DXV infection. After completing a secondary screen to confirm that these 12 lines exhibit an increased susceptibility to DXV infection, complementation tests are used to determine whether mutations are affecting the same or different genes. Viral titers will be performed to determine relative levels of virus in these mutant infected lines. These mutant lines will also be infected with the *Drosophila* C Virus to determine whether the response is specific to DXV. Additionally, the lab has shown that mutations in the *RNAi* or *Toll* pathways affect the ability of *Drosophila* to respond to DXV infection. I will determine if the new mutations identified from the screen are affecting one of these two pathways.

744C

A tolerance factor dissected from host resistance system in Drosophila. Naoaki Shinzawa[1,2], Hiroka Aonuma[1], Masayuki Miura[2], Hirotaka Kanuka[1]. 1) NRCPD, Obihiro University of Agriculture and Verterinary Medicine, Obihiro, Hokkaido, Japan; 2) Graduate School of Pharmaceutical Science, University of Tokyo, Bunkyo, Tokyo, Japan.

Host animals have resistance mechanisms against invading pathogens. In Drosophila, offensive system mainly consists of antimicrobial peptides (AMPs), phagocytosis, and melanization etc. Although stress response and homeostasis are thought to be one of the defensive factors on pathogenicity caused by pathogens, their contribution remains to be elucidated. Finding host factors that can purge bacteria from animal body could give us a new concept of host resistance system. We carried out gain-of-function screen to identify resistant or susceptible fly strains against bacterial infection (e.g. Salmonella, Listeria, S. aureus). GS system was used to find out “resistance factors” using the lethality as a marker of the resistance. About 2,500 GS lines were screened so far, and 21 resistant strains and 10 susceptible strains were obtained. Among these strains, we focused on a resistant strain GS10799 in which responsible gene is Dm p38b, one of the Drosophila genes encoding p38 MAPK. Flies overexpressing Dm p38b becomes more resistant against bacterial infection than wild-type flies. In addition, Dm p38b null mutant flies dies faster than wild-type flies. Interestingly, both gain- and loss-of-function of Dm p38b do not affect the bacterial growth since AMPs expression, phagocytotic activity of hemocytes, and melanization work normally in these flies. Additionally, these flies show as same phenotype as wild-type flies when infected with non-pathogenic bacteria (e.g. E. coli) and “acute pathogenic” bacteria (e.g. S. marcescens). Thus, we concluded that Dm p38b-related pathway has “tolerance” effect against the lethality in host resistance.

745A

Drosophila STAT (STAT92E) is anti-apoptotic by directly activating diap1 transcription. Aurel Betz[1], Hyung Don Ryoo[2], Hermann Steller[2], James E. Darnell,Jr.[1]. 1) Laboratory of Molecular Cell Biology, The Rockefeller University, NYC, NY; 2) HHMI, Laboratory of Apoptosis and Cancer Biology, The Rockefeller University, NYC, NY.

The Jak-STAT pathway is involved in a wide range of biological functions in both vertebrates and invertebrates, one of which is its role in promoting tissue proliferation and cancer. For instance, STAT3, an oncogene in mammals is thought to contribute to many endothelial malignancies and hop [tum-l] a hyperactive form of the Drosophila Jak-kinase that activates the single Drosophila STAT STAT92E leads to a form of fly leukemia. While several studies in mammals suggest a role of STATs in apoptosis, the detailed mechanisms and biological relevance remain poorly understood. Here we identify a new mechanism in flies by which STAT92E exerts a strong anti-apoptotic function in imaginal disc development. While under optimal growth conditions loss of STAT92E leads to moderate tissue reduction which may be due to reduced cell cycle activity we show that under stress conditons the degree of tissue survival is controlled by the physiological dosage of STAT92E. Furthermore the anti-apoptotic activity of STAT92E is at least in part based on its ability to directly induce the expression of the Drosophila IAP1, a critical inhibitor of cell death. diap1 regulatory sequences contain highly conserved STAT binding sites that we demonstrate to functionally contribute to diap1 transcription. These observations reveal a novel mechanism by which STAT regulates apoptosis and tissue size control during development.

746B

In vivo imaging of DIAP1 degradation during sensory organ development. Akiko Koto, Erina Kuranaga, Masayuki Miura. Dept. Genetics, Grad. Sch. Pharm., Univ. Tokyo, Tokyo, JP.

Programmed cell death or apoptosis is an essential physiological process required for normal development and maintenance of tissue homeostasis. Molecular mechanisms of apoptosis are highly conserved throughout evolution, which involve cell death execution protease, caspases and IAPs (inhibitor of apopsosis proteins). Recently, it is reported that these cell death regulators are also related with other phenomena, such as cell proliferation or cell migration. However, it is still unclear when and how the cell death signaling is activated during the development. It is significant to investigate the activation pattern of cell death signaling and to know its physiological role, we tried to visualize cell death signaling using fluorescence protein probe. *Drosophila* IAP1 (DIAP1) works as the E3 ubiquitin ligase and suppresses the caspase activation by directly binding to caspases and promoting its degradation. However once cell received cell death stimuli, DIAP1 degradation is promoted and cell death is executed by activated caspases. DIAP1 degradation is thought as the main trigger to induce cell death. We made the indicator that can detect the DIAP1 degradation, and named it as PRAP (pre-apoptosis probe by detecting DIAP1 degradation). Using PRAP, we are analysing the pattern of DIAP1 degradation during the sensory organ development. In the pupal thorax, each sensory organ precursor cell (SOP cell) divides asymmetrically to make four types of cells that compose each sensory organ. From the imaging analysis, we found that PRAP degrades asymmetrically in a distinct pattern during the sensory organ development. We will discuss the meaning of DIAP1 asymmetric degradation pattern for the sensory organ development.

747C

The *Drosophila* SUMO conjugase Lesswright regulates apoptosis and cell survival during larval hematopoiesis. Jinu Abraham[1], Liang Huang[1,2], Soichi Tanda[1]. 1) Department of Biological sciences and MCB program, Ohio university, Athens, OH; 2) Present Address: National institute of health, Bethesda, Maryland.

The *lesswright* (*lwr*) gene encodes a *Drosophila* ubiquitin-conjugating (E2) enzyme, which modifies target proteins with a ubiquitin-like modifier SUMO. In *lwr* mutants, the Toll pathway is activated, leading to over-production of larval hemocytes and formation of melanotic tumors. When *lwr* mutation was introduced into any background that induces a high hemocyte population, a significant decrease in the hemocyte count was observed. This decrease was accompanied by an increase in the percentage of apoptotic hemocytes in the *lwr* mutant background. To examine the possible link between apoptosis and the decrease in hemocyte counts, the *lwr* mutation was combined with a deficiency (H99), which removes the pro-apoptotic genes *grim, reaper* and *hid*. Introduction of H99 in *lwr* mutant background not only increased the hemocyte population but also caused a decrease in the number of apoptotic hemocytes. The E2 conjugating enzyme Ubc9 inhibits JNK mediated apoptosis in mammals. In order to examine the interaction between *lwr* and JNK pathway, the dominant negative form of *Basket* (*bsk*), the *drosophila* JNK kinase was expressed in the *lwr* mutant background. The expression of bsk^{DN} in *lwr* mutant background caused an increase in the hemocyte count along with a decrease in the number of apoptotic hemocytes compared to the *lwr* single mutants. These results suggest that lesswright protein plays an important role in cell survival. The role of *p53* in *drosophila* larval hematopoiesis will also be presented.

748A

Crystal cell rupture in *Drosophila* after injury requires Eiger, JNK and small GTPases. Gawa Bidla[1], Mitchell Dushay[2], Ulrich Theopold[1]. 1) Department of Molecular Biology and Functional Genomics, Stockholm University, Arrheniuslab F425, 10691 Stockholm, Sweden; 2) Department of Comparative Physiology, Uppsala University, Norbyvägen 18A, 75236 Uppsala, Sweden.

The prophenoloxidase-activating cascade is a key component of insect immunity. *Drosophila* prophenoloxidase (PPO) is stored in crystal cells, a specialized class of blood cells. The release of PPO involves cell rupture, but the signaling pathways involved are not yet known. Within minutes after bleeding, PPO released from crystal cells leads to visible melanization of the clot matrix. Using crystal cell rupture and melanization as readouts to screen mutants in signal transduction pathways, we show that PPO release requires TNF superfamily ligand Eiger, Jun N-terminal kinase, and small Rho GTPases. Mutations in the *imd/relish* or *Toll* pathways; the major pathways regulating transcriptional induction during immune responses, do not affect PPO release and melanization. We also show that proteolytic activation of PPO in the clot can proceed despite the presence of Spn27A, a known inhibitor of systemic PPO activation, at normal concentrations in the hemolymph. Likewise, clot melanization is independent of the recognition molecules that are required for systemic PPO activation. We provide evidence that in addition to microbial products, endogenous signals from dying hemocytes contribute to triggering and/or assembly of the PPO-activating complex, and that this process can be mimicked *in vivo* by the ectopic induction of apoptosis in hemocytes.

749B

Cricket Paralysis Virus infection of *Drosophila* reveals that the Imd pathway is involved in antiviral immune responses. Alexandre Costa, Eric Jan, Peter Sarnow, David Schneider. Dept Microbiology & Immunology, Stanford University, Stanford, CA.

The innate immune system is the first and most ancient line of defense against microbial infections. *Drosophila* has emerged as an effective system for studying innate immunity because of its powerful genetic techniques and the high degree of gene and pathway conservation. To gain insight into the interactions between viruses and the innate immune system, we are using *Drosophila* to genetically dissect the innate immune responses against viral infections. We have developed an *in vivo* host-virus model system by infecting adult flies with the Cricket Paralysis Virus (CrPV), a member of the *Dicistroviridae* family of non-enveloped, positive-stranded ssRNA viruses from invertebrates. Intra-abdominal injection of CrPV into adult flies causes a lethal infection that provides a robust assay for the identification of mutants with altered sensitivity to viral infection. To investigate the contribution of conserved immune signaling pathways to antiviral innate immune responses, CrPV was injected into isogenic mutants of different immune pathways. Genetic and molecular analyses of homozygous and transheterozygous mutants reveal that the Imd pathway is involved in antiviral immune responses.

750C

What can Drosophila teach us about tuberculosis? Marc Dionne[1,2], David Schneider[1]. 1) Dept Microbiol & Immunology, Stanford Univ, Stanford, CA; 2) Dept Craniofacial Development, Guy's Campus, King's College London, London, UK.

In order to try to understand the interaction of mycobacteria with their hosts, we have developed a system in which Drosophila melanogaster is infected with the broad-spectrum pathogen Mycobacterium marinum. This system appears to recapitulate the early stages of tuberculosis: the interaction of M marinum with the fly macrophage looks similar to the interactions between M tuberculosis and mammalian macrophages. This will be discussed briefly.

More recently, we have also found that this system has similarities to later stages of mammalian tuberculosis. In particular, cachexia is an important contributor to the lethality of mycobacterial infections in flies just as it is in humans. We show that Drosophila infected with M marinum progressively lose metabolic stores, in the form of fat and glycogen, and become hyperglycemic. Signaling via the insulin effector kinase Akt is reduced in infected animals. This reduction in Akt activation is accompanied by cleavage of activated Akt. The transcription factor FOXO, a target of negative regulation by Akt, is partly responsible for the loss of metabolic stores. These findings have clear parallels to human infection, and provide the first mechanistic link between infection-induced hyperglycemia and cachexia.

Finally, we have been examining the signaling upstream of these changes in Akt activation, and have evidence of a new interaction between insulin and wnt signaling. The precise mechanism of this interaction is not yet clear, but there are hints of similar signaling interactions in mammals.

In summary, I will discuss the similarities of mycobacterial disease in Drosophila with the early and late stages of tuberculosis in mammals, with an emphasis on the specific strengths of this system in discovery of host contributions to pathogenesis.

751A

Developmentally-regulated cell death of Drosophila salivary glands utilizes ER stress-linked apoptosis. Robert Farkas[1], Lucia Medvedova-Mentelova[1,2], Peter Low[3], Gabor Juhasz[3], Miklos Sass[3]. 1) Inst Experimental Endocrinol, Slovak Academy Science, Bratislava, SK; 2) Department of Genetics, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia; 3) Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Pazmány Sétány 1/C, H-1117 Budapest, Hungary.

Developmentally-associated programmed cell death (PCD) is a genetically encoded form of cell suicide that is required to remove incorrectly specified, superfluous or potentially dangerous cells. The Drosophila salivary glands are highly specialized secretory organs, that serve as an ideal model to study PCD as they respond to the steroid hormone ecdysone to undergo programmed histolysis during metamorphosis. Here we show that salivary gland apoptosis is linked to endoplasmic reticulum (ER) disintegration via an ER-stress mechanism. Depletion of ER calcium stores was very effective at inducing ER vesiculation and subsequent cell death. Activation of Xbp1 implicates the involvement of unfolded protein response (UPR) signaling. Genetic manipulation of ER-resident proteins, chaperones and co-chaperones resulted in a widespread and fast vesiculation of the ER, typical of that seen during the final apoptotic stage. In contrast, the genetic removal of the SERCA pump prevented tissue apoptosis. The results presented here describe for the first time the importance of cytosolic chaperones in cell death-associated UPR. Importantly, thapsigargin, an inhibitor of endoplasmic reticular Ca2+-ATPase SERCA causing depletion of ER calcium stores, induces very effective vesiculation of salivary gland cytoplasm followed by their death within 3 hr of treatment regardless of their developmental stage. Genetic manipulation by mutations or overexpression of dominant negative forms of ER proteins (Ca-P60, Crc, Cnx99A,) or chaperones and co-chaperones (Cct5, Hop, hsc3, P58IPK) resulted in strong and fast vesiculation of salivary gland cytoplasm indistinguishable from very late stages of their cell death in wild type controls.

752B

Drosophila S2 cells as a model to study E. chaffeensis infections. Alison L. Fedrow[1], Tonia Von Ohlen[1], Roman Ganta[2], Stephen Chapes[1]. 1) Biology, Kansas State University, Manhattan, KS; 2) Diagnostic Medicine & Pathobiology, Kansas State University, Manhattan, KS.

Ehrlichia chaffeensis is an obligate intracellular bacterium and the causative agent of human monocytic ehrlichiosis (HME). HME has been reported in 30 U.S. states and was designated a nationally reportable disease by the U.S. Centers for Disease Control in 1999. Although this pathogen has been shown to grow in several mammalian cell lines, no general model for the mechanism of disease pathogenesis has yet been proposed. The objective of our project is to utilize Drosophila melanogaster S2 cells and mammalian macrophages in order to identify the expression of host genes during E. chaffeensis infections. We found that S2 cells are permissive for growth of E. chaffeensis using two criteria. We saw the formation of morulae and we detected E. chaffeensis 16S gene by RT-PCR. Moreover, bacteria grown in S2 cells can reinfect mammalian macrophages. S2 cells were made non-permissive for E. chaffeensis through incubation with lipopolysaccharide. We will use this system to identify the genes involved in E. chaffeensis infections. In particular, microarray analysis and RNA interference studies will be performed on S2 cells (infected, uninfected, and non-permissive) and on mammalian macrophages. These techniques will enable us to elucidate the genes exclusively expressed by Drosophila and/or mammals during infection with E. chaffeensis and help define the genes that allow for maintenance of E. chaffeensis in its invertebrate and vertebrate hosts. This study was supported by the National Institutes of Health grants AI052206, AI55052, RR16475, RR17686, NASA grants NAG2-1274 and NAGW-1197, and the Kansas Agricultural Experiment Station.

753C

SCF ubiquitin ligase complex mediates phagocytosis through the novel F-box domain protein, Pallbearer. Nathalie Franc, Connie Au-Yeung, Emeline van Goethem, Elizabeth Silva. MRC LMCB & CBU, Univ Col London, London, GB.

Programmed cell death is a critical process for normal development. Integral to this process is the clearance of cell corpses by macrophages. We have conducted a deficiency screen to identify molecules required for this process and have found pallbearer (pall), a gene encoding a novel F-box domain containing protein. F-box domain containing proteins generally provide substrate-specificity for E3-ubiquitin ligases, the Skp/Cullin/F-box (SCF) complexes. We will present evidence demonstrating a genetic requirement for a SkpA/Cul-1/Pall complex in the engulfment of cell corpses in the Drosophila embryo, as well as genetic evidence implicating for the first time proteasomal degradation as necessary for efficient engulfment.

754A

Shaggy is required for ethanol-induced olfactory receptor neuron apoptosis. Rachael French, Ulrike Heberlein. Department of Anatomy, University of California, San Francisco, CA, 94158-2324.

It is well established that acute, or "binge" ethanol exposure causes apoptosis of both adult and developing neurons. Further, it is clear that the response of neurons to an ethanol insult is heavily influenced by genetic background, but the mechanisms behind this effect are not well understood. We will show that a single intoxicating exposure to ethanol causes apoptosis of *Drosophila* olfactory neurons, accompanied by a blackening of the third antennal segment. In addition, we will demonstrate that *shaggy*, the *Drosophila* homolog of glycogen synthase kinase 3B (GSK-3B), is required for ethanol-induced apoptosis. Finally, we will also show that the GSK-3 inhibitor lithium is protective against the neurotoxic effects of ethanol, indicating the possibility for pharmacological intervention in cases of alcohol-induced neurodegeneration. While GSK-3B has previously been implicated in the mediation of cell death under a wide variety of neurotoxic conditions, it has not been well studied in the context of ethanol-induced neurotoxicity. Furthermore, the system we describe will allow us to investigate the genetic and molecular basis of ethanol-induced apoptosis in general, and specifically to identify targets of GSK-3B in programmed cell death.

755B

Infection of *Drosophila melanogaster* with West Nile virus induces a protective RNAi response. Robert L. Glaser[1,2], Heather L. Chotkowski[1], Alexander T. Ciota[1], Jennifer L. Longacker[1], Laura D. Kramer[1,2]. 1) Wadsworth Ctr, New York State Dept Health, Albany, NY; 2) Dept Biomedical Sciences, University at Albany, State University of New York.

West Nile virus (WNV) is a mosquito-born RNA virus maintained enzootically by transmission between mosquitoes and birds, with incidental infection of humans causing encephalitic disease. We are interested in identifying host genes that modulate WNV infection, particularly genes that influence infection susceptibility of mosquitoes. RNA interference (RNAi) has been shown to provide innate antiviral immunity against a variety of RNA viruses in both mosquitoes and flies. To determine if WNV infection of an insect host induces a protective RNAi response, we developed a model of WNV infection using *Drosophila melanogaster* and characterized infection susceptibility in flies containing mutations in RNAi-related genes. *D. melanogaster* can be infected with WNV by injecting virus into the hemocoel. Flies were ~30-fold less susceptible to infection than *Culex pipiens*, a mosquito vector of WNV. Once an infection was established, the kinetics of infection were similar in the two species. Mutations in the RNAi-related genes *Dicer-2*, *piwi*, and *spindle-E* increased the frequency with which WNV infected flies and increased the rate of virus accumulation and maximum virus titers in flies once infected. In addition, WNV-homologous small interfering RNAs (siRNA) were detected in infected S2 cells. These results suggest that WNV infection of *D. melanogaster* induces a protective RNAi response. In contrast to infected S2 cells, WNV-homologous siRNAs were not detected in infected mosquito (*Aedes albopictus*) or mammalian cells (Vero), cell types in which virus titers reach levels 100-fold higher than levels in S2 cells. Since these mosquito and mammalian cells have functional RNAi pathways, these results suggest that WNV is able to evade and/or suppress the RNAi response in mosquito and mammalian hosts, while inducing a protective RNAi response in *D. melanogaster*. The underlying mechanism for this difference in host response to WNV infection is being investigated.

756C

Drosophila homologue of APP-BP1 (dAPP-BP1) interacts antagonistically with APPL during Drosophila development. Hyung-Jun Kim[1], Song-Hee Kim[1], Sang-Ohk Shim[1], Eungsik Park[1], Changsoo Kim[2], Kiyoung Kim[1], Mark Tanouye[3], Jeongbin Yim[1]. 1) School of Biological sciences, Seoul National University, Seoul, Seoul , KR; 2) School of Biological Sciences and Technology, Chonnam National University,Gwangju; 3) Department of Molecular and Cell Biology, Division of Neurobiology, University of California, Berkeley.

APP-BP1 was previously identified based upon its binding to the carboxyl terminal of beta-amyloid precursor protein. A possible role of APP-BP1 in Alzheimer's syndrome was anticipated, but has not been reported. Recently, considerable interest in APP-BP1 has come from its role in NEDD8 (ubiquitin like protein) conjugation pathway, especially as it may function in cell cycle progression and signal transduction. In this report, We have discovered that a mutation of dAPP-BP1 (Drosophila orthologue of APP-BP1) hinders tissue development, causes apoptosis in imaginal disc cells, and blocks the NEDD8 conjugation pathway. We have shown that dAPP-BP1 specifically binds the intracellular domain of APPL and APPL protein level is closely related to dAPP-BP1 protein level. Loss of dAPP-BP1 slightly decreased APPL protein levels, but overexpression of dAPP-BP1 greatly increased the amount of APPL proteins. dAPP-BP1 mutation partially suppresses the abnormal macrochaete phenotype of Appld while over-expression of dAPP-BP1 causes abnormal macrochaetes. When APPL is over-expressed, the normal bristle pattern in the fly thorax is disturbed and apoptosis is induced in wing imaginal discs. APPL over-expression phenotypes (apoptosis and abnormal macrochate) are enhanced by reducing the level of dAPP-BP1. Over-expression of APPL and its mutant forms inhibit the NEDD8 conjugation pathway. APPLsd and APPLsdΔIC over-expression induce apoptosis at imaginal discs, but only APPLsd is rescued by over-expression of dAPP-BP1. Our data suggest that APPL and dAPP-BP1 interact antagonistically during Drosophila development.

757A

Characterizing genetic elements regulating neuroblast apoptosis in Drosophila. Megumu Mabuchi[1], Wei Tang[1], Susan St.Pierre[2], Reena Patel[1], Kristin White[1]. 1) CBRC, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA; 2) The Biological Laboratories, Harvard University, Cambridge, MA.

Four Drosophila cell death regulators, rpr, grim, hid and sickle, are clustered in a 400 kb region of the third chromosome. These "RHG" genes share a short motif at their N-terminus, and appear to induce apoptosis in a similar manner. However, work by us and by others has shown that these genes are regulated by different upstream pathways. This suggests that each gene will have a unique role in inducing the death of particular cells during development. We have focused on the neuroblasts of the abdominal neuromeres as a model for understanding how the RHG genes interact to regulate the death of a particular cell during development. We have generated deletions throughout the region, and find that neuroblast apoptosis is regulated by the combined functions of more than one of the cell death genes. We have also characterized an enhancer region that is likely to regulate the expression of one or more of these genes during neuroblast apoptosis.

758B

Impact of the *Pseudomonas aeruginosa* type III secretion system on virulence and patterns of gene expression during the Drosophila immune response. Kurt McKean[1], Todd Schlenke[2], Andrew Clark[1]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biology, Emory University, Atlanta, GA.

The type III secretion system is a well-studied virulence factor common to a number of gram-negative pathogens. It is composed of a flagella-like injection apparatus used to inject a variety of effector proteins directly into host cells. Once in the host cell the effector proteins (exoenzymes) act to subvert the normal functioning of the cell often by interfering with host intracellular communication, and thus changing patterns of host gene expression, for the benefit of the bacterial cell. In this study we examine the effects of the type III secretion system of the opportunistic pathogen *Pseudomonas aeruginosa* on virulence and gene expression in *Drosophila melanogaster*. We found that virulence was decreased in transposon-mutant strains of *P. aeruginosa* lacking a functional type III secretion system. Furthermore, virulence of type III secretion system mutants varied across strains of Drosophila, indicating the presence of genetic variation in the ability to combat this virulence mechanism. We used whole-genome Affymetrix expression microarrays to compare the gene expression of flies injected with wild-type P. aeruginosa, to flies injected with strains unable to carry out type III secretion, or lacking one of the secreted exoenzymes (ExoS). Also included were both uninjected and sterile needle wound controls. Expression was examined at 1 hour and 12 hours after infection. Using a series of linear models to analyze the microarray data, we identify genes affected in their expression by the type III secretion system. This includes sets of genes both up- and down-regulated by the presence of a functional type III secretion system.

759C

Characterization of the *Drosophila Myeloid Leukaemia Factor*. Anne Plessis[1], Severine Martin-Lanneree[2], Christelle Lasbleiz[1], Matthieu Sanial[1], Herve Tricoire[1]. 1) Inst Jacques Monod, CNRS-Paris 7-Paris 6, Paris, FR; 2) Inst Cochin, U.567 Inserm/UMR8104 CNRS/UMR-S8104, Paris, FR.

In human, the Myeloid Leukaemia Factor 1 (hMLF1) has been shown to be involved in acute leukaemia, and *mlf*< related genes are present in many animals. Despite their extensive representation and their good conservation, very little is understood about their function. We previously identified *dmlf*< as an interactor of the Suppressor of fused (SU(FU)) protein (Fouix et al., 2003), a negative regulator of the Hedgehog (Hh) and Wint (WNT) signalling pathways. Independently, dMLF also was shown to interact both molecularly and genetically with the transcription factor DREF (DNA replication-related element factor) (Ohno et al., 2000), a DNA binding protein which participates in the transcriptional up regulation of many genes involved in G1/S. Last, *dmlf* over expression was also shown to suppress the toxicity caused by poly-glutamine containing protein in the *Drosophila* eye system (Kazemi-Esfarjani and Benzer, 2002).

We studied *dmlf* pattern of expression and subcellular localization and we generated the first *dmlf* mutant to study its role in fly development. Both in embryo and larva, *dmlf* is expressed in a subset of immunity cells: the crystal cells. We are analyzing the role of two conserved transcription factors lozenge and serpent, which are known to be involved in mammalian hematopoiesis, on dmlf expression. We also showed that *dmlf* is not required for crystal cell differentiation but that it acts to limit cell proliferation during haematopoiesis.

All our data argue in favour of a functional conservation of this novel family of proteins and validate the use of *Drosophila* to decipher their normal and pathological functions.

760A

Possible interactions between the JAK/STAT and the Toll pathway in *Drosophila* hematopoiesis. Ying Shen[1], Soichi Tanda[1,2]. 1) Dept Biological Sci, Ohio Univ, Athens, OH; 2) Molecular and Cellular Biology program, Ohio Univ, Athens, OH.

The Toll (Tl) and the JAK/STAT pathways are two of the major pathways regulating *Drosophila* hematopoiesis. It is still unclear whether there are any interactions between these two pathways. In $hop^{Tum\text{-}l}$ background, deleting both Dorsal (Dl) and Dorsal-related immunity factor (Dif), the two transcription factors of the Tl pathway, does not affect the total hemocyte number. In Tl^{10B} hemocytes, the Tl pathway is constitutively active resulting in a high incidence of Dl nuclear localization, whereas $hop^{Tum\text{-}l}$ hemocytes show a very low incidence of Dl nuclear localization. These results suggest that the activation of the Tl pathway is not required for the JAK/STAT-induced hemocyte overproduction. Interestingly, $hop^{Tum\text{-}l}$ Tl^{10B} double mutant combination shows a lower hemocyte count when compared with that of $hop^{Tum\text{-}l}$ single mutant. However, the double mutants still maintain a high incidence of Dl nuclear localization indicating the Tl pathway is still active. These results suggest that when the Tl pathway is activated, components downstream of the Tl pathway can suppress the hematopoietic phenotype of $hop^{Tum\text{-}l}$.

761B

Innate immunity and circadian rhythm. Michele Shirasu-Hiza, Marc Dionne, Linh Pham, Janelle Lamberton, David Schneider. Microbiology & Immunology, Stanford Univ, Stanford, CA.

Infection and disease are often correlated with disrupted circadian rhythm and sleep/wake cycles; conversely, circadian rhythm has been demonstrated to affect specific aspects of immunity. For example, in the fly, microarray analyses have revealed that circadian genes regulate transcription of several known immunity genes. However, the functional relationship between the neuronal and immune systems is highly complex and remains unclear. In particular, the effect of infection on circadian regulation has not been described previously; nor have any circadian mutant flies been tested for their immune response to pathogenic bacteria. Here we show that sick fruitflies lose circadian rhythm and that mutant flies lacking circadian rhythm are immunocompromised. Drosophila melanogaster infected with lethal doses of Streptococcus pneumoniae or Listeria monocytogenes lose their circadian rhythm several days before death. Moreover, circadian mutant flies (lacking either timeless or period) are highly sensitive to infection by these bacteria and die significantly faster than wildtype flies. Rescue of the tim01 mutation with a transposable element carrying the wild-type tim gene under the control of its own promoter rescues the phenotype. These results establish the fly as a model system for the study of bidirectional, functional communication between the neuronal system and innate immunity.

762C

RNAi analysis of serine protease inhibitors of the serpin family in *Drosophila*. Huaping Tang[1], Zakaria Kambris[2], Bruno Lemaitre[2], Carl Hashimoto[3]. 1) Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520; 2) Centre de Génétique Moléculaire, CNRS, 91198, Gif-sur-Yvette, France; 3) Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06520.

Serpins define a super family of serine protease inhibitors that are used in different cellular and developmental processes. The *Drosophila* genome encodes about 30 different serpins, of which about twenty are predicted to be inhibitory. The *Drosophila* necrotic serpin (Spn43Ac) regulates the activation of the Toll signaling pathway induced by fungal infections. The serpin Spn27A regulates the melanization reaction in immunity as well as dorsal-ventral patterning in embryonic development. Functions of the other inhibitory serpins remain largely unknown. In an effort to clarify the biological functions of these serpins, we made transgenic flies that can express hairpin RNA under control of the UAS-Gal4 system targeting each of 18 different inhibitory serpins for RNAi. For nine different serpins, ubiquitous activation of RNAi resulted in lethality or semi-lethality, indicating an essential biological role for each of these serpins. In a few cases, lethality was associated with morphological defects. For several serpins, RNAi knockdown also resulted in constitutive induction of antifungal peptide expression and the melanization reaction, suggesting that these serpins could have a role in regulating immune responses like Spn27A and Spn43Ac. Further studies will be required to reveal the specific functions of the essential serpins identified by our RNAi analysis and the biological processes that they regulate.

763A

Fork Head Controls the Tissue Selectivity of Steroid-Induced Developmental Cell Death. Michael Lehmann, Chike Cao, Yanling Liu. Department of Biological Sciences, University of Arkansas, Fayetteville, AR.

Developmental cell death during *Drosophila* metamorphosis is controlled by the steroid hormone 20-hydroxyecdysone (20E). Elements of the signal transduction pathway that triggers death are known, but it is not known why some tissues, and not others, die in response to a particular hormone pulse. We found that the tissue-specific transcription factor Fork head (Fkh) is lost from the larval salivary glands prior to death, that maintained expression of Fkh is sufficient to prevent death, and that premature loss of Fkh leads to death in response to an earlier hormone pulse. Loss of *fkh* renders the key death regulators *hid* and *reaper* hormone responsive and is, by itself, a steroid-controlled event, mediated by the 20E-induced *BR-C* gene. These results implicate the *Drosophila* FOXA ortholog Fkh with a novel function as a competence factor for steroid-controlled cell death. They explain how a specific tissue is singled out for death, and why this tissue survives earlier hormone pulses. More generally, they suggest that cell identity factors like Fkh play a pivotal role in the normal control of developmental cell death.

764B

Evolution and developmental dynamics of *Drosophila* DNA-binding transcription factors. Boris Adryan, Derek Wilson, Sarah A. Teichmann. Structural Studies Division, MRC LMB, Cambridge, GB.

DNA-binding transcription factors (TFs) are important regulators of gene expression. Despite progress in the functional annotation of the genes of *D. melanogaster* since publication of the genome over six years ago, there are many genes (~20%) of unknown function, including many putative TFs.

We set out to identify the repertoire of site-specific DNA-binding TFs in *D. melanogaster* and related species as comprehensively and accurately as possible. Our approach was to first review the literature on *D. melanogaster* TFs to extract these that bind DNA in a sequence-specific manner. Then we identified previously uncharacterized TFs using hidden Markov models of DNA-binding domains, as described in the DBD Database. This combined approach resulted in a comprehensive, annotated catalogue of *D. melanogaster* TFs that is now available online at **www.FlyTF.org**.

We structurally classified the TFs and defined families of shared domain architectures. This allowed us to systematically identify the most common domain combinations. We then studied the effect of splicing on the domain repertoire of the TFs.

In order to characterize the TF repertoire in terms of spatio-temporal expression during development, we computationally integrated gene expression information from several sources. Thus we could gain an overview of known and predicted TFs that are expressed throughout development versus those that are stage-specific, for instance. This allows us further to recognize general trends in TF usage, e.g., the temporal preference for certain TF families or the body part-specific ratio of TFs versus non-TFs.

Finally, we used phylogenetic profiling in order to analyze the evolutionary conservation of the repertoire of *D. melanogaster* TFs across invertebrates, including an analysis of lineage-specific expansions of DNA-binding domain families.

765C

Prediction of non-coding RNAs using a *Drosophila* whole-genome alignment. Yuri R. Bendana, Ian H. Holmes. Department of Bioengineering, UC Berkeley, Berkeley, CA.

The recent sequencing of twelve species of *Drosophila* provides the opportunity to perform investigations into the feasibility of using whole-genome alignments as a tool for predicting gene elements. By using comparative genomics, the functionally important conserved regions can be identified from the more variable regions.

In particular, non-coding RNAs (ncRNAs) perform important regulatory functions. However, it may be difficult to identify them by sequence alone. Since ncRNAs are assumed to display covariation in their mutations, it is hoped that multiple alignments of closely related species will display the evolutionary signals of ncRNAs more clearly.

We scanned multiple alignments of Drosophila genomes for phylogenetic signals consistent with conserved ncRNA genes. For the scan, we developed a program called Xfold, which models a Stochastic Context-Free Grammar (SCFG) with column likelihoods computed by three separate phylogenetic evolutionary models for basepairs in ncRNA genes, unpaired bases in ncRNA genes and bases in intergenic sequences. We use the likelihood computed by Xfold as a measure of how probable it is that an alignment contains a ncRNA.

For the multiple-genome alignments, we used MAVID, a program which performs a constrained multiple alignment of genomes based on known protein-coding regions. We use a MAVID alignment of the CAF1 assembly of twelve *Drosophila* species to perform a sliding-window genome scan for de novo prediction of non-coding RNAs.

We evaluate Xfold's performance using annotated ncRNAs in *Drosophila melanogaster* from Flybase. In addition, we compare Xfold's performance to RNAz, a program that predicts ncRNAs in an alignment by computing a consensus RNA structure and a measure of its thermodynamic stability.

766A

Large-scale analysis of transcriptional *cis*-regulatory modules: common features, distinct subclasses, and implications for regulatory module discovery. Marc S. Halfon[1,2,3], Long Li[1], Qianqian Zhu[1], Xin He[4], Saurabh Sinha[4]. 1) Dept. of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics and the Life Sciences, Buffalo, NY; 3) Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY; 4) Dept. of Computer Science, University of Illinois Urbana-Champaign, Urbana, IL.

Transcriptional *cis*-regulatory modules (e.g., enhancers) play a critical role in regulating gene expression. While many individual regulatory elements have been characterized, they have never been analyzed as a class. We have performed a large-scale study of *Drosophila cis*-regulatory modules (CRMs) in order to determine whether they have common features that might aid in their identification and contribute to our understanding of how they function. 280 experimentally-verified CRMs from the REDfly database were analyzed for a range of properties. These CRMs regulate gene expression in a diverse array of tissues and developmental stages and are associated with over 150 genes. Our main findings can be summarized as follows:

(1) CRMs have distinct features that as a group distinguish them from other types of DNA sequences. These include a higher GC content than other non-coding sequences (but less than coding sequences), greater evolutionary conservation than other non-coding sequences, and a tendency to be transcribed into RNA.

(2) These differences are typically not great enough to reliably classify a given unknown sequence as CRM or non-CRM.

(3) Dense clustering of transcription factor binding sites, commonly believed to be a general characteristic of regulatory modules, is rather a feature that belongs chiefly to a specific subclass.

We will discuss our development of new computational strategies for the assessment of binding site clustering and the implications of our results for regulatory element discovery, structure, function, and evolution.

767B

EDGI: new algorithm for discovery of transcriptional regulatory regions in Drosophila genome by a non-alignment method for phylogenetic footprinting. Alona Sosinsky[1,2], Barry Honig[1,2], Richard Mann[2], Andrea Califano[3]. 1) Howard Hughes Medical Institute; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 3) Department of Biomedical Informatics, Columbia University, New York, NY.

The functional annotation of the non-protein coding DNA of eukaryotic genomes is a problem of central importance. Phylogenetic footprinting methods, which attempt to identify functional regulatory regions by comparing orthologous genomic sequences of evolutionary related species, have shown promising results. The main advantage of this class of approaches is that they do not require any knowledge of the regulating transcription factors. However, a number of limitations have so far restricted their applicability. To overcome such limitations, we have developed a new method called EDGI (Enhancer Detection using only Genomic Information), which integrates a traditional motif-discovery algorithm with a novel local permutation-clustering algorithm. Together they can identify large regulatory elements (e.g. enhancers) as evolutionarily conserved, order-independent clusters of short conserved DNA sequence motifs. We tested EDGI's performance on a set of multiple available Drosophila genomes. The results of this test show that EDGI can distinguish between established sets of known enhancers and non-enhancers with 88% accuracy. This rivals predictions by methods that require knowledge of the regulating transcription factors or their DNA binding specificities. Our results demonstrate that comparative genomic analysis of multiple closely related species has substantial power to identify key functional elements without additional biological information. An EDGI Web server is available at http://luna.bioc.columbia.edu/EDGI/.

768C

FlyExpress: A growing developmental bioinformatics platform for analysis of spatial expression patterns in Drosophila embryogenesis. Bernard Van Emden[1], Hector Ramos[1], Sethuraman Paunchanathan[2], Thomas Brody[4], Stuart Newfeld[3], Sudhir Kumar[1,3]. 1) Biodesign Inst, Arizona State Univ, Tempe, AZ; 2) School of Computing and Informatics Arizona State Univ, Tempe, AZ; 3) School of Life Sciences, Arizona State Univ, Tempe AZ; 4) Neurogenetics Unit, LNC, NINDS, Natl Inst of Health, Bethesda, MD.

Translating sequence information into gene function and interaction is greatly facilitated by the growing collection of *Drosophila* melanogaster spatial and temporal gene expression patterns. Today's vast collection of diverse gene expression patterns has eclipsed the standard practice of manually inspecting the images to make biological discoveries. The FlyExpress project aims to establish a comprehensive bioinformatics framework to accelerate the use of this growing collection of expression data in the discovery of previously unknown links and components of developmental networks. The first product of this effort, www.flyexpress.net, is now on-line and contains a digital library of >40,000 spatial patterns from >3,000 genes. FlyExpress provides a Basic Expression Search Tool for images (BESTi) to enable biologists to mine genes whose spatial patterns overlap with the query gene. Here we report our progress in building the next generation of FlyExpress. We are building easy-to-use web tools for the submission of genetic/experimental attributes of images published in the peer reviewed journals and a forum for the sharing of unpublished expression images with the research community, at large. In addition, we are developing the state-of-the-art computational and statistical image analysis methods to (a) find genes with overlapping expression using a *de novo* image that eliminates the need to select a pattern existing in the database for search, (b) automate image standardization for biologically accurate image comparison in high-throughput analysis, (c) generate technologies to delineate the expression patterns from the embryo background in a consistent fashion for large-scale bioinformatics analyses, and (d) classify patterns into groups based on spatial overlaps to facilitate visual exploration of commonalities in text descriptions.

769A

Gene Targeting with zinc finger nucleases in a single generation. Kelly Beumer, Jon Trautman, Dana Carroll. Dept Biochemistry, Univ Utah, Salt Lake City, UT.

Introduction of a double-strand break (DSB) in chromosomal DNA stimulates repair by recombination in the vicinity of the break. Previously we have shown that a class of engineered nucleases with zinc finger DNA-binding domains (zinc finger nucleases, ZFNs) can make recombinagenic DSBs in the Drosophila genome and stimulate gene targeting. However, the genetics have been cumbersome. We have now shown that homologous recombinants can be recovered efficiently a single generation after injecting a plasmid encoding a "donor" DNA in the presence of RNA encoding the ZFNs.

Injection of RNA encoding each of a pair of ZFNs targeted to the *rosy* gene resulted in recovery of up 28% of progeny carrying a targeted mutation. Frequency of mutation was directly correlated to RNA concentration, while viability and fertility were inversely correlated. When the RNA was coinjected with a Bluescript plasmid carrying a 4.2 kb marked donor, 6% of all mutant offspring were the result of homologous recombination i.e., 10 of the 61 surviving, fertile injected flies gave an average of two homologous recombinants each.

We are in the process of optimizing this protocol, and testing it on two other gene targets. We will report the conditions necessary for successful F1 targeting, as well as the results of targeting experiments at other loci, and with other donor configurations. We have also designed cloning modules to ease the construction of new zinc finger combinations. These modules will be described. Zinc fingers can be derived that recognize a rather broad range of DNA sequences. With the ability to recover targeted, customized mutations at a high frequency in under four weeks with minimal genetics, this technology should be accessible to all.

770B

Manipulating large regulatory elements within the context of the bithorax complex. Carole Iampietro, Fabienne Cléard, Annick Mutero, Robert Maeda, François Karch. University of Geneva, Geneva, CH.

Abd-B is one of three Drosophila Hox genes in the bithorax complex and determines the identity of the most posterior segments. Four autonomous cis-regulatory domains (iab-5 through iab-8) regulate Abd-B expression independently in abdominal segments 5 through 8. Each cis-regulatory domain contains a set of different functional and structural elements necessary for its function. Although some of these elements have been studied in transgenic reporter constructs, thorough in situ dissection of the cis-regulatory domains has never been accomplished. By using homologous recombination and site-directed phiC31 integration, we are attempting to systematic dissect of one of these cis-regulatory domains within its native context. Thus far, using homologous recombination, we have created a new deletion in the iab-6 region where the endogenous iab-6 sequence has been replaced by a 255 bp attP integration site. Using these lines, we should be able to replace the wild-type iab-6 (and Fab-6) sequence with any DNA sequence of our choosing. Using gene conversion we have also replaced the Fab-7 boundary by a minimal 50bp attP integration site. We believe that these tools will not only help in the genetic characterization of the previously identified elements, but will also lead to the identification of exciting new elements and regulatory mechanisms within the bithorax complex.

771C

Genome-wide mapping and characterisation of protein expression and interaction in *Drosophila melanogaster* using a hybrid PiggyBac/P-element YFP gene trap system with tandem affinity tags. Ed Ryder[1], Helen Spriggs[1], John Roote[1], Emma Drummond[1], Jenny Drummond[1], Jane Webster[1], Glynnis Johnson[1], Nick Lowe[2], Kathryn Lilley[3], Svenja Hester[3], Julie Howard[3], Johanna Rees[3], Steve Russell[1], Daniel St. Johnston[2]. 1) Dept Genetics, Cambridge Univ, Cambridge, GB; 2) Gurdon Institute, Dept Genetics, Cambridge Univ, Cambridge, GB; 3) Dept Biochemistry, Cambridge Univ, Cambridge, GB.

We have initiated a screen to generate and characterise protein trap lines in *Drosophila* using a PiggyBac transposon-based strategy. The ability to generate *in vivo* tagged proteins has tremendous potential for furthering our understanding of developmental processes by allowing the characterisation of sub-cellular protein localisation and facilitating the isolation of multi-protein complexes. This is a large project involving a collaboration with over thirty UK laboratories. Our Pig/P transposons are tagged with YFP (yellow fluorescent protein), incorporated into endogenous genes via an exon-trapping strategy, thus facilitating the visualization of trapped proteins in living embryos and larvae. Correct incorporation of the fluorescent tag is a rare event, and we therefore employ an automated embryo sorter to select the insertions. Putative lines are mapped by iPCR and sequencing, with custom software predicting whether the insertion is in the correct frame, according to the current genome annotation, to produce a functional YFP fusion. The transposed exons also contain two protein affinity tags that allow the protein to be isolated in its native complex by tandem affinity purification. Complex components are identified by tandem mass-spectrometry with spectra assigned to the fly proteome via the MASCOT search engine. Positive hits, P-values and other proteomic data will be stored in the project database. To aid in the characterisation of YFP-trap lines, we have developed web-based software which allows annotation of gene expression at all stages of development and in all tissue types (including sub cellular location) using GO and the Drosophila gross anatomy ontology.

772A

Chromosomal deletion screens at the Bloomington Stock Center. Kevin R. Cook, Stacey J. Christensen, Megan E. Deal, Jill M. Gresens, Thomas C. Kaufman. Dept Biol, Indiana Univ, Bloomington, IN.

The Bloomington Stock Center has been isolating deletions with molecularly-defined breakpoints in an isogenic background using FLP-FRT technology and characterizing these deletions by molecular, genetic and cytological approaches. Our goals are to provide coverage of genomic regions lacking deletions and to provide breakpoints to further subdivide the genome. We will present an analysis of current deletion coverage and discuss gaps in coverage in terms of the distribution of haploinsufficient loci. We will describe deletions recently added to the Bloomington collection and efforts to improve the Bloomington Deficiency Kits.

773B

Characterization and functional analysis of mRNA-like non-coding RNAs in *Drosophila*. Sachi Inagaki[1], Yuji Fukuda[1], Takefumi Kondo[1], Yoshiko Hashimoto[1], Yuji Kageyama[1,2]. 1) Grad Sch Biol Sci, Nara Inst Science Technology, Ikoma, Nara, JP; 2) PREST, Japan Science and Technology Agency.

Recent progress of large-scale cDNA sequencing projects demonstrate that numerous non-coding RNAs (ncRNAs) are transcribed in eucaryotes including mammals, insects and plants. Majority of these newly identified ncRNAs can be classified into mRNA-like ncRNA, which is transcribed by RNA polymerase II, spliced and polyadenylated. Although abundance of mRNA-like ncRNAs implies importance of these molecules, in most cases, their biological functions are totally uncharacterized. In *Drosophila*, we have previously identified 33 putative mRNA-like ncRNAs in embryogenesis (MREs) by combination of *in silico* filtering processes and *in situ* expression analysis. Since the majority of MREs are expressed in tissue specific manners, MREs may play important roles in developmental events.

To ask if these RNAs are functional molecules, we tried to characterize molecular and functional properties of MREs. We first examined lengh of these MREs *in vivo*. Northern blot analyses of 33 MREs revealed that only six of the original MRE cDNAs are full-length and independent transcripts. Among them, two MREs contains small but highly coserved ORFs, suggesting that they function as protein-coding mRNAs. The rest of the transcripts, MRE3, 16, 31 and 32, are possible candidates for untranslatable RNAs. To elucidate physiological functions of these RNAs, we established loss-of-function and gain-of-function strains for all of the four MRE genes. Results of genetic analyses of these fly strains will be presented as well.

774C

spineless: CENTRAL ROLE IN BUILDING THE RETINAL MOSAIC REQUIRED FOR COLOR VISION IN *DROSOPHILA*. Preet Lidder, Claude Desplan. Dept Biol, New York Univ, New York, NY.

Color vision requires the comparison of the output of photoreceptor cells (PRs) that respond to different wavelengths of light. In *Drosophila*, color vision is achieved by the R7 and R8 'inner' PRs, while the outer PRs (R1-R6) are responsible for motion detection. Although all ommatidia appear morphologially identical, they can be grouped into several functional subclasses, based on the rhodopsin content of their inner PRs. The pale (p) and yellow (y) ommatidia are distributed in a stochastic manner with a conserved ratio of 30% (p) to 70% (y). Evidence from our lab has suggested that the gene *spineless* (*ss*) is responsible for the formation of the ommatidial mosaic. *ss* is specifically expressed during pupal life in a subset of R7 cells where it induces the y ommatidial fate. The remaining R7 cells that do not express *ss* choose the pR7 fate. R8 cells receive signals from R7 to acquire their p or y fate. We are studying how the stochastic expression of *spineless* in a subset of PRs leads to the creation of the complex retinal mosaic used for color vision. Towards this end, we used DNA microarray analysis to identify mRNAs that were elevated/repressed in *ss* loss- or gain-of-function retinas compared to wild type. These experiments have been important for identifying the downstream effectors critical for conferring different PR subtypes. Gene expression profiles for the targets of *spineless* have also been compared to uncover common regulatory characteristics that may be potentiated through a particular pathway. Since the mechanisms of eye development and differentiation are conserved from flies to humans, ultimately, a more detailed knowledge of the fly retinal mosaic will lead to a better understanding of the vertebrate retina.

775A

Sex-specific expression of alternative transcripts in Drosophila. Lauren McIntyre[1], Lisa Bono[2], Anne Genissel[3], Marina Telonis-Scott[1], Larry Harshman[4], Marta Wayne[1], Artyom Kopp[2], Sergey Nuzhdin[2]. 1) University of Florida, Gainesville, FL; 2) The Ohio Univeristy; 3) UC Davis; 4) Univeristy of Nebraska.

Background Many genes produce multiple transcripts due to alternative splicing or utilization of alternative transcription initiation/termination sites. This 'transcriptome expansion' is thought to increase phenotypic complexity by allowing a single locus to produce several functionally distinct proteins. However, sex, genetic and developmental variation in the representation of alternative transcripts has never been examined systematically. Here, we describe a genome-wide analysis of sex-specific expression of alternative transcripts in Drosophila melanogaster. Results We compared transcript profiles in males and females from eight Drosophila lines (OregonR and 2b, and 6 RIL) using a newly designed 60-mer oligonucleotide microarray that allows us to distinguish a large proportion of alternative transcripts. The new microarray incorporates 7,207 oligonucleotides, satisfying stringent binding and specificity criteria that target both the common and the unique regions of 2,768 multi-transcript genes, as well as 12,912 oligonucleotides that target genes with a single known transcript. We estimate that up to 22% of genes that produce multiple transcripts show a sex-specific bias in the representation of alternative transcripts. Sexual dimorphism in overall transcript abundance was evident for 53% of genes. The X chromosome contains a significantly higher proportion of genes with female-biased transcription than the autosomes. However, genes on the X chromosome are no more likely to have a sexual bias in alternative transcript representation than autosomal genes. Conclusion Widespread sex-specific expression of alternative transcripts in Drosophila suggests that a new level of sexual dimorphism at the molecular level exists.

776B

Identification of genes involved in color vision using Affymetrix GeneChips. Tamara Mikeladze-Dvali, Preet Lidder, Claude Desplan. Dept Biol, New York Univ, New York, NY.

Color vision in Drosophila, is achieved by comparing inputs of two species of inner photoreceptors (R) with different spectral absorbencies. In each ommatidium the proximal R8 and the distal R7 share one optical path. Two subsets of inner photoreceptors play a role in color vision. The yellow (y) subset expresses rhodopsin (rh)4 in R7 and rh6 in R8. In the pale (p) subset R7 expresses rh3 and R8 rh5. The rh's are expressed in mutually exclusive and a highly regulated manner. The p and y subsets are stochastically distributed throughout the 800 ommatidia of the eye with a 30:70% ratio. It is thought that R7 makes the choice to express the y or p rh. It then tells R8 to express the corresponding rh. We have previously shown that melted (melt) and the large tumor suppressor gene (lats or warts) form a bistable loop to specify the p and y subsets in the R8. warts is activating rh6 expression and repressing rh5 in the yR8. melt is thought to respond to the pR7 signal and down-regulate warts, allowing differentiation of the pR8. By genetically manipulating the wart/melt loop we can lock the R8 in either the p or y state. Here, we present data where, by using Affymetrix GeneChips on wart and melt gain- and loss-of-function retinas, we have identified genes involved in R8 subset specification.*melted melt lats warts rhodopsin rh*.

777C

Variation in transcript abundance of chemoreceptors in adult and larval *Drosophila melanogaster* assessed by cDNA expression microarrays. Shanshan Zhou[1,4], Christina Grozinger[3,4], Trudy Mackay[2,4], Robert Anholt[1,2,4]. 1) Zoology, NC State University, Raleigh, NC; 2) Genetics, NC State University, Raleigh, NC; 3) Entomology, NC State University, Raleigh, NC; 4) W. M. Keck Center for Behavioral Biology, NC State University, Raleigh, NC.

Chemosensory behavior in *Drosophila melanogaster* presents an excellent model system for assessing environmental effects on behavioral phenotypes, as transcriptional adjustments in expression levels of chemoreceptors occur rapidly in response to changing environmental conditions. We have constructed expression arrays that represent 50 *Odorant binding protein (Obp)*, 56 *Odorant receptor (Or)*, and 59 *Gustatory receptor (Gr)* genes, genes that encode other antennal specific proteins, genes encoding components of neurotransmitter pathways, and several house-keeping genes to assess variation in expression levels among these genes in adults and larvae of the co-isogenic Canton S (B) strain. Transcripts for *Obp*s, *Or*s and *Gr*s were detected with high sensitivity after normalization and background correction with virtual absence of dye effects. There was excellent concordance between fluorescent signal intensity on the array and abundance of transcript detected by RT-PCR. We observed considerable variation in transcript abundance in both larvae and adults among *Obp*, *Or* and *Gr* genes, including those located in clusters in the genome (*e.g. Obp56, Obp57, Or85, Gr22*). While many *Or* and *Gr* transcripts were not detectable or expressed at low levels, some generated strong signals and many *Obp* transcripts generated hybridization signals that exceeded the signals observed with *Or*s and *Gr*s by an order of magnitude, as predicted for abundantly secreted gene products. Transcriptional profiles between larvae and adults (males and females combined in equal proportion) revealed both adult-specific and larva-specific expression of chemoreceptors. Thus, the chemosensory microarrays we constructed will be valuable tools for future studies in which we can assess the plasticity of the chemosensory window through which the fly smells its chemical environment.

778A

Genome-wide analysis of embryonic mRNA localization pathways. Eric Lecuyer[1], Hideki Yoshida[1], Neela Parthasarathy[1], Christina Alm[1], Pavel Tomancak[2], Tomas Babak[1], Timothy R. Hughes[1], J. Timothy Westwood[3], Henry M. Krause[1]. 1) Donnelly CCBR, University of Toronto, Toronto, ON, CA; 2) Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, GER; 3) Canadian Drosophila Microarray Centre, University of Toronto, Mississauga, ON, CA.

The localization of mRNA molecules is a well known mechanism for targeting proteins to specific cellular compartments. However, the overall prevalence and variety of mRNA localization patterns is unknown. To assess this issue, we have initiated a genome-wide screen, using a sensitive high-throughput Fluorescent In Situ Hybridization procedure, to identify localized mRNAs during the early stages of Drosophila embryogenesis. Thus far, analyses of mRNAs from over 4000 genes have demonstrated that an unexpectedly high proportion of expressed transcripts (70%) are subcellularly localized. The screen has revealed many novel varieties of subcellular localization patterns, while also providing a dynamic view of transcript localization and degradation events occurring during embryogenesis. Furthermore, computational analyses reveal that distinct classes of localized transcripts are enriched for mRNAs encoding functionally related proteins, suggesting that mRNA localization, and localized mRNAs, control the assembly of diverse cellular modules. These results will provide the basis for further functional and bioinformatic analyses of associated regulatory mechanisms and biological roles.

779B

FISH based method for automated quantification of nascent and cytoplasmic mRNA transcript numbers in fixed *Drosophila* embryonic cells. Adam C Pare[1], Derek Lemons[1], David Kosman[1], William Beaver[2], Yoav Freund[2], William McGinnis[1]. 1) Cell and Developmental Biology, UC San Diego, La Jolla, CA; 2) Computer Science, UC San Diego La Jolla, CA.

Determining mRNA concentration within a population of cells has been a difficult problem for molecular biologists. Traditionally the problem has been addressed using Northern Blot analyses, RT-PCR, and most recently microarrays. While relatively simple to carry out these methods all have their shortcomings. Most importantly they are limited by poor temporal and spatial resolution, especially in complex tissue samples. We report here the detection and automated quantification of single cytoplasmic mRNA transcripts using FISH in fixed *Drosophila* embryos. We also describe quantification of expression at nascent transcriptional sites in the nucleus. It is interesting to note that these sites of transcription colocalize with TATA Binding Protein (TBP) domains within nuclei, while they are largely excluded from heterochromatic domains (revealed by dense DAPI signal). Using this technique we constructed a detailed "expression profile" of the developmentally important Hox gene *Sex Combs Reduced (Scr)* during early embryogenesis. This simple and exquisitely sensitive method should allow for accurately time-resolved and quantitative expression profiles of dynamically expressed genes in complex tissues.

780C

CORRELATION OF MOCROSATELLITES OF DNA WITH ENZYME VARIATION AT THE MDH LOCUS IN DROSOPHILA MELANOGASTER. DOMINGO ALIRIO MONTANO. GENETICA, UNIVERSIDAD ANTONIO NARINO, BOGOTA, BOGOTA, CO.

The Malate Deshidrogenase system in Drosophila melanogaster is characterized by an unusually high level of natural-occuring polymorphism both for electrophoretically detectable allozymic variation and for variation in levels of enzyme expression.Morover, these variants are characterized by a distinctive geographic pattern. The purpose of the present study was to test whether variation detected at the level of enzyme activity and electroforetical movility is refleted in variation detected at the DNA level. A sample of twenty Drosophila strains were chosen to represent the widest possible variety of enzymes phenotypes and geographic origins. The results indicate strong associations between enzyme polymorphism and microsatellites polymorphism; in addition there is a significant non random association between different DNA polymorphisms. We also observed allelic variants of DNA specific geographic correlated more strong rather than with allozymic variation.

781A

The Tucson Drosophila Species Stock Center: Resources for the Drosophila Community. Stacy Mazzalupo[1], Sergio J. Castrezana[1], Therese A. Markow[1,2]. 1) Arizona Research Labs, Univ Arizona, Tucson, AZ; 2) Ecology & Evolutionary Biology, Univ Arizona, Tucson, AZ.

The *Drosophila* Species Stock Center located in Tucson, Arizona is home to 260 species of Drosophila. Over 36 species groups are represented. Although the bulk of our collection consists of wild-type strains collected from diverse geographical areas, we are quickly acquiring many mutants and transgenic flies of a range of species. Our collection now includes P-element insertion stocks created by other labs (*e.g. D. mauritiana, D. simulans*). Some of these insertions have been mapped by inverse PCR (Araripe, L.O., Hartl D & Tao, Y., D.I.S., submitted). We completed several EMS screens with both *D. erecta* and *D. persimilis* species. This yielded useful markers such as *yellow* and *Curly* and the hope of obtaining *white*. There are currently 12 genomes sequenced and thus non-*melanogaster* species are becoming more valuable resources. The *Drosophila* community would like access to more transgenic stocks and more genetically-marked stocks. Also, there is a huge need for balancer chromosomes in all these species. We hope to continue to provide an essential resource for the fly community and expand our services in the coming years.

782B

A new family of Drosophila balancer Chromosomes with a w- dfd-GMR YFP marker. Gregory Beitel[1], Tien Le[1], Zhiguo Liang[2], Heeren Patel[1], Marcus Yu[1], Gitanjali Sivasubramaniam[1], Matthew Slovitt[1], Guy Tanentzapf[3], Nihar Mohanty[1], Sarah Paul[1], Victoria Wu[1]. 1) BMBCB, Northwestern Univ, Evanston, IL; 2) Department of Microbiology and Immunology MC790, University of Illinois-Chicago, Chicago IL 60612-7344; 3) Dept. of Cell and Systems Biology, University of Toronto, Toronto, ON M5S 3G5.

Most fluorescently marked balancer chromosomes suffer from one or more limitations in that they have poor embryonic visibility, bear a Gal4 driver that improves visibility but causes undesired expression of UAS transgenes on the balanced chromosome, or bear a w+ transformation marker that interferes with scoring of transgenes on the balanced chromosomes. To overcome these limitations, we created a new fluorescent marker construct in a w- P-element vector that expresses a nuclear-localized Yellow Fluorescent Protein under the direct control of the dfd and GMR enhancer elements. dfd-driven expression is scorable as fluorescence near the head and mouth hooks from stage 13 through the end of larval development, and as fluorescence at the end of the proboscis in adults. GMR-driven expression is scorable in larval and pupal eye discs, and in adult eyes. The utility of this marker is enhanced by identification of an anti-GFP/YFP serum that is compatible with heat fixation. This dfd-GMR-YFP marker has been hopped to TM3 Sb, TM6B Sb Tb, CyO and FM7 balancer chromosomes, and stocks of these balancers are being made available through the Bloomington stock center or by contacting the Beitel lab (beitel@northwestern.edu). Further details of the construction of this marker can be found in Le et al. Genetics (in press).

783C

CaSpeR5, a family of Drosophila transgenesis and shuttle vectors with improved multiple cloning sites. Gregory Beitel, Tien Le, Marcus Yu, Brandon Williams, Sagar Goel. Dept BMBCB, Northwestern Univ, Evanston, IL.

Although the pCasper1-4 and pUAST vectors have been the mainstay of Drosophila transformation vectors, their multiple cloning sites (MCS) have a limited number of unique restriction sites. Further, neither of the MCSs in pCaSpeR or pUAST are present in small shuttle or cloning vectors, which is problematic because the large size (> 8 kb) of the transgenesis vectors requires sequence manipulations such as site-directed mutagenesis or deletion dropouts to be done in small plasmid vectors, and the modified DNA to be moved to the transgenesis vectors. The lack of matching shuttle vectors further constrains the usable cloning sites and can complicate moving large genomic fragments between a cloning vector and a transgenesis vector. To overcome the above limitations, we engineered a new MCS based on the pCaSpeR4 MCS that adds five new six-base cutter sites, but most importantly flanks the entire MCS by two eight-base cutters on each side. We call this improved vector pCaSpeR5 since the new vector retains all the restriction sites of the pCaSpeR4 MCS in their original order. We also created pUAS-C5 by replacing the MCS of pUAST with a modified version of the pCaSpeR5 MCS (C5 MCS) that lacked the ATG-containing SphI site. Although the pUAST vector has many common six-cutter sites in its backbone, the C5 MCS nonetheless adds five new six-cutter and the flanking four eight-cutter sites to the pUAS expression vector. To facilitate clone manipulation, we created small ampicillin- and kanamycin-resistant shuttle vectors by replacing the MCSs of pBlueScript and pHSX with the C5 MCS. These vectors will be contributed to the DGRC and are also available from the Beitel lab (beitel@normthwestern.edu).

784A

The Transcriptional Landscape. Susan Celniker, Joseph Carlson, Mark Stapleton, Bhaveen Kapadia, Soo Park, Kenneth Wan, Richard Weiszmann, Charles Yu, Ann Hammonds. Berkeley Dros Genome Ctr, Lawrence Berkeley National Lab, Berkeley, CA.

Determining the genomic sequence of an organism, including the heterochromatin, is only the first step in understanding its biology. Accurate annotation of genomic sequence requires the identification of the complete set of genes, including alternative transcripts and non-coding sequence, the identification of cis-regulatory elements that regulate gene expression and the determination of temporal and spatial patterns of gene expression. We have used inter-species comparative sequence analysis and molecular studies to aid in deciphering the *Drosophila melanogaster* genome. To characterize the transcriptional landscape, we have produced and are continually extending the Drosophila Gene Collection (DGC). The collection now contains 14,152 sequenced clones of which 8,456 contain the complete annotated coding sequence (ORF) and match the gene annotations including 734 strain polymorphisms. In the collection, we have clones representing 831 alternate transcrips that produce alternate proteins. The genes not yet represented by clones are those that are rarely expressed or produce large transcripts. We have conducted directed screening starting with the transcription factors to identify and sequence clones for these transcripts, many of which alter existing gene annotations. Using this strategy we have captured clones representing 1,515 genes. Using microarray data we are continuing this directed strategy in libraries that have yet to be interrogated. To identify transcription start sites of these rare messages we are using RACE.

To provide a resource for high throughput proteomics applications, we used the set of validated full-length clones as the source of ORFs to generate expression ready clones. To date we have made 4,293 constructs for C-terminal fusions and 3,168 constructs for N-terminal fusions. We have also produced over 400 clones for expression in tissue culture and flies. These clones will be used as part of a large project to generate a protein complex map of Drosophila.

785B

Textpresso for Fly: a Literature Search Engine for Researchers and Curators. Hans-Michael Muller[1], Beverley Matthews[2], Susan Russo[2], Eimear Kenny[3], Arun Rangarajan[1], William Gelbart[2], Paul Sternberg[1]. 1) California Institute of Technology, Pasadena, CA; 2) Harvard University, Cambridge, MA; 3) Rockefeller University, New York, NY.

Textpresso is a text-mining system for scientific literature whose capabilities go far beyond those of a simple keyword search engine. Its two major elements are a collection of the full text of scientific articles split into individual sentences, and the implementation of categories of terms for which a database of articles and individual sentences can be searched. The categories are classes of biological concepts (e.g., gene, allele, cell, etc.) and classes that relate two objects (e.g., association, regulation, etc.) or describe one. The corpus of articles and abstracts is marked up to identify terms of these categories. A search engine enables the user to search for one or a combination of these tags and/or keywords within a sentence or document. As the ontology allows word meaning to be queried, it is possible to formulate semantic queries. Full text access increases recall of biological data types from 45% to 95%, and using category searches can yield a 3-fold increase in search efficiency. Originally developed for C. elegans literature, we have adapted Textpresso for Drosophila literature, by installing a corpus of 5000 full-text articles and 11000 abstracts, and by implementing fly-specific categories such as 'body part', 'developmental stage', 'gene (D. melanogaster)' and 'transposon'. Some Drosophila gene names (e.g., 'for', 'not', 'a') are the same as common English words, and we are applying automated methods to disambiguate word senses accordingly. Textpresso for Fly is available at http://www.textpresso.org/fly.

786C

Genome Sequence and Analysis of *Tribolium castaneum*, the Red Flour Beetle. Stephen Richards, Yue Liu, Kim C. Worley, Erica Sodergren, Steven E. Scherer, Catherine M. Rives, Donna M. Muzny, George Weinstock, Richard A. Gibbs, The Tribolium Genome Consortium. Human Genome Sequencing Center, Baylor Col Medicine, 1 Baylor Plaza, Houston, TX.

We have sequenced the genome of the first Coleoptera, *Tribolium castaneum*, the red flour beetle. The assembled sequence comprises 165Mb, of which 85% has been anchored to the 10 chromosomes. A number of gene modeling software packages have been used to identify gene models. Data based annotation pipelines (ensembl pipeline ran at HGSC, gnomen ran by NCBI) produce ~9,500 data supported gene models. To get the remaining genes we also ran abinitio gene prediction software including FgenesH, Augustus, Genscan, Geneid, and gnomen HMM. Glean (Aaron Mackay) was used to produce a consensus gene model set of 16,365 gene models. This gene model set has been further enhanced by selective manual annotation by members of the Tribolium genome consortium. Over one thousand genes have been manually annotated, using web-based tools provided by the HGSC.The biological analysis of the genome is ongoing, and includes, genes involved in systemic RNAi, chitinases, identification of vertebrate genes not found in *D. melanogaster*, expansions found in the gustatory and olfactory receptors, and others.We are also sequencing the parasitic wasp (*Nasonia Vitripennis, giraulti* and *longicornis*) genome, and the pea aphid (*Acyrthosiphon pisum*) genomes, and will provide updates on these projects. All sequence data is available though NCBI genbank, and also via the HGSC website: http://www.hgsc.bcm.tmc.edu/.

787A

A Genome-Wide RNA Interference Screen to Identify New Components of the RAS/MAPK Pathway. Dariel Ashton-Beaucage, Marc Therrien. Institut de Recherche en Immunologie et Cancerologie, Université de Montréal, Montreal, Quebec, CA.

The RAS/MAPK pathway participates in a wide array of cellular processes including proliferation, differentiation and survival. The principal components of the pathway, RAS, RAF, MEK and MAPK, form an evolutionarily conserved signalling unit called the MAPK module. This module has been the object of many studies in a variety of model organisms. Despite the fact that many new elements have been found to act on the pathway, the means by which signal specificity can be generated is still poorly understood. In order to identify new components of the pathway, we have developed an RNA interference (RNAi) high-throughput screening method in S2 cells. S2 cells are well adapted to RNAi screening as they directly absorb double-stranded RNA (dsRNA) added to the culture medium. A stable cell line expressing a constitutively active form of RAS (RASV12) is used to induce a strong pathway activity state. The cells and double-stranded RNAs (dsRNAs) are prepared in 96 well plates on a robotic platform and are examined using an automated microscopy system. An immunofluorescence staining procedure is used to measure the level of phosphorylated MAPK. The average cell intensity is then measured and compared to a GFP dsRNA control. The depletion of known components of that pathway such as RAF, MEK, MAPK, KSR and CNK produces a signal intensity of less than 10% of the control signal. Conversely, depletion of PTP-ER, a known negative regulator of the pathway, produces a signal of roughly 200% of that of the control. Using this system we will test the effect of the depletion of each of the genes of the drosophila genome using the dsRNA set generated at IRIC (over 15000 individual dsRNAs) from DNA templates purchased from Open Biosystems. A preliminary test of 320 dsRNAs of the set (amongst which were KSR and RAF) has demonstrated the robustness of the assay. We are currently in the process of screening the full RNAi set. Preliminary results from the screen will be presented.

788B

Using viral suppressors of RNA silencing to explore the diversity and functions of small RNAs in *Drosophila*. Bassam Berry[1], Delphine Fagegaltier[1], Ronald van Riji[2], Raul Andino[2], Jean-Luc Imler[3], Olivier Voinnet[4], Christophe Antoniewski[1]. 1) Developmental Biology, CNRS / Institut Pasteur, Paris, FR; 2) University of California, San Francisco, CA 94143-2280; 3) CNRS-UPR9022, IBMC, 15 rue René Descartes, 67084 Strasbourg Cedex, FR; 4) CNRS - IBMPC, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France.

Plants employ RNAi as a virus defense mechanism. In response, plant viruses encode proteins that can suppress RNA silencing. Silencing suppressor proteins of different virus genera are highly diverse in sequence and structure and inhibit different steps in the RNAi pathways including both the siRNA and miRNA mediated silencing pathways. These features make them invaluable tools to unravel many key questions in our understanding of RNAi silencing mechanisms in virtually any organism. We have established Drosophila transgenic lines enabling in vivo expression of several RNA silencing suppressors, including B2, P15, P19, P21, P25, P38, P0, Hc-Pro and Tas. We have studied the effect of these suppressors on siRNA, miRNA and virus-induced silencing, as well as on fly fertility. Our results provide the demonstration of an effect of plant and insect viral inhibitors in distinct small-RNA mediated silencing pathways in the context of a whole developing animal.

789C

HDAC inhibitors and Drosophila - A fruitful team. Marc Hild, Haidi Yang, Dan Garza. Novartis Institutes for BioMedical Research (NIBR), Cambridge, MA.

While the existence of an 'epigenetic code' involved in regulation of gene expression has become apparent only in the last years, the effects of inhibiting histone deacetylation have been studied for decades. Histone deacetylases (HDACs) play key roles in maintaining chromatin structure and gene expression. As an essential part of the machinery that keeps genes inactive, HDACs have been implicated in multiple cellular processes, such as cellular memory, apoptosis, muscle development, and early embryonic development. Recent studies indicate that abnormal HDAC activity may lead to aberrant expression of oncogenes and/or tumor suppressors resulting in cancer. In addition, abnormal histone acetylation has been implicated in cardiac hypertrophy. Due to its excellent RNAi system, we have established Drosophila cells as an in vitro system to study the mechanism of action (MoA) for inducing apoptosis of two Novartis pan-HDAC inhibitors. Genome-wide RNAi screens in tandem with HDACi treatment have identified 208 suppressors (170 with human homolog) and 85 enhancers (70 with human homolog). In order to identify the modifiers specific for HDAC inhibition and to exclude genes simply enhancing cytotoxicity we perform screens with other HDACi (same / different chemical class) as well as cytotoxic compounds. The further study of these modifiers will be useful for understanding the MoA of HDACi, and also provide a basis for developing biomarkers predictive for the success of HDAC treatment in patients. Finally, these modifiers can be useful novel targets for compounds synergizing with HDACi.

790A

Design of a second-generation RNAi library for Drosophila. Thomas Horn[1], Jeff Reid[2], Wolfgang Huber[3], Amy Kiger[2], Michael Boutros[1]. 1) German Cancer Research Center, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany; 2) Department of Cell and Developmental Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA; 3) EMBL - European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge CB10 1SD, UK.

RNA interference (RNAi) is a powerful approach to systematically deplete transcripts on a genome-wide scale. Due to the penetrance and efficiency of RNAi in invertebrates, model organisms such as *Drosophila* and *C. elegans* have contributed important insights with the identification of novel components of diverse biological pathways. A key issue in genome-wide RNAi experiments is the design and coverage of available libraries.

The rational design and stringent quality control of long dsRNAs remains an important topic both in small and large-scale RNAi experiments. Design rules of long dsRNAs have to take into account both restrictions based on the properties of the contained siRNAs, such as their target specificity and predicted efficiency, and experimental limitations, such as the design of functional primer pairs to amplify the dsRNA template by PCR of genomic sequences.

We developed a bioinformatics approach that automates the required tasks for the de-novo design of long dsRNAs for RNAi in single experiments and at a genome-wide scale. The software predicts specific and efficient target sites for appropriate amplicon sequences. We will show data on the redesign of a second-generation RNAi library with improved specificity and coverage that targets all predicted genes in the *Drosophila* genome. The synthesis of the RNAi library was optimized through a two-step PCR using re-amplifiable tags. Further, the plate layout was designed with a molecular and phenotypic barcode that allows unambiguous identification of assay plates and with empty wells for additional assay-specific controls. Information on used primer sequences and quality information on dsRNAs of the library are being made accessible online (http://rnai.dkfz.de).

791B

Rapid construction of RNAi transgenes using pRISE, a transformation vector exploiting Gateway Technology. Yuji Kageyama[1,2], Takefumi Kondo[1], Sachi Inagaki[1]. 1) Grad Sch Biol Sci, Nara Inst Sci Tech, Ikoma, Nara 630-0192, JP; 2) PREST, Japan Science and Technology Agency.

RNA interference (RNAi) has been developed as a powerful tool to elucidate gene function in diverse organisms, including mammals, insects and plants. In each case, RNAi is achieved by using double-stranded RNA (dsRNA) as a 'trigger' molecule that recognizes a complementary 'target' sequence and eventually induces gene silencing mediated by the RNAi inducing silencing. In *Drosophila*, RNAi is commonly achieved by establishment of transgenic lines expressing a long inverted repeat sequence, which leads to formation of hairpin-type dsRNAs *in vivo*. However, construction of plasmids including a long inverted repeat of a target sequence is often problematic; for example, it is widely known that plasmids with a long inverted repeat frequently undergo internal deletion during bacterial culture. Another difficulty arises from the fact that cloning two identical sequences in opposite orientations limits the use of the multiple cloning sites of a recipient vector plasmid.

To accomplish rapid construction of transgenes expressing dsRNA in *Drosophila*, we developed a novel transformation vector, pRISE, which contains an inverted repeat of the *attR1-ccdB-attR2* cassette for in vitro recombination and a pentameric GAL4 binding site for conditional expression. These features enabled us to construct RNAi transgenes without a complicated cloning scheme. In cultured cells and transgenic flies, pRISE constructs carrying dsRNA transgenes induced effective RNAi against an EGFP transgene and the endogenous white gene, respectively. These results indicate that pRISE is a convenient transformation vector for studies of multiple *Drosophila* genes for which functional information is lacking.

792C

Genetic dissection of the rhabdomyosarcoma oncoprotein PAX-FKHR in a Drosophila model. Tiana Endicott[1], Rene Galindo[1,2]. 1) Molecular Biology, UT Southwestern Med Center, Dallas, TX; 2) Pathology, UT Southwestern Med Center, Dallas, TX.

Rhabdomyosarcoma (RMS), the most common childhood soft tissue tumor, is a family of malignant tumors that demonstrates skeletal muscle-type differentiation. Of the RMS sub-types, the alveolar variant (ARMS) is particularly aggressive and carries a poor prognosis. ARMS is a translocation-based tumor, uniquely associating with one of two non-random chromosomal translocations, t(2;13)(q35;q14) and t(1;13)(p36;q14). Both translocations are analogous, disrupting loci of the PAX (Pax3 on chromosome 2, Pax7 on chromosome 1) family of transcription factors, with subsequent in-frame fusion to the FKHR (fork head in rhabdomyosarcoma) gene on chromosome 13. PAX-FKHR misexpressed in mouse myofibers can cause rhabdomyosarcoma at a low frequency, suggesting that skeletal muscle is an ARMS tissue of origin. The mechanisms by which PAX-FKHR alters the biology of differentiated muscle are unknown. To further explore this issue, we are utilizing Drosophila as an animal model for PAX-FKHR pathogenicity. We have shown that PAX-FKHR expressed in fly muscle causes potent muscle phenotypes, including muscle fiber dysmorphology and the ectopic presence of mononucleated myogenic-type cells, and lethality. Importantly, these phenotypes are intermediate in sensitivity and are susceptible to genetic modifier screening. We are performing a deficiency screen to identify dominant modifiers of PAX-FKHR pathogenicity. Utilizing the Bloomington Drosophila Deficiency Kit spanning the left arm of chromosome 2, we have identified five deficiencies that strongly suppress PAX-FKHR and four that strongly enhance. We are presently utilizing smaller, overlapping deficiencies to further delineate the critical chromosomal regions that modify PAX-FKHR activity, and testing lines mutant for individual loci from within these regions. We are also expanding the screen to include the right arm of Chromosome 2. The identification of new PAX-FKHR cofactors and gene targets will infer critical information about PAX-FKHR pathogenicity.

793A

Drosophila as a model of the childhood malignancy rhabdomyosarcoma. Rene Galindo[1,2], Jay Allport[2], Eric Olson[2]. 1) Dept Pathology, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept Molecular Biology, Univ Texas SW Medical Ctr, Dallas, TX.

Alveolar rhabdomyosarcoma (ARMS) is an aggressive myogenic-type tumor and a gain-of-function disease, caused by misexpression of the PAX3-FKHR or PAX7-FKHR fusion oncoprotein from chromosomes structurally rearranged due to non-random chromosomal translocation [t(2;13)(q35;q14) and t(1;13)(p36;q14)]. PAX3-FKHR misexpressed in terminally differentiating mouse myofibers can cause rhabdomyosarcoma at a low frequency, suggesting that skeletal muscle is an ARMS tissue of origin. Since patterned muscle is widely viewed as irreversibly syncytial, questions persist, however, regarding this potential pathogenetic mechanism for ARMS tumor initiation. To further explore this issue, we generated transgenic Drosophila lines that conditionally express human PAX-FKHR, realizing that PAX and FKHR signaling is conserved between mammals and files. We postulated that serial imaging of PAX-FKHR transgenics with muscle-specific Green Fluorescent Protein reporters would allow us to uncover previously unseen PAX-FKHR phenotypes. We found that PAX7-FKHR causes nucleated cells to form and separate from syncytial myofibers, which then spread to non-muscular tissue compartments, including the central nervous system, and that wild-type PAX3 demonstrates similar potential, showing that the critical moieties originate from the PAX portion of the protein fusion. We further show that Ras, which is known to interfere with the differentiation of myogenic cells, genetically interacts with PAX7-FKHR: constitutively activated Ras enhances PAX7-FKHR phenotypes while loss-of-function ras alleles dominantly suppress PAX7-FKHR activity, including rescue of lethality. These results show that PAX-FKHR can drive the generation of discrete nucleated cells from differentiated myofibers in vivo, argues for syncytial muscle as an ARMS tissue of origin, and demonstrates that Drosophila provides a powerful new system to screen for genetic modifiers of PAX-FKHR. New PAX-FKHR cofactors and gene targets will infer critical information about PAX-FKHR pathogenicity and open new avenues for directed drug therapy of ARMS.

794B

aPKCζ and Lgl play a key role in *Drosophila* and human epithelial architecture. Daniela Grifoni[1,2], Flavio Garoia[1], Paola Bellosta[4], Federica Parisi[2,4], Dario De Biase[3], Dennis Strand[5], Sandro Cavicchi[1], Annalisa Pession[2,3]. 1) Biology Dept. Alma Mater Studiorum, Bologna, Italy; 2) Pathology Dept. Alma Mater Studiorum, Bologna, Italy; 3) Oncology Dept., Pathology Section, Bellaria Hospital, Bologna, Italy; 4) Biology Dept. CUNY City College, NY, USA; 5) First Dept. Internal Medicine, Johannes Gutenberg University, Mainz, Germany.

Atypical PKC (aPKC) and Lethal giant larvae (Lgl) regulate apical-basal polarity in *Drosophila* and mammalian epithelia. At the apical domain, aPKC displaces Lgl that, in turn, maintains aPKC inactive at the basolateral region (1). The mutual exclusion of these two proteins seems to be crucial for the epithelial structure and function; indeed, Lgl inactivation and/or aPKC overexpression lead to tissue architecture disruption and massive overgrowth (2). Here we show that a cytoplasmic aPKC overexpression does not induce epithelial disorders in *Drosophila*; a cortical distribution is strictly required to perturb cell polarity and proliferation. One aPKC isoform exists in *Drosophila*, named aPKCζ, while two isoforms are present in humans, namely aPKCζ and aPKCι. Since we previously demonstrated that *Drosophila* Lgl is the functional homologue of the Human giant larvae-1 (Hugl-1) protein (3), we argued if aPKC and Hugl-1 interplay could be impaired in human epithelial disorders and investigated aPKCι, aPKCζ and Hugl-1 localization in cancers deriving from ovarian surface epithelium (OSE). Both in the mucinous and serous histotypes, Hugl-1 and aPKCι showed a membrane-to-cytoplasm release while aPKCζ showed an apical-to-cortical redistribution, perfectly recapitulating the *Drosophila* model. Though some recent works support a causal role for aPKCι overexpression in human carcinomas (4, 5), our results suggest a key role for aPKCζ in apical-basal polarity impairment, a mechanism that seems to be driven by changes in protein localization rather than in protein abundance. (1) Hutterer et al., Dev. Cell 2004; (2) Rolls et al., JBC 2003; (3) Grifoni et al., Oncogene 2004; (4) Eder et al., PNAS 2005; (5) Regala et al., JBC 2005.

795C

Ras, Raf, and PI3-Kinase activities in peripheral glia regulate perineurial glial cell number in larval peripheral nerves. William Lavery, Michael Stern. Dept Biochemistry & Cell Biol, Rice University, Houston, TX.

Drosophila peripheral nerves, structured similarly to their mammalian counterparts, comprise three cell layers: motor and sensory axons, wrapped by an inner peripheral glia (analogous to the mammalian Schwann cell), and an outer perineurial glia (analogous to the mammalian perineurium). We previously reported that activated Ras^{V12} expressed specifically in the peripheral glia promotes perineurial glial growth, and this growth promotion is mediated by the Ras effector PI3-Kinase (PI3K), but not the Ras effectors Ral or Raf. To determine if this increase in perineurial glial thickness results from increased cell size or increased cell number, we visualized perineurial glial nuclei with the Hoechst DNA dye and counted the number of nuclei per unit length. We found that activated Ras in the peripheral glia increased perineurial glial cell number two-fold, but had no effect on cell size. The increase in perineurial glial cell number might result from increased recruitment of precursor cells into the perineurial sheath. In contrast, expressing either activated PI3K or activated Raf in the peripheral glia each increased perineurial glial cell number slightly less than two-fold. Activating Raf in the peripheral glia decreases perineurial glial cell size, whereas activating PI3K in the peripheral glia increases perineurial glial cell size. Our results are consistent with the possibility that the peripheral glia regulates perineurial glial cell size and cell number by distinct mechanisms. In mammalian peripheral nerves, the Schwann cell releases several growth factors that can affect the proliferative and migratory properties of neighbors. Some of these factors are oversecreted in Schwann cells defective in *Nf1*, which encodes the Ras-GTPase activator Neurofibromin and is the gene responsible for the disease Type 1 Neurofibromatosis. All individuals with this disease exhibit neurofibromas, which are tumors of peripheral nerves. Our results raise the possibility that growth of these tumors might be regulated at least in part by the activities of Ras, Raf, and PI3K within the Schwann cell.

796A

PDCD2/Zfrp8 in Drosophila and human hematopoiesis. Svetlana Minakhina, Ruth Steward. Dept Molec Biol & Biochemistry, Waksman Inst, Rutgers Univ, Piscataway, NJ.

PDCD2 (programmed cell death 2) is a highly conserved protein in humans and Drosophila. We characterized the Drosophila *PDCD2* gene, *zfrp8* (zinc finger RP8) and found that the gene has an important role in blood development. *zfrp8* alleles cause enormous hyperplasia of the hematopoetic organs, the lymph glands, and over-proliferation of hemocytes. Using genetic and cell biological approaches we find that *zfrp8* has little or no connection to programmed cell death. Instead it regulates cell proliferation.

The function of PDCD2 in humans is largely unknown, but we found that PDCD2 is expressed differently in normal and cancerous tissues. We suggest that PDCD2 may serve as a biomarker of a number of hematological malignancies, including Acute Myelogenous Leukemia (AML) and Acute Lymphocytic Leukemia (ALL).

Genetic interaction experiments in Drosophila and analysis of large-scale human DNA microarray expression profiles, currently available online, suggest that the gene is connected to several signaling/morphogenetic pathways, including the TNF-, and the TGFβ-GATA pathways. We propose that PDCD2/Zfrp8 present a new link between these pathways and cell cycle regulation.

797B

A structure-function analysis for the *Drosophila* fragile X protein. Paromita Banerjee, Thomas C. Dockendorff. Zoology, Miami University, Oxford, OH.

Fragile X syndrome is a prominent form of mental retardation that is observed in all human populations. The fragile X mental retardation protein (FMRP) is an RNA-binding protein that regulates transport and translation of select RNAs and is essential for normal neuronal development and behavior in all species in which its function has been examined. Several RNA binding domains are conserved between FMRPs from different species and include Tudor-related domains, two KH domains, and an RGG box. Since the vast majority of fragile X cases arise from transcriptional silencing of the *FMR1* gene, little is known about the *in vivo* contributions of individual RNA binding domains to FMRP function. To address this issue, we have generated point mutations and deletions in the *Drosophila* fragile X gene and characterized phenotypes associated with these mutations. Point mutations in the individual KH domains that are predicted to disrupt protein-RNA interactions result in partial loss of function for all neural development and behavior phenotypes examined. These observations are consistent with biochemical studies of FMRP showing that individual RNA binding domains are capable of binding substrates, and demonstrate that other domains of dFMR1 have a significant contribution to its function. In contrast, a mutation in a C-terminal domain that is not directly implicated in RNA binding results in complete loss of function for a subset of phenotypes that are associated with a null allele. This result suggests that the C-terminal domain regulates the activity of dFMR1 in a manner that is necessary for distinct functions of the protein.

798C

Determining a molecular role for *Drosophila fragile X related gene (dfmr1)* in metabotropic glutamate receptor (mGluR) signaling. Balpreet Bhogal, Thomas Jongens. Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Fragile X syndrome is the most common heritable form of mental retardation. Patients with this disease display mild to severe mental retardation, behavioral traits such as developmental delay, autistic-like behavior, sleep disorders and attention deficit hyperactivity disorder, as well as prominent physical features. This disease is caused by loss of function of the *Fragile X mental retardation (FMR1)* gene, which encodes an RNA binding protein. Although previous studies suggest that Fragile X functions as a translational regulator, the molecular function, as well as its regulatory targets, remain to be elucidated.

Recent studies suggest that metabotropic glutamate receptor (mGluR) signaling is hyperactivated in the absence of Fragile X. Our lab is interested in determining a molecular function for Fragile X in mGluR signaling using *Drosophila melanogaster* as a model organism. In *Drosophila*, there is a single gene, *Drosophila fragile x related (dfmr1)*, that is orthologous to FMR1. Our lab has developed a model to study Fragile X syndrome in *Drosophila* based on a null mutation of *dfmr1*. This model displays several phenotypes that bear similarity to symptoms observed in human Fragile X patients. Recent pharmacological studies performed using our fly model and other Fragile X mouse models have shown that some behavioral defects are rescued when mGluR signaling activity is diminished, suggesting a role for Fragile X in the regulation of this pathway. Our lab is currently interested in elucidating a regulatory molecular role for Fragile X in mGluR signaling. We are also interested in determining regulatory targets of Fragile X that could impact mGluR signaling.

799A

Regulation and function of Myotubularin phosphoinositide phosphatase in development. Jared Dennis, Amy Kiger. Dept Biological Sci, Univ California, San Diego, La Jolla, CA.

The localized regulation of distinct phosphoinositide phosphates (PIPs) is important to distinguish membrane compartments and spatially control cellular processes. Mutations in different PIP phosphatases are associated with human disorders, presumably due to misregulated responses. Human mutations in Myotubularin (MTM) phosphatases, MTM1 and MTMR2, are associated with myotubular myopathy and neuropathy of Charcot-Marie Tooth disease, respectively, but the cellular bases are not understood. MTMs encode a large conserved family of phosphatases with *in vitro* activity against PI(3)P and PI(3,5)P_2; however, mounting evidence suggests that different MTMs *in vivo* act on different or spatially-distinct PIP pools. We identified *myotubularin (mtm)*, the *Drosophila* homolog of human MTM1 and MTMR2, as important for an induced cell shape change. Using RNAi in cells, we characterized two defects in endolysosomal homeostasis and actin stability that coincided with a failure in cellular morphogenesis, and demonstrated that PI(3)P is a likely substrate that mediates both *mtm* functions. Using *mtm* RNAi hairpins, we discovered that *mtm* is essential for survival through pupal development, and that lethality could be rescued by co-expression of a wildtype *mtm* cDNA. As predicted from our cell-based studies, *mtm* mutant pupal wings exhibited enlarged endolysosomes and PI(3)P domains, and we are currently testing the significance of these phenotypes to arrested wing morphogenesis. Importantly, we found that adult eclosion failed upon *mtm* RNAi in muscles, suggesting conserved *mtm* developmental roles to those disrupted in MTM-related human myopathy. Given the non-redundant roles for overlapping expression of MTMs in humans, we are exploring endogenous Mtm spatial regulation. Interestingly, we found that Mtm-GFP is primarily cytoplasmic but differentially localized during development. We are using RNAi screens to identify factors that regulate and respond to Mtm functions, which will provide further insight into PIP regulation important for cell spatial control and disease.

800B

Studying the Molecular Bases of *O*-mannosylation in *Drosophila* Model System. Dmitry Lyalin, Naosuke Nakamura, Haiwen Li, Vladislav Panin. Dept Biochemistry & Biophysics, Texas A&M University, College Station, TX.

O-mannosylation is a specific form of glycosylation, a post-translational protein modification with *O*-linked mannose attached to serine or threonine residues. *O*-mannosylation is involved in some crucial biological processes such as neuronal and muscle development, cell adhesion and cell migration in humans. Two *O*-mannosyltransferase genes have been described in mammalian genomes so far, *POMT1* and *POMT2*. Mutations in both of these genes have been linked to the most severe form of muscular dystrophy in humans, the Walker-Warburg Syndrome.

Just like vertebrates, *Drosophila* has two *O*-mannosyltrasferase genes, *DPOMT1* (*rt*) and *DPOMT2* (*tw*), which share significant similarity with their mammalian counterparts. Mutations of both *DPOMT1* and *DPOMT2* cause the “rotated abdomen” phenotype, a clockwise rotation of abdominal segments.

Previously, we characterized the pattern of expression of the *rt* and *tw* gene during embryogenesis. *In situ* hybridization revealed that *rt* and *tw* have very similar expression patterns during embryonic development. Both proteins are localized in the ER compartment within the cell and co-expression of *rt* and *tw* is required for normal development. Phenotypic analysis of double mutants shows a statistically significant repression of *rt* mutant phenotype by tw^1 allele.

In order to investigate the possible mechanism of RT and TW molecular interaction, we over-expressed tw^1 cDNA using the UAS/Gal4 system. Experimental data suggest several possible mechanisms that there could account for the specific mode of interaction between tw^1 and *rt*. We are using a combination of genetic, and biochemical strategies to discriminate between these possibilities and to elucidate the molecular features of RT/TW interactions. The results of these experiments will be discussed from the perspective of using *Drosophila* as a model system for investigation of molecular bases of *O*-mannosylation.

801C

***Drosophila* Dystroglycan as a potential target of O-mannosylation by two protein O-mannosyltransferases, RT and TW.** Naosuke Nakamura, Dmitry Lyalin, Michiko Nakamura, Olga Lavrova, Haiwen Li, Vladislav Panin. Dept. Biochem. & Biophysics, Texas A&M University, College Station, TX.

Dystroglycan is one of the most important components in the dystrophin-glycoprotein complex (DGC). In mammals, glycosylation of alpha-dystroglycan is thought to be essential for DGC function because hypoglycosylation of alpha-dystroglycan leads to loss of its extracellular ligand binding activity and inability of DGC to function as a link between extracellular matrix and cytoskeleton. Recently, several severe human congenital muscular dystrophies were found to be associated with genetic defects in glycosyltransferases including O-mannosyltransferases, POMT1 and POMT2. Recent studies demonstrated that *Drosophila* has two O-mannosyltransferase genes, *rotated abdomen (rt)* and *twisted (tw)*, and most of the genes encoding DGC-associated proteins, including *Dystroglycan (Dg)*. These indicate that *Drosophila* might be a suitable model system for studying the molecular mechanisms underlying O-mannosylation and several human congenital neuromuscular diseases. However, until now, it has not been known whether Dystroglycan (DG) is O-mannosylated by these enzymes in *Drosophila*. Thus, our research is focused on characterizing glycosylation of *Drosophila* DG, including its potential O-mannosylation. To this end, we successfully produced flies ectopically expressing DG, RT, and TW proteins under control of the UAS-Gal4 system. Interestingly, our preliminary data indicate that ectopic expression of these proteins during development does not result in any significant morphological abnormalities, and the overexpressing flies are viable and fertile. We plan to purify DG protein with FLAG tag from the transgenic flies and to analyze its O-mannosylation. We will present data from our combined *in vivo* and *in vitro* approaches and will discuss the results in the light of potential involvement of O-mannosylation in DG functioning in *Drosophila*.

802A

Indirect flight muscles of *Drosophila* as a model system to study abnormal protein aggregate myopathies. Upendra Nongthomba[1], Shital Salvi[1], Divesh Thimmaiya[1], John Sparrow[2]. 1) MRDG, Indian Institute of Science, Bengalooru, IN; 2) Dept. of Biology, University of York, YO10 5DD, UK.

The Indirect Flight Muscles (IFMs) form the bulk of the *Drosophila* thorax musculature. IFMs because of their close structural and functional resemblance to human skeletal muscles provide, in a genetically tractable organism, a unique system for the genetic dissection of the orthologous human genes implicated in myopathies, dystrophies and neuromuscular disorders. Many muscle diseases (myopathies) are due to mutations in those structural proteins that assemble to form the muscle sarcomere. However, little is known about the aetiology of these diseases. Most sarcomeric proteins exhibit high structural and sequence conservation and perform similar functions in human and *Drosophila* muscles. We are using the IFMs to address the roles of specific structural proteins in sarcomere assembly and formation of the nemaline structures and abnormal protein aggregates that typify human myopathies. This group of genetic diseases are typically due to mostly thin filament protein mutants. In *Drosophila* the molecular genetic analysis of these mutants is readily achieved *in vivo*. Our recent findings will be presented and discussed with the emphasis on developing IFMs as a versatile model tissue to study the human muscle diseases. The study of IFMs development and function in flies has the potential to provide invaluable insights into the basic mechanisms involved in sarcomere assembly and human muscle diseases.

803B

Understanding the role of Drosophila Fragile X Mental Retardation Protein (FMRP) as a translational regulator. Anita Pepper, Thomas Jongens. Dept. of Genetics, Univ. of Pennsylvania SOM, Philadelphia, PA.

The loss of fragile x mental retardation protein (FMRP) is responsible for the most common heritable form of mental retardation, Fragile X Mental Retardation Syndrome. FMRP has been implicated in translational regulation of specific mRNAs through localization, stabilization, or as a member of a ribonucleoprotein complex. Recent data suggests that FMRP acts as a localized translational regulator of target mRNAs at the synapses, although the biomolecular mechanisms that underlie FMRP function are unknown.

Drosophila is an excellent model organism to study Fragile X syndrome because it has a single fragile x related gene (*dfmr1*) that encodes the *Drosophila* fragile x related protein (dFMRP). In order to further our understanding of dFMRP's molecular role we have created S2 cell lines and transgenic fly strains expressing a C-terminal tandem affinity purified (TAP) tagged version of dFMRP (dFMRP-cTAP). We have used a genomic fragile X construct with the endogenous promoter to maintain near normal levels of the expressed protein. Using lysates from both the cell lines and heads and ovaries of the fly strains we are purifying dFMRP interacting protein complexes and identifying components using Mass Spectrometry. From these experiments we have isolated and validated *in vivo* interactors of dFMRP that have been previously published such as Argonaute 2. In addition, we have isolated newly identified interactors of dFMRP that are currently being validated. We are encouraged that these interactions may shed some light on Fragile X's molecular role in vivo.

804C

***detached* encodes *Drosophila* Dystrophin, which acts with other members of the Dystrophin Associated Protein Complex to regulate cell signalling in developing wing veins.** Robert Ray, Christina Christoforou, Claire Greer, Benjamin Challoner, Dimitris Charitzanos. School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG United Kingdom.

The two most common forms of human muscular dystrophy are Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD), both of which are associated with mutations in the *dystrophin* gene. Dystrophin is a component of a complex of proteins, the Dystrophin Associated Protein Complex (DAPC), that is thought to provide a mechanical link between the cytoskeleton and the extracellular matrix, the loss of which leads to muscle fragility, contraction-induced damage, and necrosis. The Dystrophin protein is highly conserved, and studies on a variety of systems have revealed a range of functions for Dystrophin and the DAPC in muscle and muscle-associated junctional complexes. In this report, we present the characterization of *detached (det)*, and show that this gene encodes the *Drosophila* ortholog of Dystrophin. In contrast to the functions that have been described for Dystrophin in other systems, the phenotype associated with *det* is a defect in wing vein patterning, and we show that this phenotype is the only morphological defect associated with a Dystrophin null in the fly. Our data support the conclusion that loss of Dystrophin does not lead to muscular dystrophy phenotypes in flies. We show that the vein function of Dystrophin involves two other components of the DAPC, Dystroglycan and Laminin A, thus, the entire link between cytoskeleton and extracellular matrix has been co-opted for this atypical DAPC function. Analysis of the effect of *det* mutations on vein specification shows that the complex is required for maintenance, but not initiation, of BMP signalling, suggesting a role for Dystrophin in the signalling events that lead to vein specification. As such, the *Drosophila* wing is an ideal model system for characterizing the role of the DAPC in mediating intercellular signalling processes.

805A

The *Drosophila* orthologue of the mouse autoimmunity gene *roquin* is an essential gene. Peter Smibert, Vicki Athanasopoulos, Robert Saint. CMGD, Australian National University, Canberra, ACT, Australia.

We have been using the *Drosophila* model system to examine developmental roles for the sole *Drosophila* ortholog of the mouse *Roquin* and *MNAB* genes. Mouse *Roquin* was identified in an ENU mutagenesis screen as the gene mutated in the *san roque* mouse strain, which develops autoimmunity due to an accumulation of autoreactive T cells. The *roquin* gene encodes a protein product that is predicted to be an E3 ubiquitin ligase by virtue of a conserved RING domain at the amino terminus. Roquin also contains a conserved C3H Zn finger, which is commonly found in RNA binding proteins. *Drosophila roquin* transcripts are maternally deposited, zygotically expressed in the embryonic salivary gland and increase in abundance throughout larval growth. We have generated mutant alleles of *Drosophila roquin* and found that flies homozygous or transheterozygous for the *roquin* alleles predominantly die at the 2nd and 3rd instar larval stages. Endogenous mouse Roquin localises to cytoplasmic stress granules, as does ectopically expressed mouse Roquin and *Drosophila* Roquin in HeLa cells. We conclude, therefore, that *roquin* is an essential *Drosophila* gene that plays a conserved role in the regulation of mRNA turnover.

806B

Characterization of the *torp4a* gene, a *Drosophila* homolog of human *DYT1* (Torsin A) associated with early-onset dystonia. Noriko Wakabayashi-Ito, Nicole Smith, Jo-Chen Chou, Vijaya Ramesh, James Gusella, Naoto Ito. Center for Human Genetic Res., Massachusetts General Hosp, Boston, MA.

Dystonia is a neurological movement disorder characterized by involuntary muscle contraction that forces certain parts of the body into abnormal movements or postures. Most cases of early-onset dystonia are associated with the mutation of the *DYT 1* (Torsin A) gene. In the *Drosophila* genome, one torsin-like gene, which is named *torp4a*, has been identified. We examined expression of *torp4a* in early embryo by whole mount *in situ* hybridization. RNA is detected in very early stage (stage 1, 2) embryos. At a later stage, transcripts are also detected in presumptive mesoderm. For further analysis, we generated an antibody against Torp4a. Torp4a is expressed in CNS and PNS at a later stage. Robust expression was observed in the follicle cells of the ovary. In order to determine normal function of *torp4a*, we used the technology developed by Gong and Golic (2003) and Gordon, *et. al.* (2005). We have obtained candidate lines that have white marker on X chromosome. We are currently determining the structure of *torp4a* region of these candidate lines.

807C

Studying the circadian defect observed in the *Drosophila* model of Fragile X Syndrome. Yan Wang[1], Amita Sehgal[2], Thomas A. Jongens[1]. 1) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Neuroscience, HHMI, University of Pennsylvania School of Medicine, Philadelphia, PA.

Fragile X syndrome is the most common inherited mental retardation disease. Similar to sleep disorders in Fragile X patients, flies with loss of *dfmr1* gene function display arrhythmic free-running locomotor activity. As the central clock molecules still oscillate in the *dfmr1* mutants, it is implicated that dFMR1 plays a role in regulating circadian output. PDF is the main circadian output factor in flies and its cycling at the LNvs terminals has been suggested to mediate activity regulation from the central clock to downstream neurons. Overexpression of *dfmr1* or *pdf* in the LNvs neurons leads to lengthened period with delayed nuclear entry of PER, suggesting some similar role of dFMR1 and PDF in circadian output and a link from output to input on the clock. By using Gal4/UAS system, we further study the role of dFMR1 in specific subsets of neurons for circadian output, which will elucidate some specific output neural circuit with dFMR1's function in it.

808A

Genetic analysis of Rothmund-Thomson syndrome homolog in *Drosophila*. Jianhong Wu, Carrie Marean-Reardon, Christopher Capp, Tao-shih Hsieh. Biochemistry Department, Duke University Medical Center, Durham, NC 27710, USA.

Rothmund-Thomson Syndrome (RTS) is a recessive disorder characterized by shorter stature, skeletal abnormalities, poikiloderma, premature aging, and predisposition to cancers, especially osteogenic sarcoma. Mutation of RecQ4, which is a member of the evolutionarily conserved RecQ DNA helicase family, causes a subset of RTS cases. Members in this family play a critical role in maintaining the genomic integrity of organisms from bacterium to human. To probe the genetic function of RecQ4 in *Drosophila*, we obtained *Drosophila RecQ4* mutants *recq4*^{7470} and *recq4*^{19}, a hypomorph and a null mutant, respectively. Homozygous flies for *recq4*^{7470} are viable, but female sterile. These females lay fewer eggs with eggshell defects, which are specifically caused by a reduction of the chorion gene amplification of follicle cells, resulting in thin and fragile eggshell. While homozygous animals for *recq4*^{19} can survive through embryonic development, which is assumed due to the maternal loading, this null mutation causes fragmentation of chromosomes, dramatically reduced mitotic index, and lethality of larvae. Both DNA endoreplication and diploid cell DNA replication in larvae homozygous for *recq4*^{19} are impeded. Our data implicate a critical role of RecQ4 in DNA replication.

809B

Development of a *Drosophila melanogaster* model for drug screening in Parkinson's disease. Lori A. Hrdlicka[1], Joost Schulte[2], Hsin-Pei Shih[1], Christopher J. Cummings[1], James K. T. Wang[1]. 1) EnVivo Pharmaceuticals, Inc., Watertown, MA; 2) The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA.

There are currently no treatments for Parkinson's disease (PD) and Huntington's disease (HD) patients that attenuate the progression of these neurodegenerative disorders. EnVivo Pharmaceuticals is discovering and developing drugs for these diseases using a proprietary whole organism drug screening method based on *Drosophila* disease models and phenotypic analysis. Using this *in vivo* platform, different focused small molecule libraries, including drugs approved by the FDA for other indications, were screened in *Drosophila* HD models. Several hits were identified and a lead compound for HD is presently in preclinical development. To adopt this successful and novel drug discovery approach for PD, it is necessary to develop a *Drosophila* PD model that is amenable to compound screening. Thus, we created transgenic *Drosophila* that express human *alpha-synuclein* alleles in the nervous system. *Alpha-synuclein* is genetically linked to autosomal dominant familial PD. Its gene product is a major component of Lewy bodies and cytoplasmic inclusions, which are pathological characteristics of PD. *Drosophila* expressing high levels of human alpha-synuclein in the nervous system showed a shortened lifespan and a detectable motor phenotype. In addition, the expression of alpha-synuclein in the dopaminergic system and the eyes resulted in disruption of a dopaminergic neuronal cluster and a loss of rhabdomeres, respectively. These data support the feasibility of establishing transgenic *alpha-synuclein Drosophila* models with progressive phenotypic deficits that may be utilized for high-throughput compound screening. The achievements of such an approach in HD exemplify its potential to develop novel disease-modifying treatments for PD.

810C

Putting the toolbox to work: A role for HDACs in disease. Ranjani Padmanabhan, Jian Chen, Florian Gmeiner, Marc Hild, Dan Garza. Drosophila Genetics Unit, Novartis Institutes for Biomedical Research (NIBR), Cambridge, MA.

The maintenance of chromatin architecture is essential for regulation of gene expression in vertebrates and invertebrates. Histone acetylation provides a conserved, post-translational mechanism for chromatin modification. Hyperacetylation of histones by Histone Acetylases (HATs) has been shown to increase levels of transcription of distinct genes, whereas hypoacetylation by Histone Deacetylases (HDACs) is associated with decreased transcriptional activity. As an essential part of the machinery that interacts with the nucleosome, HDACs have been implicated in a range of essential cellular processes, such as cellular memory, apoptosis, muscle development, and early embryonic development.

Our primary focus is to elucidate the role of HDACs in disease and development. HDACs have been known to associate with oncogenes and tumor suppressors, and studies show that HDAC inhibitors are able to selectively kill cancer cells. Abnormal histone acetylation has been implicated in cardiac hypertrophy, suggesting a role of HDACs in muscle development. In the last meeting, we presented our HDAC toolbox which includes multiple knock-down (RNAi), and gain-of-function HDAC fly lines for the set of five HDACs that are present in *Drosophila*, as well as antibodies that allowed us to detect HDAC expression in a tissue-specific manner. Here, we will show how our HDAC reagents have been a valuable tool for *in vivo* validation of two genome-wide RNAi screens performed in our lab, as well as asking the critical questions for HDAC function in development. Our data suggests a distinct role for HDACs in mitochondrial function, chromatin remodeling, as well as in stress response.

811A

The use of a Drosophila model of Spinal Muscular Atrophy for small molecule high-throughput drug discovery. Natasha Thomas[1], Paul Overton[1], Marcel van den Heuvel[2], Jon Tinsley[1], Emmanuel Dequier[1]. 1) VASTox plc, 91 Milton Park, Abingdon, OX14 4RY, UK; 2) MRC FGU, OCGF, University of Oxford, Oxford, OX1 3QX, UK.

The current drug discovery paradigm is initiated by the identification of biological targets associated with disease, which are used to derive *in-vitro* biochemical tests for high throughput drug screening. This approach is resource intensive and can take many years. In addition, there is no guarantee of success, and only 2% of hits isolated in *in-vitro* tests lead to clinically approved drugs. In VASTox we are re-designing the drug discovery pipeline by using *in-vivo* models of human disease generated in small model organisms such as Drosophila and zebrafish to (1) reduce the cost of drug discovery programs and (2) shorten the bench to bed time period required to develop a new drug. To this end VASTox is integrating high-throughput screening, toxicology and chemistry platforms to reduce the risk associated with *in-vivo* drug discovery screens. Our drug discovery programs have developed strategies to perform high content screening of large libraries of compounds, and our first full scale screen utilises a Drosophila model of Spinal Muscular Atrophy (SMA).

SMA, a recessive neuromuscular disease, is linked to mutations in the *Survival Motor Neuron (SMN) gene*. SMA patients either do not acquire or eventually lose the ability to move and death occurs primarily through respiratory insufficiency. SMA is the leading genetic cause of infant mortality in the world, affecting 1 in 6,000 newborns. A Drosophila mutant with a mis-sense mutation in the *smn* gene similar to those found in SMA patients has been isolated. Prior to death, *smn* mutant animals show progressive loss of mobility and increasingly uncoordinated movement.

The first active compounds which improve the symptoms of SMA in an *in-vivo* Drosophila screen designed to model the disease have been isolated and are now undergoing lead development in the VASTox drug discovery Platform.

812B

Copper Homeostasis & tau-mediated Neurodegeneration in Drosophila. Kirsten E Allan[1], James Camakaris[1], Richard Burke[2]. 1) Department of Genetics, University of Melbourne, Melbourne, AU; 2) School of Biological Sciences, Monash University, Melbourne, AU.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in humans and is the most common dementia in the elderly. AD is characterised by the formation of intracellular neurofibrillary tangles (NFTs) and extracellular senile plaques containing beta-amyloid. The tangles are composed of abnormally hyperphosphorylated tau microtubule binding protein. Copper has been implicated in the etiology and/or progression of AD, however the exact role this essential trace element plays in the disease is unknown. The tau protein has been shown to directly bind copper *in vivo* leading to a conformational change in the protein into a beta-sheet structure [1]. Beta-sheet structures are thought to be the precursor to protein aggregation and thus formation of NFTs. Thus it is hypothesised that copper plays an important role in tau aggregation and in AD.

A *Drosophila* model of tau pathologies has been well characterised in the literature [2]. We are examining the role copper may play in AD, using this *Drosophila* model. In order to do this we are genetically manipulating copper levels in specific tissues by over-expression of known copper homeostasis genes, DmATP7 (copper efflux) and DmCtr1A (copper uptake). Additionally, feeding experiments with copper or copper chelators are being performed to determine if copper enhances or abolishes the tau phenotype observed. This study should lead to an understanding of the role copper plays in AD and other tauopathies. 1. Ma, Q.F., et al., Peptides, 2006. 27(4): p. 841-849. 2. Wittmann, C.W., et al., Science, 2001. 293(5530): p. 711-714.

813C

Toxicity caused by Alzheimer's Abeta peptides is associated with abnormal regulation of cell cycle genes. Weihuan Cao, Tina Gangi, Mary Konsolaki. Deartment of Genetics, Rutgers University, Piscataway, NJ.

Flies have been extensively used as a model system for the analysis of molecular pathways involved in various neurodegenerative diseases. Our laboratory uses Drosophila in order to study the toxic effects of Alzheimer's disease beta-amyloid (Abeta) peptides. In our previously generated model system, transgenic flies expressing Abeta peptides in their nervous system exhibit phenotypes that mimic aspects of Alzheimer's disease pathology (Finelli et al, 2004). The severity of these phenotypes is progressing with age and correlates well with accumulating levels of Abeta. Recently, it has been proposed that abnormal regulation of cell cycle genes is an early event that precedes neurodegeneration associated with Alzheimer's disease. Although the molecular pathways that lead to such abnormal regulation are not understood, it is generally accepted that the expression of cell cycle genes in post-mitotic cells would cause major disruptions of cellular functions and lead to cell death. In order to better understand the events that lead to cell death in response to overexpression of Abeta, we examined the expression of cyclin genes in our Abeta transgenic flies. We have found that Abeta expression can induce expression of cyclins in a dose dependent way. Moreover, we have tested mutations in various cyclin genes and found that they can modify Abeta-induced phenotypes. It is currently unclear whether cyclins mediate the toxic effects of Abeta by mobilizing the cell cycle machinery of the cell or whether they have other functions, unrelated to their role in cell cycle progression. In order to address this question, we are testing mutations in kinases that are known to form complexes with cyclins, for their effects on Abeta phenotypes.

814A

The *Drosophila Palmitoyl Protein Thioesterase 1 (Ppt1)*, a homologue of the Batten Disease PPT1 gene, is required for normal embryonic neural development. Quynh Chu-LaGraff, Erika Selli, Cassandra Denefrio. Dept Biology, Union College, Schenectady, NY.

The gene *Drosophila Palmitoyl Protein Thioesterase 1 (Ppt1)* was previously identified to be a homolog of the human PPT1 gene. Defects in human PPT1 leads to the pediatric neurological disease called Infantile Neuronal Ceroids Lipofuscinosis (INCL). It is known that removal of the fly Ppt1 protein results in neural phenotype reminiscent of the human disease, the accumulation of autofluorescence deposits and decreased lifespan in adults. Since INCL patients die at a young age, earlier developmental neural defects due to the loss of PPT1 are postulated but have yet to be elucidated. Our results indicate that *Drosophila* may provide insights into how the loss of Ppt1 function results in developmental neural defects. In the absence of Ppt1, the earliest neural phenotype is detected as early as stage 11 embryogenesis with altered expression in an identified motoneuron lineage, the RP2 motoneuron. Ppt1 mutant embryos exhibit partial loss of even-skipped expressing GMC4.2a and later, RP2 neuron, in addition to a decreased in the number of EL neurons. Neural defects are not restricted to just this motoneuron: Ppt1 mutants also display abnormal CNS and PNS development as detected by BP102 and 22C10 immunohistochemistry. We are currently investigating whether these axonal defects are due to the death of the RP2 motoneuron as well as to neurons in other identified neuronal lineages.

815B

Functional dissection of Orthodenticle in the *Drosophila* eye: a model to understand human cone-rod dystrophy and Leber congenital amaurosis. Pierre Fichelson, Franck Pichaud. Cell Biology, MRC LMCB, London, GB.

In humans, cone-rod dystrophy and Leber congenital amaurosis (LCA) are diseases causing deterioration of the cone and rod photoreceptor cells, leading to blindness. Mutations in the *cone-rod homeobox* (*crx*) gene have been shown to be associated with these two retinopathies. *crx* belongs to a conserved gene family encoding transcription factors containing a PAIRED-class homeodomain. This gene family plays a strikingly conserved role in the morphogenesis of anterior head structures including the central nervous system. In *Drosophila*, *orthodenticle* (*otd*) is the only member of this gene family. Interestingly, in the compound eye, *otd* has been implicated in photoreceptor morphogenesis and rhodopsin expression. This offers a unique opportunity to establish an *in vivo* model in a genetically amenable system such as *Drosophila* to understand human cone-rod dystrophy and LCA. We present a detailed characterization of the *otd* mutant eye phenotype in respect to the morphogenesis of the photoreceptor apical organelle. We also present preliminary data regarding the function of Otd is this cell type.

816C

Genetic Modifiers of Prion Disease in Drosophila. Brendan Gavin[1], James Geoghegan[2], Nathan Deleault[2], Maria Dolph[1], Vikram Khurana[3], Mel Feany[3], Surachai Supattapone[2], Patrick Dolph[1]. 1) Dept Biol, Dartmouth Col, Hanover, NH; 2) Department of Biochemistry, Dartmouth College, Hanover, NH; 3) Department of Pathology, Division of Neuropathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Gerstmann-Straussler-Scheinker (GSS) syndrome is a fatal neurodegenerative disorder linked to genetic mutations in the host prion protein gene (PRNP). GSS is characterized by cerebellar ataxia and dementia as well as the neuropathological presentation of amyloid plaques consisting of protease resistant PrP fragments. The most common form of GSS disease is associated with a proline to leucine mutation at codon 102 of PRNP. This mutant gene encodes for a prion protein (PrP) that is more likely to misfold and accumulate as a toxic form of PrP. We have developed a fruitfly model of GSS disease by expressing either the wildtype (WT) or mutant (P102L) murine PRNP gene specifically in cholinergic or dopaminergic neurons using the GAL4-UAS system. Flies expressing the mutant P102L, but not the wildtype, copy of prion protein are characterized by behavioral defects, poor coordination, and shortened lifespans. Brains of flies expressing the mutant prion gene protein display striking neuropathology and contain a misfolded form of prion protein. Work will be presented describing candidate genetic enhancers or suppressors of the P102L phenotype.

817A

Drosophila X11 regulates the amyloid precursor protein and plays an essential role during neurodevelopment. Garrett Gross[1], Renny Feldman[1], Volker Hartenstein[2], Ming Guo[1]. 1) Neurology, University of California, Los Angeles, CA; 2) Molecular, Cellular and Developmental Biology, University of California, Los Angeles, CA.

One of the pathological hallmarks of Alzheimer's disease (AD) is the deposition of beta-amyloid (Abeta) in the brain. Abeta is generated from the Amyloid Precursor Protein (APP) by two proteolytic events, one of which is mediated by gamma-secretase. Mammalian cell-based studies suggest that the X11 protein family can regulate Abeta secretion, likely by modulating gamma-secretase function. Additionally, X11 has been shown to physically interact with APP's intracellular domain (AICD), a fragment of APP produced following gamma-cleavage. The X11 protein family contains one PTB and two PDZ domains. We have studied X11 in Drosophila to gain insight into its function. We identified X11 from a screen for gamma secretase and APP regulators using a previously published APP and gamma-secretase cleavage-based reporter (hereafter called GAMAREP; Guo et al. Hum Mol Genet 12:2669 (2003)). Overexpression of X11 strongly suppresses the small and rough eye phenotypes of GAMAREP flies. Moreover, overexpression of a mutant form of X11 devoid of the PTB domain fails to suppress the reporter. We provide evidence that X11 suppresses the phenotypes of GAMAREP flies by interacting with the AICD, which is consistent with results from mammalian studies. Finally, overexpression of human X11 in flies also suppresses GAMAREP eye phenotypes, suggesting that human and fly X11 are functionally conserved.

To explore X11's function in Drosophila, we generated deletion mutants of X11, which are viable. X11 has a homologous protein, X11B, in the Drosophila genome. Removal of both forms results in flies that are embryonic lethal, suggesting these two homologs carry out at least partially redundant functions. Preliminary studies suggest that the lethality is due to defects during neurodevelopment. Further characterization of the double mutant's phenotypes are currently underway.

818B

Circadian rhythms as model systems to study the effects of transcriptional dysregulation in MJD-afflicted Drosophila. Amy B Hart, John M Warrick. Biology, University of Richmond, Richmond, VA.

Machado Joseph disease (MJD) is a member of the family of human polyglutamine diseases that is caused by an increased number of CAG repeats that occur in the DNA sequence and encode an expanded polyglutamine repeat. Transcriptional dysregulation due to the inactivation of transcriptional regulators has been proposed as a mechanism of pathology in human polyglutamine disease. Circadian rhythms drive the daily locomotor activity of humans and Drosophila. These rhythms are regulated by tightly controlled transcriptional feedback loops. Analysis of daily locomotor rhythm in Drosophila may serve as a model system in which to study the role of transcriptional dysregulation in the disease pathology. Expression of full-length mutant protein Ataxin-3 (ATX-3) causes disruption in circadian activity, while the normal ATX-3 has no effect on circadian activity. A substantial rescue of circadian behavior was observed in behavioral assays of flies expressing human heat shock protein, a molecular chaperone. Presently, the localization and levels of expression of circadian proteins per and clk in the brain are being studied to explore the possible link between protein aggregation and transcriptional dysregulation leading to the disease.

819C

Mutational analysis reveals different aggregation propensity of Aβ42 is associated with distinct intraneuronal accumulation profile and pathological phenotypes in *Drosophila*. Koichi Iijima[1,3], Hsueh-Cheng Chiang[3], Stephen Hearn[3], Inessa Hakker[3], Amy Leung[3], Kanae Iijima-Ando[2,3], Yi Zhong[3]. 1) Laboratory of Aging and Neuroproteinopathies,; 2) Laboratory of Neuronal Protein Misfolding, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Alzheimer's disease (AD) is a fatal neurodegenerative disease characterized by memory impairment followed by global cognitive deficits. Although the pathogenic mechanisms are not fully understood, extra- and intraneuronal accumulation of Aβ42 has been suggested to play a central role in the pathogenesis of AD. Previously, we demonstrated overexpression of Aβ42 in *Drosophila* recapitulated many features of AD including memory defects, locomotor dysfunction, neurodegeneation and premature death accompanied with intraneuronal accumulation of Aβ42 (Iijima, K. et al. 2004).

Recent literature suggests that structural polymorphism of Aβ42 may confer its multifaceted toxicities and a complex AD pathogenesis. We are motivated to elucidate this hypothesis in *Drosophila*. To manipulate the aggregation propensity of Aβ42, we developed new transgenic flies expressing mutant Aβ42 that either enhance (Arctic mutation from familial AD; Aβ42Arc) or suppress (artificial mutation; Aβ42art) amyloid fibril formation *in vitro*. At a cellular level, Aβ42 accumulates both in the cell body and neurites and damages both structures, while Aβ42Arc mainly deposits in the neuronal soma and causes more severe cell body degeneration than Aβ42. In contrast, Aβ42art preferentially accumulates in the neurites and induces more severe neurite degeneration. At a behavioral level, Aβ42Arc enhances memory defects, locomotor dysfunction and premature death. Intriguingly, Aβ42art causes more severe memory defects but milder locomotor dysfunction or premature death. These results demonstrate that Aβ42 with different aggregation propensity can produce behavioral and pathological heterogeneity *in vivo*.

820A

Effects of N-terminal fragments of huntingtin harboring expanded polyglutamine stretch on CREB activity in *Drosophila*. Kanae Iijima-Ando[1,3], Koichi Iijima[2,3]. 1) Laboratory of Neuronal Protein Misfolding, Farber Institute for Neurosciences,; 2) Laboratory of Alzheimer's Disease and Neuroproteinopathies, Farber Institute for Neurosciences,; 3) Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA.

Expansion of CAG repeat coding polyglutamine (polyQ) stretch causes neurodegenerative diseases such as Huntington's Disease (HD). Disruption of cAMP-response element-binding protein (CREB) activity is suggested to be involved in the pathogenesis of HD. In previous study, we reported that expression of 108 polyglutamine peptides with Myc-Flag tag (Q108myc/flag) in neurons reduces CREB activity in *Drosophila* using *in vivo* reporter. Expression of non-toxic, short polyQ peptide(Q22myc/flag) does not attenuate CREB activity. Therefore, attenuation of CRE-Luc activity depends on the length of the polyQ that is overexpressed. Recent study with animal models of HD revealed that the length of huntingtin (htt) fragments harboring polyQ has dramatic effect on their toxicity. htt produces various lengths of N-terminal fragments in vivo. We hypothesized that each fragment has different impact on CREB activity. We compared CREB activity in the flies with expression of N-terminal fragment (1-548) of htt with 128Q (httN548Q128) and that of exon1 with 93Q (httexon1Q93) in neurons. We found that CREB activity was decreased in httN548Q128 flies, but not in httexon1Q93 flies. Under this experimental condition, httN548Q128 or httexon1Q93 flies showed the similar level of premature death. This difference does not seem to be solely attributable to length of polyQ stretch, because expression of Q127 polyglutamine peptides with HA tag does not dampen CREB activity. These results suggest that each fragment of htt with expanded polyQ stretch causes toxicity by different mechanism and targeting multiple pathways may be required for HD treatment.

821B

The role of *Drosophila* EDEM in misfolded protein degradation. Min-Ji Kang, Hyung Don Ryoo. Department of Cell biology, NYU School of Medicine, New York, NY.

Expression of mutant proteins that misfold in the endoplasmic reticulum (ER), activates a signaling network known as the unfolded protein response (UPR). UPR reduces total misfolded proteins in the ER through the attenuation of protein synthesis, increase in folding capacity, and accelerated degradation of misfolded proteins. UPR is implicated in many diseases, here, we examined a *Drosophila* model for Retinitis Pigmentosa (RP), ninaE^{G69D}, which has a missense Rhodopsin-1 mutation. We found that the UPR pathway involving the endonuclease ire-1 and the transcription factor xbp1 becomes active in the retina of ninaE^{G69D} flies. Correlating with this, the level of Rhodopsin-1 is diminished significantly. This has prompted us to examine the mechanism of misfolded Rhodopsin-1 degradation in the RP *Drosophila* model and in response to UPR. EDEM protein has been proposed to act as a "degradation lectin" for endoplasmic reticulum-associated degradation (ERAD) of misfolded glycoproteins. We isolated two EDEM homologs encoded in the *Drosophila* genome, annotated as CG3810 (EDEM1), and CG5682 (EDEM2), respectively. The expression of EDEM2 was induced after ER-stress. We found that *Drosophila* EDEMs accelerate glycoprotein ERAD in transfected S2 cells and *Drosophila* imaginal discs, as shown by increased degradation of misfolded 1-antitrypsin variant (null Hong Kong), a well known ERAD substrate. Furthermore, the levels of Rhodopsin-1 in ninaE^{G69D} mutant were diminished in *Drosophila* retina expressing EDEMs. Our experiments suggest that *Drosophila* EDEMs are important for misfolded glycoprotein degradation, likely to contribute to ER homeostasis in stressed cells. Furthermore, these tools will allow us to assess whether reducing misfolded Rhodopsin-1 changes the course of retinal degeneration in this disease model.

822C

Reduced histone methyltransferase activity due to pathogenic and polymorphic genotypes. Parsa Kazemi-Esfarjani[1,3,4], Zahra Fayazi[1,3,4], Jason M. Myers[2,3,4], John M. Aletta[2,3,4]. 1) Dept Physiology & Biophysics; 2) Dept Pharmacology & Toxicology; 3) Center for Neuroscience; 4) School of Medicine & Biomedical Sciences, Univ Buffalo, Buffalo, NY.

Reduced gene transcription caused by reduced histone acetylation may contribute to neurodegeneration in Huntington's disease and other polyglutamine (polyQ) disorders. Since gene transcription is also regulated by histone methylation, we examined the effect of proteins with expanded polyQ tracts on histone methylation in various *Drosophila* models of polyQ toxicity. To measure histone methyltransferase activity, exogenous histone H3, protein extracts of fly heads and ^{3}H-SAM were combined in vitro and the resulting methylproteins were analyzed by SDS-PAGE and fluorography. Extracts from w^{1118} control flies and flies expressing shorter repeats of 20Q or 41Q in the retina gave rise to equivalent radiolabeling of H3. Flies expressing 63Q or 127Q, or a truncated huntingtin with 120Q exhibited greatly reduced H3 labeling. A fly line called $ash1^{B1}$ carries a mutation in *ash1* locus, encoding an enzyme that methylates lysine 4 and 9 of H3 and lysine 20 of histone H4. Extracts from heterozygous $ash1^{B1}$ produced H3 labeling similar to that of 63Q or 127Q flies, hence the mutation appeared dominant. To see whether genetic background contributes to the reduced labeling, we tested iso-1, $ash1^{B1}$'s original genetic background, and two wild-type laboratory strains of *Drosophila melanogaster*, Canton Special (CS) and Oregon-R (OR). Compared to w^{1118}, labeling was similar in iso-1, very weak in CS, and intermediate in OR. Therefore, genetic background does not seem to underlie the weak labeling in $ash1^{B1}$ extracts, and reduced labeling in extracts with expanded-polyQ proteins may indeed be due to ASH1 inhibition. Furthermore, these results indicate that the reduction in this H3 methyltransferase activity may occur as an apparently benign polymorphism among *Drosophila* strains, and hence may not be relevant to the pathogenesis of polyQ disorders. We plan to employ microarray expression profiling and H3 protein proteolytic mapping to identify the methyltransferase(s) involved.

823A

Mitochondrial-targeted mRNAs as a novel gene therapy for encephalomyopathies. Nicole Kotchey[1,2], Michael Palladino[1,2]. 1) Department of Pharmacology, University of Pittsburgh SOM, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh SOM, Pittsburgh, PA.

Mutation of the *mtATP6* gene, which encodes a subunit of the F_0F_0-ATP Synthase, also known as Complex V in mitochondria, is known to cause a group of related encephalomyopathies. The F_0F_0-ATP Synthase acts as an hydrogen ion transporter that couples hydrogen ion dissipation with ATP production. Diseases including NARP (neuropathy ataxia and retinitis pigmentosa) and MILS (maternally inherited Leigh's syndrome) are caused by missense mutations in the *ATP6* gene. Flies bearing a mitochondrial *ATP6* missense mutation have been isolated with progressive neuromuscular impairment and degeneration that model NARP/MILS disease. We have developed a transgenic strategy where allotopic expression of a mitochondrial-targeted *ATP6* mRNA may serves as a potential gene therapy for mitochondrial disease. Mitochondria in metazoans are known to import a nuclear encoded 5S rRNA, which is essential for mitochondrial protein synthesis. We discovered 100 distinct 5S rRNA genes in the 56 D-F regions of the right arm of *Drosophila* chromosome 2. Sequence comparisons revealed 17 groups of genomic variants and 14 processed rRNA variant counterparts. RT-PCR was used to determine which of the 5S rRNAs are localized to the mitochondria. We aim to use 5S rRNAs, or a smaller functional component of the 5S rRNA, to target *5S::ATP6* mRNA into mitochondria and assess the ability of allotopic mRNA expression to rescue the *mtATP6[1]* mitochondrial mutation *in vivo*.

824B

α-synuclein Mediates Dopamine Homeostasis and Oxidative Stress Susceptibility in *Drosophila*. Hakeem Lawal, Faiza Ferdousy, Glen Douglas, Zhe Wang, Janis O'Donnell. Dept Biol, Univ Alabama, Tuscaloosa, AL.

Parkinson's Disease is the second most common neurodegenerative disorders having both a sporadic and a hereditary form caused by genetic and environmental factors. α-synuclein is the major component of Lewy bodies in Parkinson's and Lewy Body Diseases. In Parkinson's Disease, selective degeneration of dopaminergic neurons in the midbrain is the major neuropathological hallmark. While the mechanism for this is unclear, recent reports indicate that mutations in human α-synuclein have been linked to Parkinson's Disease and α-synuclein has potential dopamine regulating functions in mammalian models. Exposure to several environmental toxins such as pesticides and herbicides, acting through the generation of oxidative stress, have been shown to be risk factors in PD. We have established a PD model by using herbicide paraquat which generates oxidative stress, neurological/behavioral symptoms and induces neurodegeneration in *Drosophila*. Here we explore a link between exogenous α-synuclein expression, oxidative stress and consequences for dopamine homeostasis. We report that induction of oxidative stress correlates with a perturbation of dopamine levels and that exogenous α-synuclein expression alters dopamine biosynthesis, reflecting similar regulatory interactions in mammalian neurons. Further, we present results of a genetic analysis of α-synuclein and dopamine regulatory components. Our findings confirm, in an in vivo, organismal model, previous reports implicating α-synuclein in both the oxidative stress response and in dopamine homeostasis.

825C

Behavioral analysis on a *Drosophila melanogaster* Alzheimer's disease model. Matt B. Mahoney[1], Devin Keefe[1], Winnie Lee[1], Emily Lund[1], Jimmy Symonds[1], Akshay Yeshokumar[2], Lori A. Hrdlicka[1], François Huet[1], Phil O'Neil[1], Joost Schulte[3], Hsin-Pei Shih[1], Eric Sigel[1], Carol M. Singh[1], Christopher J. Cummings[1], James K.T. Wang[1], Michael Ahlijanian[1], Gerhard Köenig[1]. 1) Discovery, EnVivo Pharmaceuticals, Inc., Watertown, MA; 2) William E. Simon Graduate School of Business Administration, University of Rochester, Rochester, NY; 3) Picower Institute for Learning and Memory, MIT, Cambridge, MA.

Neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases result in progressive loss of cognition and motor control, leading to death. No available treatments slow the progression of such diseases. EnVivo Pharmaceuticals is committed to discovering and developing treatments for neurodegenerative diseases. We have developed a medium-throughput behavioral screening platform utilizing *Drosophila* as a model system for drug discovery. Our platform identifies compounds that rescue deficits in the climbing behavior of flies over-expressing human disease genes, such as *Htt 128Q*, the mutation in the *huntingtin* gene that results in Huntington's disease. This platform has generated multiple discovery programs, currently at various stages of pre-clinical development for Huntington's disease. Based on the success with the Huntington fly, we have expanded our screening capabilities to include testing chemical entities in *Drosophila* expressing various combinations of human Alzheimer's disease genes (tau and APP derivatives). These lines display a variety of phenotypes, including locomotor and behavioral deficits and developmental aberrations. They also display hallmarks of human disease, such as amyloid-beta production and protein aggregation. It is anticipated that screening compounds in these Alzheimer's disease models will be an equally productive strategy in accelerating drug discovery as we have demonstrated in our effort in Huntington's disease.

826A

Improving scFv Intrabody suppression of Huntington's Disease Pathology in a fly model. Julie Mclear[1], Danielle Lebrecht[1], Allison Dumas[1], Anne Messer[1,2], William J. Wolfgang[1,2]. 1) Wadsworth Center, Albany, NY; 2) Dept. of Biomedical Sciences University at Albany Albany NY.

Intracellular expression of an engineered antibody fragment (intrabody) selected against exon 1 of huntingtin (htt), anti-htt-scFv-C4 (C4), has been shown to ameliorate cellular pathology in tissue culture and organotypic brain slice-models of Huntington's Disease (HD). We have extended these results to the intact functioning nervous system of Drosophila. In flies, neuronal expression of human Htt- exon 1 with a 93 polyQ repeat causes neurodegeneration and premature death. Co-expression of the C4 intrabody reduces pathology in the nervous system of HD flies. The proportion of HD flies surviving through metamorphosis to adulthood increases from 23% to 100% in the presence of C4, while a control scFv shows no benefit. C4 also prolonged the lifespan of adult HD flies, and slowed neurodegeneration and aggregate formation. These results demonstrate for the first time the therapeutic value of intrabodies for correction of a neurodegenerative disorder in vivo. Because rescue of disease pathology was only partial, we are undertaking three independent strategies to improve therapeutic outcome. 1) Characterization of second site mutations that improve longevity, to identify additional targets for intervention. Using the deficiency kit has identified two deficiencies that dramatically improve HD fly survival. 2) Combining intrabody and the previously validated therapeutic drugs, Congo Red, LiCl, or Vitamin B3, led to a dose dependent additive increase in neuronal survival but no increase in adult longevity. 3) For the chaperone HSP70, increased or decreased levels improved or reduced HD fly survival but showed no additive effects with C4 intrabody. Support: NSF to AD, HDSA and NIH to AM, and NIH to WJW.

827B

Over and underexpression of nejire in a Drosophila model of Machado-Joseph Disease. Ravi J. Nagraj, Brendan J. Thelen, John M. Warrick. Department of Biology, University of Richmond, Richmond, VA.

Spinocerebellar Ataxia 3, or Machado-Joseph Disease (MJD) is an inherited human neurodegenerative disease characterized by an expanded repeat of glutamine residues in the Ataxin 3 protein. It is a member of the polyglutamine family of diseases, of which Huntington's Disease is also a member. We are using transgenic flies expressing both full-length and truncated mutant and normal protein as a model for MJD. Currently we are using this model to study the role of modifiers in disease progression. One such modifier is CREB binding protein (CBP), which acts as a transcription activator and has histone acetyltransferase activity. With our model, we have seen that CBP is colocalized with mutant MJD into insoluble aggregates in cell nuclei. In order to see the effects of lowered CBP on disease expression, we crossed our full-length MJD expressing flies to hypomorphic nejire mutants, nej^3 and nej^p. Nejire is the Drosophila homologue of human CBP. We also have overexpressed Drosophila CBP (dCBP) with the EP950 and EP1179 lines. Overexpression of CBP causes severe degenerative developmental effects. To limit these, we used a Rhodopsin-1 Gal4 driver (Rh1-Gal4). The Rhodopsin system drives expression late in pupal development so that developmental problems are relieved. By lowering and raising the expression of dCBP, we will show how levels of CBP affect MJD pathogenesis.

828C

Atlastin is a novel ER protein involved in Golgi-ER transport. Genny Orso[1,2], Jessica Tosetto[2,3], Diana Pendin[2], Andrea Daga[2,4]. 1) Dept of Genetics, Trinity College, Dublin, Ireland; 2) E. Medea Scientific Institute, Conegliano,Italy; 3) Dept of Pharmacology, University of Padova, Italy; 4) Dulbecco Telethon Institute, Italy.

The hereditary spastic paraplegias encompass a diverse spectrum of disorders which are characterized by progressive spastic weakness of the lower extremities. The SPG3A gene, encoding the novel protein atlastin, has been identified as the locus responsible for a form of paraplegia characterized by the earliest onset. Atlastin protein sequence shows homology to large GTPases of the dynamin superfamily. The function of atlastin is unknown, and consequently the pathological mechanism underlying disease remains unknown. We have identified and cloned the fly homologue (D-atlastin) of human Atlastin and raised an antibody against the fly protein. We carried out a detailed analysis of the embryonic and larval expression patterns as well as the subcellular localization of D-atlastin. D-atlastin is ubiquitously expressed during all stages of Drosophila development. At the subcellular level D-atlastin is highly enriched in and co-localizes with Endoplasmic Reticulum (ER) markers. A small amount of signal is also detected in the Golgi apparatus suggesting an involvement of this protein in vesicular trafficking between ER and Golgi. To clarify the biological role of D-atlastin we generated transgenic flies for its overexpression and knockdown in vivo. Ubiquitous overexpression of D-atlastin causes death around stage 13 of embryonic development. Analysis of tissues overexpressing D-atlastin show morphological alterations of the Golgi which disassembles and colocalizes with markers for endoplasmic reticulum. In contrast to overexpression, loss of D-atlastin allows survival of few escapers with most individuals dieing at pupal stage. Eclosed adults have a short lifespan and an obvious reduction of size. Our results show that Atlastin is important for vesicular transport between ER and Golgi and necessary for proper development.

829A

Characterization of genetic modifiers of *spastin* in *Drosophila* models of Autosomal Dominant- Hereditary Spastic Paraplegia. Emily F. Ozdowski, Sophia F. Gayle, Nina T. Sherwood. IGSP, Duke University, Durham, NC.

Drosophila *spastin* (*spas*) is the orthologue of the human SPG4 gene, in which mutations result in Autosomal Dominant- Hereditary Spastic Paraplegia (AD-HSP). AD-HSP is a neurodegenerative disease characterized by loss of lower limb sensation and reduced or abolished mobility. Null mutations in *spas* cause similar behavioral phenotypes in adult *Drosophila*, such as disrupted walking and flying, as well as changes in synaptic strength and bouton number in the larval NMJ. Molecularly, *spas* is an AAA ATPase that colocalizes with microtubules and demonstrates ATP-dependent microtubule cleaving activity in *in vitro* experiments. We are thus employing both reverse and forward genetic approaches to identify modifiers of *spas* function and further understand its cell biological role in the nervous system and other tissues. Nearly all known missense mutations in SPG4 are found within the critical C-terminal ATPase domain. However, one common change associated with AD-HSP, S44L, is a putative N-terminal intragenic modifier that, when in trans with mutations affecting the catalytic domain, correlates with an acceleration of disease onset. While the mechanism for such exacerbation is unknown, *in silico* analysis suggests that S44 is a site for phosphorylation, and may be a target of Cdk activity. We have generated UAS-*spas*^{S44L} and UAS-*spas*^{K388R} (catalytic domain mutation) fly lines to engineer genetic ratios similar to the human disease state. We will test the role of S44L as a modifier of *spas* function in the fly, and whether it behaves as a Cdk phosphorylation site. To identify additional genetic partners of *spas*, we screened genomic deletions for dominant enhancers and suppressors of a *spas* overexpression eye phenotype. Three areas of interaction were identified on chromosome III: one rescues the effects of *spas* when deleted, while two others cause more severe defects. The three regions are currently being narrowed to the genes responsible using overlapping deletions and P insertions. Overall, identifying modifiers of *spas* is addressed more easily with *Drosophila* genetics.

830B

Drosophila model of human inherited TPI deficiency glycolytic enzymopathy. Michael Palladino[1,2], Adam Frank[1,2], Jacquelyn Seigle[1,2], Alicia Celotto[1,2]. 1) Department of Pharmacology University of Pittsburgh School of Medicine Pittsburgh, PA 15261; 2) Pittsburgh Institute for Neurodegenerative Diseases University of Pittsburgh School of Medicine Pittsburgh, PA 15260.

Heritable mutations, known as inborn errors of metabolism, cause numerous devastating human diseases, typically as a result of a deficiency in essential metabolic products or the accumulation of toxic intermediates. We have isolated a missense mutation in the Drosophila *Tpi* gene that causes phenotypes analogous to symptoms of TPI (triosephosphate isomerase) deficiency. TPI deficiency is a familial human disease characterized by anaerobic metabolic dysfunction resulting from pathological missense mutations affecting the encoded TPI protein. In *Drosophila*, *sugarkill (Tpi[sgk1])* mutants revealed severely reduced longevity, progressive locomotor deficiency and neural degeneration. Biochemical studies demonstrate that mutation of this glycolytic enzyme gene does not result in a bioenergetic deficit, suggesting an alternate cause of enzymopathy associated with TPI impairment. The *Tpi[sgk1]* mutation affects an amino acid known to exist at the dimer interface, however, size exclusion chromatography demonstrates the mutant and wildtype proteins both exist as homodimers under native conditions. Analysis of protein stability revealed a striking temperature-dependent reduction in protein stability that appears to underlie disease pathogenesis.

831C

Na+/K+ ATPase Alpha Isoform Specificity and Neuropathogenesis. Michael Palladino[1,2], Rosie Miller[1,2]. 1) Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Mutations resulting in haploinsufficiency of the human sodium pump genes *ATP1A2* and *ATP1A3* result in familial hemiplegic migraine (FHM) and rapid-onset dystonia parkinsonism (RDP), respectively. The Na+/K+ ATPase has essential cell junction functions and is integral in maintaining the cellular resting membrane potential, normal neural neurotransmission, and drives many important secondary processes in neuronal and non-neural tissues. Dominant and loss-of-function mutations in *Drosophila ATPalpha*, the only known gene that encodes the alpha subunit of the Na+/K+ ATPase, result in shortened lifespan, locomotor impairment, and neurodegeneration. *ATPalpha* is alternatively spliced, with at least 12 different wildtype protein isoforms. Transgenic expression of eight major wildtype ATPalpha isoforms was used to determine the origin of the mutant phenotypes and to elucidate ATPalpha isoform specificity *in vivo*. It has been suggested that sodium pump impairment may secondarily modulate the Na+/Ca++ exchanger, calX, which may underlie pathogenesis associated with reduced sodium pump function. Overexpression of *calX* has previously been shown to rescue retinal degeneration associated with TRP channel mutations in *Drosophila*. We examined the affect of modulating *calX* expression with HS-CalX and a UAS-CalX transgenes on neuropathogenesis in *ATPalpha* mutants.

832A

New continuous cell culture from RQ2 transgenic *Drosophila* as *in vitro* model of pathogenesis of human neurodegenerative diseases. Dmitriy Panteleev[1], Abraham Grossman[4], Konstantin Pyatkov[3], Natalia Schostak[2], Elena Zelentsova[2], Boris Andrianov[1], Michael Evgen'ev[2]. 1) Mol Genet of Eukaryotes, Vavilov Institute of Gen Genet, Moscow, RU; 2) Engelhardt Inst Mol Biol, Moscow, RU; 3) California Inst Technol, Pasadena, CA 91125, USA; 4) Q-RNA inc., 3960 Broadway, New York, NY 10032, USA.

Protein misfolding and aggregation into insoluble fibrils and plaques are the main pathology signs of human neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington prion protein associated diseases. These abnormal protein assemblies may be the result of impaired chaperone system of protein folding or the presence of pathogenic chaperons. Nucleic acids are able to have chaperon-like activities (Biro 2005). As it was shown before inducible expression of specific highly structured RNA triggers neurodegeneration accompanied by decline in learning and memory retention of adult flies with formation of intracellular congophilic aggregates in brain of transgenic flies after heat-shock treatment (Savvateeva-Popova 2007). For detailed investigation of molecular mechanisms of protein misfolding the new continuous cell culture was established. For this purpose we used traditional protocols (Kakpakov 1969, Ecshalier 1970) with some modifications which allowed us to achieve fast cell division in two month after primary culture initialization as well as to obtain continuous cell cultures using only few primary ones (less than ten). After the first passage the culture was subcloned into three lines which differ by cell morphology, rates of cell division and adhesive properties. One subclone named RQ2(b) after 30 passages reached stable growth parameters with approximate doubling time 48 hours. PCR showed positive transgene signal. The level of transgene expression is being investigated.

833B

Developmental functions of two acyl-CoA synthetases, Bubblegum and Double Bubble, in Drosophila. Anna Sivatchenko, Anthea Letsou. Human Genetics, University of Utah, Salt Lake City, UT.

The *Drosophila melanogaster* homologous genes *bubblegum (bgm)* and *double bubble (dbb)* code for very-long-chain fatty-acid (VLCFA) acyl-CoA synthetases that are required for degradation of VLCFAs. We identified *dbb* gene as a ventrally restricted transcript in our high-throughput patterned expression screen (Simin et al, 2002). Sequence similarity of the *bgm* and *dbb* genes and their close proximity in the genome led us to the hypothesis that they have overlapping functions in embryogenesis. We have shown that *dbb* and *bgm* exhibit overlapping expression profiles and are co-regulated by the Dorsal pathway that is essential for specification of ventral cell fates during dorsoventral patterning in Drosophila embryos. Amorphic mutations in either *bgm* or *dbb* are homozygous viable. In agreement with our hypothesis that *bgm* and *dbb* play redundant roles during embryonic stages of development, we observed RNAi-induced embryonic lethality in double mutant embryos but not in *bgm* or *dbb* single mutants. We have identified a short, highly conserved sequence in the chromosomal region between the *bgm* and *dbb* coding regions. In agreement with our hypothesis that this conserved sequence comprises an essential regulatory element for the *bgm* and/or *dbb* genes, we observe down-regulation of both transcripts in mutants homozygous for the P-element insertion that maps immediately upstream of this sequence and which is associated with lethality in postembryonic stages of Drosophila development. It has been previously shown that mutations in *bgm* lead to neurodegeneration and accumulation of VLCFAs, suggesting a link to the human disease X-adrenoleukodystrophy (Min, K. T. & Benzer, S. 1999), however, bgm mutant flies recapitulate only some aspects of X-ALD. The redundant function of *bgm* and *dbb* allowed us to speculate that a better X-ALD disease model can be provided by *bgm dbb* double mutant flies. We are currently generating knock-out animals to define the functional relationship between the bgm and dbb genes and to elucidate their roles in *Drosophila* embryogenesis and nervous system development.

834C

A protective role for *PTEN induced putative kinase 1 (PINK1)* in a Drosophila model of Parkinson's disease. Amy M. Todd, Brian E. Staveley. Dept Biology, Memorial University of Newfoundland, St. John's, NL, CA.

Parkinson's disease (PD) is a common progressive neurodegenerative disorder characterized by a loss in the dopamine producing-neurons of the brain. Recently, it has been found that mutations in *PTEN induced putative kinase 1 (PINK1)* cause a form of autosomal recessive Parkinson's disease. PINK1 is thought to protect against mitochondrial dysfunction by operating upstream of the protective parkin E3-ubiquitin ligase. Previous work in our laboratory has shown *parkin's* ability to rescue an *α-SYN* induced PD-like phenotype in Drosophila, presumably through targeting the α-SYN protein for degradation.

Using Drosophila *PINK1*, we have performed longevity, mobility and histological studies to investigate the effects of overexpression of *PINK1* and its ability to rescue the *α-SYN* phenotype. Overexpression of *PINK1* in the DA neurons results in normal longevity and locomotion. However, overexpression of *PINK1* along with *α-SYN* in the DA neurons results in the rescue of the *α-SYN* dependent PD-like phenotype. These findings suggest that PINK1 is able to protect against degeneration, presumably via activation of parkin resulting in the tagging of α-SYN and its subsequent degradation. In addition, overexpression of *PINK1* in the eye with *α-SYN* results in a phenotype with visible overgrowth in the ommatidia. This suggests that PINK1 may have a pro-survival / pro-growth role in the cell with the ability to shift the cell fate away from cell death during eye development. As overgrowth was not observed when *PINK1* is expressed without *α-SYN*, this may suggest an aspect of the subtle control involved in neuronal survival. These findings demonstrate the importance of *PINK1* in the protection against apoptosis and in the pathogenesis of Parkinson's disease. *Support contributed by: Parkinson's Society of Canada grant and NSERC Discovery grant to B.E. Staveley.*.

835A

Ataxin-1, a spinocerebellar ataxia type 1 disorder protein, causes cytotoxicity by perturbing nuclear receptor signaling. Xin Tong, Chih-Cheng Tsai. UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Emerging evidence indicates that several polyglutamine disease proteins are functionally connected with gene transcriptional regulation, and that nuclear localization is necessary for these proteins to manifest neurotoxicity. Thus far, however, the specific transcriptional regulatory pathways that are affected by these polyglutamine disease proteins have not been elucidated. For ataxin-1 (ATXN1), whose mutant form causes spinocerebellar ataxia type 1 (SCA1), we recently established that ATXN1 participates in transcriptional repression through its interactions with a class of conserved histone deacetyalse-associating transcriptional co-repressors, including SMRT (Silencing Mediator of Retinoid and Thyroid hormone receptors) in vertebrates and SMRTER (SMRT-related ecdysone receptor interacting factor) in fly. Because SMRT and SMRTER are nuclear receptor corepressors, we probed whether ATXN1-mediated cytotoxicity involves nuclear receptors. Our results reveal that the ecdysone signaling pathway in fly and, in parallel, the thyroid hormone signaling pathway in human cells are both perturbed by ATXN1. Connecting polyglutamine diseases such as SCA1 to aberrant nuclear receptor signaling has major implications, because it raises the possibility that intervention with steroid or thyroid hormones can be exploited to provide an effective therapy for treating SCA1.

836B

A role for the Batten disease gene Cln3 in endosomal trafficking at the synapse. Richard Tuxworth, Guy Tear. MRC Centre for Developmental Neurobiology, Kings College London, UK.

The Neuronal Ceroid Lipofuscinoses (Batten disease) are the most common inherited neurodegenerative disorder of childhood. They are characterised by early onset epilepsy, retinal degeneration and a progressive neurodegeneration that is ultimately fatal. At the cellular level, NCLs are characterised by an accumulation of autofluorescent lipoprotein deposits in the lysosomes although the neurobiology of disease is not well understood. The most common form of Batten disease, Juvenile NCL, is caused by mutations in Cln3, encoding an endosomal-lysosomal transmembrane protein. Cln3 is ubiquitously expressed but only neural tissues suffer degeneration in patients. The normal cellular functions of Cln3 are unclear and need to be elucidated in order to understand the disease process and to suggest potential avenues for intervention. With this aim, we have been studying the Drosophila homologue of Cln3. We demonstrate that, like its mammalian counterpart, Drosophila Cln3 is resident in vesicular compartments at the synapse where we suggest it may play a role in endosomal trafficking. Overexpression of Cln3 in motor neurons leads to locomotory defects in larvae, whilst overexpression in imaginal discs leads to patterning defects in the eye and wing that are indicative of a role in endocytosis. Furthermore, these phenotypes can be modified by genes encoding known regulators of endosomal processing at the synapse. An unbiased modifier screen of the overexpression phenotype is currently at an advanced stage and we will present the results. We have identified nine point mutations in the Cln3 gene that leads to coding changes. Several of these are predicted to lie within transmembrane domains and map close to known human disease-causing mutations. We are currently analysing these mutations for defects in synapse development and function, for signs of adult neurodegeneration, accumulation of autofluorescent deposits and for affects on lifespan.

837C

SWISS CHEESE/NTE, A Gene Involved In Neural Integrity and Organophosphate Toxicity. Jill S. Wentzell[1], Alexandre Bettencourt da Cruz[1], Max Mühlig-Versen[1], Paul Glynn[2], Doris Kretzschmar[1]. 1) Center for Research on Occupational and Environmental Toxicology, Oregon Health & Sciences University, Portland, OR; 2) MRC Toxicology Unit, University of Leicester, Hodgkin Building, Leicester LE1 9HN, United Kingdom.

The Drosophila swiss cheese (sws) mutant is characterized by progressive degeneration of the nervous system. Cell-specific expression reveals that neurons and glia depend autonomously on SWS expression. The human ortholog of SWS is Neuropathy Target Esterase (NTE), the molecular target in organophosphate-induced delayed neuropathy that is caused by many pesticides and chemical warfare agents. The mechanism of interaction between organophosphates (OPs) and SWS/NTE remains unknown. We are now using the Drosophila system in vivo and in primary cell culture to investigate these mechanisms. In addition to SWS/NTE involvement in OP induced toxicity, we are investigating the normal physiological function of SWS, as this function is currently not understood. It is unknown which domains of SWS are essential for its physiological function. Expression of constructs with mutations in various protein domains has helped to elucidate this question. A construct deleting the N-terminally located transmembrane domain showed severe degeneration without any in vitro catalytic activity. This construct suggests that SWS has a function independent of the C-terminal catalytic domain. Normal function has also been investigated by yeast-two-hybrid experiments that suggest SWS interacts specifically with the PKA catalytic subunit C3. Genetic interactions with deficiencies and PKA overexpression support this finding. We are currently investigating this interaction more thoroughly.

838A

Deciphering the role of protein aggregation in polyglutamine pathogenesis in Drosophila. Alan S.L. Wong[1,2], Edwin H.Y. Chan[1,2,3]. 1) Laboratory of Drosophila Research; 2) Molecular Biotechnology Programme; 3) Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, China.

Polyglutamine (polyQ) diseases belong to a group of progressive neurodegenerative disorders, caused by glutamine-coding CAG codon expansion in the affected genes. The translated gene products carry an expanded polyQ tract which highly favors protein aggregation as well as triggers degeneration of affected neurons. PolyQ protein aggregates were reported to exhibit roles in both neuronal toxicity and protection. The founding role of protein aggregation in polyQ degeneration remains unclear to date. Machado-Joseph disease (MJD) is one of the most studied polyQ diseases. Here, we establish a temperature-sensitive, inducible Drosophila model transgenic for polyQ-expanded MJD gene fragment. Expression of mutant MJD protein fragment led to progressive accumulation of sodium-dodecyl sulfate (SDS)-insoluble aggregates followed by apoptotic degeneration of retinal neurons. We will examine the association between polyQ protein aggregates and polyQ-induced degeneration.

839B

Differential regulation of immune related genes in *Drosophila melanogaster* Schneider 2 sells transfected with human *OTK18*. Kimberly Carlson, Sarah Marshall, Cole Spresser. Biology, University of NE at Kearney, Kearney, NE.

OTK18 is a novel human transcriptional suppressor with 13 zinc finger motifs and both Krüppel-associated boxes A and B. This transcriptional regulator is suspected to play multiple roles within human development and the innate immune response. As has been done for many human genes, further characterization of *OTK18* could be done in a simpler model system. Therefore, the main thrust of the proposed research was to utilize the *Drosophila melanogaster* embryonic Schneider 2 (S2) cell line as model system to characterize *OTK18* gene interactions. cDNA microarray analysis was performed on total RNA extracted from S2 cells that had been transfected with *pEGFP-OTK18* (plasmid with an enhanced green fluorescent protein promoter) for 6, 12, and 24 hours. The cDNA microarray analysis revealed differential expression of genes known to be important in regulation of *Drosophila* immunity and apoptosis. The levels of two of these genes (*Metchnikowin* and *CG16708*) were verified by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). *Metchnikowin* was down-regulated, and *CG16708* up-regulated, which corresponds with gene expression profiles seen in human monocytic cells in which *OTK18* is over-expressed. In the future, we hope to define a mechanism for *OTK18* gene regulation of immunity and developmental processes.

840C

Insulators flank the latency-associated transcript (LAT) promoter in HSV-1. Qi Chen, Lan Lin, Sheryl Smith, Jing Huang, Shelley Berger, Jumin Zhou. Wistar Institute, Philadelphia, PA.

HSV-1 infection involves both latent and lytic phases of infection. In the latent phase of infection, the LAT region encodes for multiple transcripts, including the 8.3-kb primary transcript and two stable introns of 2.0 kb and 1.5 kb. 2.0 kb LAT intron is the only abundant transcript found during the latent phase. Recent studies demonstrated that histones in the LAT promoter and intron regions contain histone modifications permissive for transcription, whereas those associated with the lytic-specific immediate-early (IE) gene ICP0, only about 5 kb away, contains histone modification typical of silenced chromatin. How this active chromatin is kept separate from the repressed chromatin in the nearby ICP0 region remains crucial to the understanding of HSV lytic cycle. In this study, we show that 2.0 kb LAT intron contains insulator element. An 800 bp region from the LAT intron can block enhancer-promoter interactions in both Human K562 cells and Drosophila embryos. The LAT insulator also protects the transgene from positional effects in the Drosophila eye tissue. EMSA revealed that nine copies of 16 bp repeats located in the center of the 800bp insulator interact with both human and Drosophila CTCF. In vivo CHIP assay demonstrated that mouse CTCF interacts with these repeats in latent infected mouse trigeminal ganglion cells. The deletion of these repeats impaired insulator activity in Human K562 cells and Drosophila embyos. Additional, preliminary tissue culture study also identified another enhancer blocking activity from a 1.4 kb region upstream of the LAT promoter. These results strongly suggest that insulators flank the LAT promoter, separating active chromatin from repressed chromatin during latency. This study demonstrates that Drosophila presents as an elegant system to analyze insulator elements from diverse organisms.

841A

Anthrax EF and LF toxins affect several signaling pathways in Drosophila. Annabel Guichard, Beatriz Cruz-Moreno, Abby Cooper, Ethan Bier. Dept Biol, Univ California, San Diego, La Jolla, CA.

Anthrax is a severe and widely distributed disease that primarily affects cattle. Occasionally, anthrax affects humans when contact occurs with sick animals, or, as in the case of the 2001 attack, when weaponized spores are inhaled. *Bacillus anthracis* achieves infectivity mainly through the secretion of three toxins, PA (Protective antigen), EF (Edema Factor), and LF (Lethal Factor). After binding to surface receptors present on most mammalian cells, PA gets endocytosed and promotes the entry of EF and and LF into the cytoplasm. EF is a potent Calmodulin-dependent Adenylate cyclase, and LF is Zinc metalloprotease that cleaves most human Mitogen Activated Protein Kinase Kinases (MAPKK), and possibly unknown targets. We have shown previously that LF and EF expressed in transgenic flies can induce developmental phenotypes reflective of their known activities in mammals, suggesting that the effects of these toxins on host organisms can be examined in model invertebrate systems such as *Drosophila*. For example, we found that LF inhibits dorsal closure during embryogenesis, and most likely acts at the level of Hemipterous, the Drosophila MAPKK acting in the JNK pathway involved in this process. EF also induced expected effects such as a *hedgehog*-like phenotype in the wing, consistent with the known role of cAMP-dependent PKA in inhibiting hh signaling. Here we show that LF and EF have additional effects that are likely to be mediated by components that were not known to be targeted by these anthrax toxins. Moreover, we find tat LF and EF can act in a synergistic fashion in certain settings. We also present genetic and biochemistry approaches aimed to identify novel targets of the LF protease.

842B

Modeling hypercapnia in *Drosophila*: physiological and molecular effects of CO_2. Iiro Helenius[1,2,5], Thomas Krupinski[1,5], Douglas Turnbull[3], Neal Silverman[4], Eric Johnson[3], Jacob Sznajder[2], Greg Beitel[1]. 1) BMBCB Department, Northwestern University, Evanston, IL; 2) Division of Pulmonary and Critical Care Medicine, Northwestern University, Chicago, IL; 3) Institute of Molecular Biology, University of Oregon, Eugene, OR; 4) Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA; 5) Co-first authors.

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the US. The decreased gas exchange in COPD often results in elevated CO_2 levels, a condition termed hypercapnia. Neither the role of elevated CO_2 in disease manifestation nor the mechanisms by which non-neuronal cells sense and respond to CO_2 are known. We are studying the effects of elevated CO_2 on *Drosophila* to identify hypercapnia response pathways. Our results reveal that high but non-anesthetic CO_2 levels reduce fecundity (up to ~90%), egg hatching (up to ~95%) and movement. These phenotypes should enable us to screen for genetic backgrounds or pharmacological agents which reduce potentially harmful effects of hypercapnia. Microarray and RT-PCR analysis of S2 cells identified multiple gene families that are up- or downregulated in response to high pCO_2, independently of extracellular pH. In contrast to hypoxia and reactive oxygen stress, numerous immunological defense and heat response genes are downregulated during hypercapnia. Accordingly, induction of the antimicrobial peptide Diptericin in S2 cells challenged with *E. coli* peptidoglycan was also markedly compromised. These data may help explain the susceptibility of COPD patients to infections and are consistent with reports that hypercapnia impairs cytokine production in human macrophages. Our findings suggest *Drosophila* will be suitable for modeling hypercapnic injury and the strong responses of S2 cells to CO_2 should allow high throughput RNAi screening to define cellular pathways mediating CO_2 responses.

843C

Altered respiration in the *Drosophila* Bang-sensitive paralytic mutant *easily-shocked*. Daniel Kuebler, Brian Burke. Department of Biology, Franciscan University of Steubenville, Steubenville, OH.

The Bang-sensitive (BS) paralytic mutants are susceptible to seizures following a variety of insults. The seizure activity that occurs in the BS mutants is characterized by violent uncoordinated contractions of the legs, wings and abdomen that cause the flies to spin violently. Despite the fact that they all share this seizure susceptibility phenotype, there is no common physiological defect that is shared by all members of the BS mutant family. There are, however, a number of BS mutants that have defects in genes involved in mitochondrial metabolism. In fact, previous work has found that some of these mutants have significantly lower ATP levels than normal flies. Given the link between metabolism and human seizure disorders, we further examined this connection by measuring respiration rates in wild type and BS mutant flies. One BS mutant, *easily shocked* (*eas*), exhibited an elevated metabolic rate as measured by carbon dioxide production over time. The eas flies produced 4593 ± 198 ppm of CO_2 per gram of flies over a 6.5 hour period while CS flies produced 3250 ± 137 ppm. To rule out the possibility that the elevated metabolic rate in the *eas* flies was simply due to an increased level of activity, we monitored activity levels by tracking the path length traversed by individual *eas* and CS flies in a small confined arena. Under these conditions, we found that individual *eas* flies did not display increased motor activity when compared to CS flies. Taken together, these results suggest that the *eas* flies may be metabolically less efficient than wild type flies, a phenotype which may contribute to their seizure susceptibility.

844A

Mapping the stress response in the *Drosophila* brain. Wendi Neckameyer. Dept Pharmac & Physiol Sci, St Louis Univ Medical Ctr, St Louis, MO.

Stress elicits both neurochemical and behavioral changes in neurotransmitter signaling pathways, which have been implicated in the etiology of mental illness, drug abuse, and neurodegenerative disorders. To understand the role of stress in brain function and malfunction, it is critical to identify the neural circuitry involved. Our studies demonstrate that different circuitry is activated in response to stress, dependent upon gender, sexual maturity, reproductive status and type of stress. We have now identified brain regions critical in modulating the stress response in *Drosophila*, using viable strains with defined abnormalities in specific anatomical structures. These mutations are in a wild-type background to permit direct comparisons between populations. Animals from different populations (sexually immature versus sexually mature; male versus female; virgin versus mated females) were assayed for behavioral changes in response to starvation and oxidative stress. Deviations from the normal stress response in different mutant lines identified regions comprising the stress circuitry in specific populations. Given the high degree of conservation of neurotransmitter signaling pathways, elucidation of the stress response in *Drosophila* should offer substantial insight into the role of stress in both adaptive fitness and brain function.

845B

Obesity and regulation of energy homeostasis in *Drosophila melanogaster*. Tania Reis, Iswar Hariharan. MCB, UC Berkeley, Berkeley, CA.

We are increasingly aware that obesity is not simply an effect of excessive food intake. The complexity of this disorder is illustrated by the lack of effective targets for treatment, and the scarcity of known regulators coordinating energy storage and usage. Maintaining energy balance requires coordination of the nervous system (sensing satiety and regulating feeding behavior) and energy storage tissues (storing energy as fat/sugar or mobilizing it). We are studying the effects of modified diets on the types and levels of energy storage, and using genetic screens to identify pathways regulating energy homeostasis, including which organs sense and process food sources and physiological changes in energy levels. With this work we aim to define a regulatory network for energy homeostasis.

846C

Functional analysis of EFHC1, a gene involved in Juvenile Myoclonic Epilepsy, in Drosophila. Maria Giovanna Rossetto[1,2,3], Genny Orso[2,4], Erica Zanarella[2,3], Stefano Casonato[3], Andrea Daga[2,3]. 1) Pharmacology, University of Padova, Padova, IT; 2) E. Medea Scientific Institute, Conegliano, Italy; 3) Dulbecco Telethon Institute, Italy; 4) Department of Genetics, Trinity College, Dublin, Ireland.

Juvenile Myoclonic Epilepsy (JME), the most common cause of grand mal seizures, accounts for 3-12% of all epilepsies. We have identified two Drosophila homologs (CG8959 and CG11048) of myoclonin/EFHC1, a gene recently linked to JME in humans. We are now using Drosophila as a model to study the functions of this gene in normal development and in pathology to define the mechanisms whereby mutation of myoclonin causes human disease. We have generated a paradigm for overexpression of both fly EFHC1 homologs and determined that neuronal expression of both transgenes causes locomotory deficit in adult flies and at the subcellular level produces morphological defects in the neuromuscular junction. We have generated antibodies against both proteins and immuhistochemical analysis of third instar larvae has demonstrated that both CG8959 and CG11048 are endogenously present at neuromuscular junctions. However, while expression of CG11048 is found at synaptic terminals innervating all larval muscles, expression of CG8959 is restricted to terminals that innervate exclusively muscle 12. This result suggests that EFHC1 proteins are synaptic and thus likely to exert a function in synaptic structure and/or function.

847A

Drosophila melanogaster genetic based resistance and susceptibility to Bacillus cereus. Tiffany E. Schwasinger, Wanda Layman, Lawrence Harshman. Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

Laboratory selection was conducted on *D. melanogaster* for resistance and susceptibility to infection by *B. cereus*, a gram-positive bacterium. Replicate lines consistently exhibited a direct response to selection; in every pair of selected and control lines divergent selection for resistance or susceptibility to *B. cereus* was observed. Indirect responses to selection were investigated including fecundity and age specific survival. There was no consistent effect of selection on fecundity. The overall trend in the data indicated that lifespan was inversely correlated with immune response.

848B

A *Drosophila* model of Spinal Muscular Atrophy reveals a function for SMN in striated muscle. Rajendra Thimmappaiaha, Michael Walker, Graydon Gonsalvez, Karl Shpargel, A. Gregory Matera. Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

Mutations in human *SMN1* cause Spinal Muscular Atrophy (SMA). The etiology of SMA is unclear, although patient-derived SMN mutations display defects in assembly of small nuclear ribonucleoproteins (snRNPs) in vitro. We have developed a *Drosophila* system to model SMA in vivo. Larval-lethal null mutations in fruitfly *Smn* show no detectable reduction in spliceosomal snRNP levels, making it unlikely that these animals die from snRNP deprivation. In contrast, hypomorphic *Smn* mutations result in reduced levels of dSMN protein in the adult thorax, causing flightlessness and acute muscular atrophy. Mutant flight muscle motoneurons display pronounced axon routing and arborization defects. Act88F, the flight muscle-specific actin isoform, was not expressed in the mutants, a finding that is consistent with a loss of motoneuron innervation. In wildtype muscles, dSMN protein colocalized with sarcomeric actin and forms a complex with α-actinin, the thin filament crosslinker. Analysis of mutant muscles showed that while thin filament formation was compromised, thick filament formation was relatively unperturbed. Thus the *Smn* hypomorphs phenocopy null mutations in *Act88F*. Importantly, the sarcomeric localization of Smn protein was conserved in mouse hindlimb muscles. Collectively, these observations strongly suggest a muscle-specific function for human SMN and underscore the potential importance of muscle cell dysfunction in modulating the severity of SMA.

849C

Life span extension by diet restriction and insulin/IGF signaling: Observations of mutual dependence and independence. Kyung-Jin Min, Rochele Yamamoto, Marc Tatar. Department Ecology and Evolutionary Biology, Brown University, Providence, RI.

Diet restriction (DR) and insulin/IGF signaling (IIS) are major interventions to extend life span in yeast, worm, flies, rodents and possibly in primates. How DR modulates life span in any species is unclear, although it is widely speculated that reduced IIS induced by DR is a primary regulator of the postponed aging. Here we describe analyses with Drosophila aimed to understand whether and how DR modulates aging through insulin signaling. We over-expressed dFOXO, the insulin responsive transcription factor, in adult fat bodies. Expressed from the head fat body dFOXO extends lifespan only when adults were fed yeast-rich diets. Expressed from the abdominal fat body, dFOXO extends lifespan only when fed restricted diets. When FOXO is expressed from either fat body the level of dilp2 mRNA from MSN is decreased. Interestingly, diet restriction itself does not affect the level of dilp2 message, but decreases message of dilp5. These results suggest that dFOXO interacts with the nutrient state to affect adult aging. To determine whether this interaction is indicative of a role for IIS in longevity extension by DR we reduced transcript levels of insulin like peptides by tissue specific expression of RNAi. Our initial observations show that DR is equally able to increase lifespan in adults with and without nutrient responsive dilps production. Likewise, preliminary data shows that DR strongly increases lifespan with FOXO null mutations. Overall data indicate that DR in flies is likely to control aging through pathways that are both dependent and independent of IIS.

850A

chico rescues mitochondrial defects in Drosophila longevity extension under dietary restriction. David Rand, Rebecca Wagaman, Jeffrey Hofmann. Ecology & Evolutionary Biol, Brown Univ, Providence, RI.

Mitochondria are believed to be a primary cause of aging due to damage from free radicals produced as byproducts of oxidative phosphorylation (OXPHOS). The enzyme complexes that carry out electron transport and OXPHOS are jointly encoded by nuclear- and mtDNA-encoded proteins that physically interact and should coevolve to maintain function. In an effort to examine direct genetic effects of mtDNA variants on variation in longevity, we introduced mtDNAs from Drosophila simulans into the nuclear genetic background of D. melanogaster to generate 'hypomorphic' mitochondrial genotypes ("sim-mel" strains). There are ~90 amino acid and over 400 silent site changes between the mtDNAs from these two species. These disrupted mito-nuclear genotypes show reduced performance in fitness and physiological assays. However, on normal food, the sim-mel strains do not show significantly reduced longevity, establishing that they are not generally sick genotypes. When sim-mel and control mtDNAs are subjected to dietary restriction (DR) of yeast, there is a very clear main-effect of mtDNA under the conditions that shows the greatest longevity extension (D. simulans mtDNAs reduce, but do not eliminate, longevity extension on 2% yeast). These data establish that mitochondrial genes are in the DR-longevity extension pathway, and strongly implicate protein as a key component of this mitochondrial effect. When paired with the chico mutation (an insulin signaling pathway hypomorph that extends longevity), the mtDNA-dependent defect in the DR response is significantly rescued. This establishes that mitochondrial genes connect the longevity extending pathways of DR and insulin signaling. Our goals are to use these mito-nuclear disruption genotypes to dissect the complex epistatic interactions between mitochondrial and nuclear genomes that regulate longevity in whole animals.

851B

Dynamics and age-dependence of resistance to environmental stresses in diet restricted Drosophila. Isabell J. Scherer[1], Dae-Sung Hwangbo[1], Joep M.S. Burger[1,2], Daniel E.L. Promislow[1]. 1) Department of Genetics, University of Georgia, GA; 2) Department of Biology, University of Fribourg, Fribourg, Switzerland.

Dietary restriction (DR) is a nutritional manipulation whereby food or caloric intake is reduced without causing malnutrition. Thus far, it is the only non-genetic tool proven to extend lifespan in almost all species tested so far. Although it has been almost a century since lifespan extension by DR was first reported, the mechanisms underlying DR still remain elusive. One common explanation is that DR animals reduce investment in reproduction, enabling them to allocate more resources into extended lifespan. However, recent studies demonstrate that DR can extend lifespan of flies independent of any effect on reproduction. Another widely accepted hypothesis postulates that DR extends lifespan by increasing resistance to environmental stressors, specifically starvation and oxidative stress. Even though previous studies suggest that DR can increase resistance to these stressors, these studies have only analyzed stress resistances at a single age, typically early in life. Here we present the dynamics of stress resistance in diet restricted flies over the entire lifespan, focusing in particular on starvation resistance. Previous studies have demonstrated that an acute switch of dietary regime from a rich diet to DR or vice-versa completely switches the mortality trajectory. We further test if stress resistance, a functional trait highly related to lifespan, also follows this pattern.

852C

Functional analysis of a juvenile hormone esterase binding protein in *Drosophila melanogaster*. Zhiyan Liu, Narinder Pal, Russell Jurenka, Bryony Bonning. Department of Entomology and Program in Genetics, Iowa State University, Ames, IA.

A putative juvenile hormone esterase (JHE) binding protein, P29, was isolated from the tobacco hornworm *Manduca sexta* (J. Biol. Chem. 275(3): 1802-1806). A possible *Drosophila melanogaster* homolog of P29 encoded by CG3776 was identified by sequence alignment, and binding of recombinant *Drosophila* P29 to JHE was confirmed. P29 mRNA and an immunoreactive protein of 25 kDa were detected in *Drosophila* larvae, pupae and adults although the predicted size of the protein is 30kD. *Drosophila* P29 is predicted to localize to mitochondria (MitoProt; 93% probability) and has a 6kD N-terminal targeting sequence. Subcellular organelle fractionation and confocal microscopy of *Drosophila* S2 cells confirmed that the immunoreactive 25kD protein is present in mitochondria but not in the cytosol. Expression of P29 without the predicted N-terminal targeting sequence in High Five™ cells showed that the N-terminal targeting sequence is shorter than predicted, and that a second, internal mitochondrial targeting signal is also present. An immunoreactive protein of 50 kDa in the hemolymph does not result from alternative splicing of CG3776 but may result from dimerization of P29. Many, if not all of the phenotypes resulting from hyperexpression of *Drosophila* P29 are consistent with the effects of a reduced juvenile hormone (JH) titer: Hyperexpression of P29 during the early larval stages was lethal, while hyperexpression during the third instar resulted in reduced size of adult flies. Hyperexpression of P29 in adult flies resulted in hyperactivity and reduced longevity. Hyperexpression in females resulted in reduced fecundity and decreased production of courtship pheromone, cis,cis-7,11-hepta cosadiene. Hyperexpression of P29 in males resulted in male-male courtship behavior and in decreased production of the aggregation pheromone, cis-vaccenyl acetate. In addition, hypoexpression of P29 resulted in increased egg production, which is consistent with increased JH titer. Hypomorphic flies also had reduced longevity. Elucidation of the function of P29 in relation to JHE is ongoing.

853A

The regulation of lifespan by *falafel*. Brian Sage[1], Xi Lou[2], Li Qian[3], Rolf Bodmer[3], Heinrich Jasper[2], Marc Tatar[1]. 1) Dept Ecol & Evol Biol, Brown Univ, Providence, RI; 2) Dept of Biology, Univ of Rochester, Rochester, NY; 3) Center for Neurosciences and Aging, The Burnham Institute, La Jolla, CA.

Conserved pathways contribute to the regulation of aging. The *C. elegans* gene *Suppressor of Map Kinase* (*SMK-1*) is required for longevity extension upon reduced insulin signaling. To elucidate how *SMK-1* participates in aging regulation we investigated its homolog in *D. melanogaster*, *falafel* (*flfl*). Overexpression of *flfl* extends lifespan when diet is restricted but not with *ad libitum* diets; *flfl* is either parallel or independent of the mechanism by which diet restriction extends longevity. Expressed in the adult heart, *flfl* retards cardiac aging, suggesting that *flfl* participates in autonomous processes of functional aging. Through examination of apoptosis in the developing eye, we find *flfl* interacts with *dFoxo* and *JNK*. Given these data, and the suggestion that *flfl* is a regulatory subunit of a protein phosphatase complex, we propose a model for how *flfl* integrates known modulators of aging.

854B

The dUSP36 Ubiquitin Specific Protease is required for larval growth and moulting. Emmanuel Taillebourg, Marie-Odile Fauvarque. Laboratoire Transduction du Signal EMI 104 INSERM CEA, Département de Réponse et Dynamique Cellulaires, CEA-Grenoble.

Posttranslational modification of proteins by the small molecule ubiquitin is emerging as a key regulatory event involved in many cellular processes. Besides targeting proteins for proteosomal degradation, ubiquitylation is known to regulate gene transcription, DNA repair, intracellular trafficking and signal transduction. While the ubiquitin ligases, the enzymes which catalyze the conjugation of ubiquitin to substrates, have been the focus of many studies, our understanding of the role of the deubiquitinating enzymes, which mediate the removal and processing of ubiquitin, is less developed.

In order to address this issue, we have obtained and characterized a null mutant of the drosophila USP36 gene which encodes a deubiquitinating enzyme homologous to the human USP36 protein. dUSP36 mutant larvae are severely impaired in growth and die during the larval stages. Moreover, they undergo the first larval moult more slowly than wild-type larvae and only a fraction of them undergo the second larval moult. The delayed first larval moult can be partially rescued by feeding ecdysone to the mutant larvae. These data indicate that dUSP36 is required for larval growth and moulting and that a step upstream of ecdysone release is affected in dUSP36 mutants.

Insect moulting occurs in response to the periodic release of ecdysone which is synthesised and secreted by the prothoracic gland. To investigate a role of dUSP36 in this process, we specifically silenced dUSP36 in the prothoracic gland using an RNAi construct. The specific inactivation of dUSP36 in the prothoracic gland completely inhibits pupation, which is due to a high titre peak of ecdysone at the end of the third larval instar. Our data suggest that dUSP36 inactivation in the prothoracic gland has impeded this ecdysone peak and that dUSP36 acts in the prothoracic gland to promote ecdysone release.

855C

The Regulation of Lipid Storage by Insulin in Drosophila melanogaster. Justin DiAngelo[1], Morris Birnbaum[1,2]. 1) Dept Medicine, Univ Pennsylvania, Philadelphia, PA; 2) Howard Hughes Medical Institute.

Insulin regulates both cell growth and metabolism by signaling through a highly conserved pathway. In *Drosophila*, the regulation of cell growth by insulin has been well established; however, how insulin regulates nutrient storage is quite unclear. Although adult flies mutant for the *insulin receptor (dInR)*or *chico* have increased whole body triglycerides, it has been recently reported that larval fat body clones overexpressing dInR or the catalytic subunit of PI3-kinase (Dp110) accumulate more fat than controls. Therefore, to clarify how insulin regulates nutrient storage in *Drosophila*, we took a tissue-specific approach to manipulate insulin signaling in the fat body, the major triglyceride storage organ of the fly. Activating insulin signaling by ectopically expressing a constitutively active dInR (dInRCA) or the kinase dAkt in the larval fat body led to an increase in whole larval triglyceride levels compared to control animals as measured by a quantitative assay. Comparable results were obtained when examining fat bodies alone. Conversely, inhibiting insulin signaling by overexpressing the lipid phosphatase dPTEN or a dominant negative Dp110 in the larval fat body decreased whole larval triglyceride levels compared to controls. Additionally, activating insulin signaling in the adult fat body by overexpressing dInRCA or an active form of dAkt (myrAkt) led to a 2-3 fold increase in whole animal triglyceride levels. This data supports the hypothesis that insulin signaling via dAkt promotes lipid storage in both the larval and adult fat body and adds support for the use of the fruit fly to identify novel genes involved in nutrient storage.

856A

Sympatric *Drosophila simulans* flies with distinct mtDNA types show difference in mitochondrial metabolism. Subhash D. Katewa[1,2], J. William O. Ballard[1]. 1) Ramaciotti Centre for Gene Function Analysis, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia; 2) Department of Radiation Oncology, University of Iowa, Iowa City, IA 52242, USA.

Drosophila simulans is an ideal fly system to study the effects of mitochondrial DNA (mtDNA) variation on the functioning of mitochondria. It harbors three distinct mtDNA haplogroups (siI, -II, -III) with no evidence of nuclear subdivision. Two of the mtDNA types (siII, -III) occur together in Tanzania, Kenya, Madagascar, and Reunion Island. A pair wise comparison of the complete mtDNA genomes of an siII line and an siIII line from Madagascar shows a total of 27 amino acid differences. We compared mitochondrial respiration, proton leak and hydrogen peroxide production in D. simulans male flies with distinct mtDNA haplogroups (siII and -III) that were collected in sympatry in Kenya. We found that repeatable bioenergetic differences exist between 11 d old males harboring siII and siIII mtDNA. In mitochondria isolated from thoracic muscles, flies with siIII mtDNA type showed higher mitochondrial respiration rates and higher complex IV activities. Males harboring siIII mtDNA also had lower hydrogen peroxide formation by both complexes I and III, lower proton leak and lower mitochondrial ATPase activities. In combination, the results suggest mitochondria isolated from siIII mtDNA harboring males have more efficient metabolism than siII mtDNA harboring flies.

857B

Screen for genes controlling appetite and adiposity reveals genes that potentially effect lifespan. Sergiy Libert, Emmeline Peng, Jessica Zwiener, Danielle Skorupa, Scott Pletcher. Dept CMB, Baylor Col Medicine, Houston, TX.

Dietary restriction (DR) is known to extend lifespan in many species. In natural populations, DR are likely to be common, due to limited food resources and fierce competition for them. In human societies, however, individuals from the majority of countries are able to obtain virtually unlimited amounts of food. Obesity and health hazards associated with it are becoming major concerns in developed countries While eating habits might be learned it is recognized that genetic components of appetite and adiposity are important. We screened 1900 (single p-element insertion) gene mutations in fruit flies for altered food consumption and triglyceride content. All mutations were contained in an isogenic background. All strains were grown and aged under highly controlled conditions (food concentration, density of larvae, temperature, humidity, light:dark cycling). Ten day old adult flies were subsequently placed on food containing inert dye for 6 hours and were frozen afterwards. We used a robotic high-throughput tissue processor to analyze the amount of food consumed in last 6 hours (indicative of appetite) and triglyceride content of male and female flies. We found that majority of mutations had small effect on both appetite and food accumulation; mainly (but not exclusively) in sex independent manner. However, we identified several mutations that caused flies to significantly increase food consumption without changes in triglyceride content. We also identified genes that influenced animals base-level of triglycerides, with and without alteration of appetite. Detailed analysis of one interesting gene, which we named ponchik, has begun. We re-verified that mutation in the ponchik gene or its promoter caused male and female flies to accumulate an excess of fat, without significantly altering their appetite or food consumption. Preliminary data suggests that ponchik mutants have altered lifespan. Additional alleles of ponchik were generated and lacZ reporter strains were obtained. Detailed analysis of its aging phenotypes, stress resistance and responsiveness to DR treatment are underway.

858C

Processing of SREBP in *Drosophila* lacking Scap. Krista Matthews, Robert Rawson. Molecular Genetics, Univ Texas SW Medical Ctr, Dallas, TX.

Regulation of sterol synthesis in mammals is controlled through protein interactions of the sterol regulatory element binding protein (SREBP) with the SREBP cleavage activating protein (Scap) and Insig, all of which are integral membrane proteins. The *Drosophila* genome contains orthologs of SREBP and Scap, but not of Insig. Previously, we have shown that SREBP is an essential gene in *Drosophila*. Larvae lacking SREBP fail to transition from second to third instar owing to a deficiency in overall fatty acids levels.

We generated a Scap loss-of-function allele and find that by contrast to SREBP null mutants, flies lacking Scap are viable, showing only a slight developmental delay when compared to heterozygous siblings. As in the mammalian system, *Drosophila* Scap appears to stabilize full length SREBP; precursor levels are significantly reduced in Scap mutants. However, nuclear SREBP is detectable in larvae lacking Scap. Furthermore, in flies lacking both Scap and site-2 protease, the intermediate form of SREBP is present. This indicates cleavage by the site-1 protease in the Golgi. Our results suggest that in *Drosophila* Scap is not absolutely necessary for transport of SREBP from the endoplasmic reticulum to the Golgi for processing and, ultimately, activation of transcription.

859A

Roles for the DHR96 nuclear receptor in lipid metabolism and the starvation response. Matt Sieber, Carl Thummel. Human Genetics, University of Utah, salt lake city , UT.

DHR96 is a member of the nuclear receptor phylogenetic group that includes the human *LXR* sterol receptor and *SXR* xenobiotic receptor. *DHR96* is expressed in the fat body, Malpighian tubules, and regions of the midgut, all of which are key metabolic centers in the fly. Previous studies have also shown that *DHR96* plays a role in xenobiotic detoxification and the regulation of lipid metabolic genes. We have found that *DHR96* null mutants display enhanced starvation sensitivity, decreased glucose levels under starvation conditions, a lean fat body phenotype by Nile red staining, and a decrease in total TAG levels. Conversely, transgenic overexpression of *DHR96* results in starvation resistance, elevated glucose levels, an obese phenotype in the fat body by Nile Red, and increased levels of total TAG. These data suggest a role for DHR96 in TAG mobilization/storage and that *DHR96* may repress genes involved in the lipid metabolic response to starvation. Studies will be presented that use metabolic profiling by GC/MS and other assays to test this model in an effort to define the role of *DHR96* in lipid metabolism and energy homeostasis.

860B

The Impact of SIR2 and Novel Candidate Genes on Triglyceride Homeostasis. Danielle Skorupa[1], Beverly Patuwo[2], Sergiy Libert[1], Jessica Zwiener[1], Pletcher Scott[1]. 1) Huffington Center on Aging, Baylor College of Medicine, Houston, TX; 2) Center for Educational Outreach, Baylor College of Medicine, Houston, TX.

We have established that, in *Drosophila*, nutrient availability induces significant changes in lipid storage and metabolism. However, the extent to which such changes are directly responsible for the effects of diet on lifespan are unknown. The Sir2 protein, a novel NAD+ dependent histone deacetylase, is emerging as an evolutionarily conserved regulator of adult lifespan. Like the yeast and worm Sir2 genes, *Drosophila* Sir2 has been reported to be required for lifespan extension resulting from dietary restriction (Rogina & Helfand, 2004). SIRT1, the mammalian homologue of Sir2, has also been implicated in the repression of adipogenesis and 3T3-L1 adipocytes fail to properly mobilize their triglyceride (TG) stores upon nutrient deprivation (Picard et *al.*, 2004). Our lab is interested in investigating Sir2 null and other mutant fly strains for *in vivo* differences in TG storage and mobilization. We first examined the role of Sir2 in this process by comparing the TG content of mutant Sir2 and wildtype flies that were starved and subsequently refed. Despite the *in vitro* evidence for a role of Sir2 in the deployment of mammalian lipid stores, our results contradict the prediction that the Sir2 gene plays a considerable role in fat mobilization in *Drosophila*. So that we may identify additional metabolically important molecules, we are in the process of screening flies mutated with retrotransposition (P-element) to determine which genes are important in fat homeostasis and triglyceride dynamics. By measuring TG levels in response to starvation and refeeding treatments we can identify mutants with not only changes in steady-state levels of TG but also those with differing rates of storage and/or utilization. Following the preliminary screening procedure, a secondary screen will be conducted to determine whether or not mutants with fat metabolism phenotypes can be utilized to make predictions about longevity, DR responsiveness, and overall fitness of the organism.

861C

Alterations in the HIF signalling pathway in median neurosecretory cells induces a diabetic phenotype in *Drosophila melanogaster*. Cathy Slack[1], Jake Jacobson[1], Colin Selman[2], Dominic J Withers[2], Linda Partridge[1]. 1) Department of Biology, University College London, London, UK; 2) Centre for Diabetes and Endocrinology, Department of Medicine, Rayne Institute, University College London, London, UK.

The von Hippel-Lindau (VHL)/hypoxia-inducible factor (Hif) pathway is a master regulator of gene transcription in mammals. Under normoxic conditions the VHL protein captures and ubiquitylates HIFα subunits, targeting them for proteasomal degradation. When oxygen supply to cells decreases, HIFα subunits are protected from VHL-mediated destruction, dimerise with HIF-1β and induce transcription of a range of target genes, many of which encode proteins that mediate adaptive responses to reduced oxygen availability. In mammals, these responses range from increased glycolysis to angiogenesis and erythropoeisis. Moreover, in humans von Hippel-Lindau disease, a rare hereditary cancer syndrome characterised by highly vascular tumours, is caused by mutation of the vhl gene. As transcriptional targets of the VHL/HIF pathway encode proteins that mediate glucose-handling in mammals and *Drosophila* models of insulin signalling currently command great interest, we directed RNAi against the *Drosophila Vhl* transcript in order to further investigate these pathways in the fly. We found that knockdown of VHL in the median neurosecretory cells (MNCs, the producers of *Drosophila* insulin-like peptides) produced larvae and adults with altered glucose homeostasis. In addition, we observe effects on both feeding and fecundity, suggesting that HIF signalling in the MNCs is upstream of a cascade of physiological and behavioural responses.

862A

Cloning and characterization of *lot's wife (lwf)*, a mutation that disrupts food processing and digestion. Edward M. Blumenthal. Dept Biol Sci, Marquette Univ, Milwaukee, WI.

Proper digestive function is critical for animal survival, but few *Drosophila* mutants have been reported that affect digestion or food processing. We have isolated an X-chromosome EMS mutant, *lwf*, which appears to disrupt the normal operation of the digestive system. Mutant flies begin dying 2-3 days post-eclosion and show 100% lethality within 7 days. By the time they die, *lwf* flies exhibit grossly distended crops, suggesting that they are able to eat. However, little food appears to move into and through the midgut and hindgut, as shown by an 80% reduction in the production of fecal spots by *lwf* males, compared with wild-type, during the second and third days of adult life. We hypothesize that mutants are starving to death despite having free access to food. We mapped *lwf* by meiotic recombination to the interval between *g* and *sd*, and the location was further restricted to 13A by the failure of 12 deficiencies in this region to uncover the phenotype. Sequencing identified an A to T transversion in the third exon of *CG33968*; this is predicted to be a nonsense mutation in codon 181 (out of 827). Consistent with the nonsense-mediated decay that should result from this mutation, real-time RT-PCR showed a lack of *CG33968* mRNA in *lwf* flies. To confirm that the mutation in *CG33968* is the cause of the *lwf* phenotype, we tested for complementation with $CG33968^{CB6275\text{-}3}$, which carries a P-element just upstream of the transcriptional start. While $CG33968^{CB6275\text{-}3}$ flies showed no obvious survival phenotype, transheterozygous *lwf*/ $CG33968^{CB6275\text{-}3}$ females displayed 80% mortality within 7 days post-eclosion. This failure to complement strongly suggests that *CG33968* is the *lwf* gene. *CG33968* is predicted to encode an integral membrane protein; it belongs to a 19-member gene family that is heretofore uncharacterized in *Drosophila*. Interestingly, a *C. elegans* homolog of *CG33968*, *nrf-6*, was isolated in a screen for fluoxetine (Prozac) resistance (Choy and Thomas, 1999), providing a potential link between Prozac and digestive physiology. *Supported by NIH grant R21 DK60860 and Marquette University.*.

863B

Rearing media as a variable in *Drosophila melanogaster* fecundity: an activity to introduce scientific methods of inquiry to biology students. Darby Carlson[1], Laura Wollard[1,2], Benjamin Klein[1], Kimberly Carlson[1]. 1) Biology, University of Nebraska at Kearney, Kearney, NE; 2) Winfield Middle School, Winfield, KS.

A major challenge in teaching the process of science to students is designing and implementing laboratory activities that emulate what is actually done in a research laboratory. To facilitate this effort, science educators have been encouraged to design exercises that span multiple laboratory periods, encourage independent thinking, promote hypothesis-driven experimentation, and data collection and analysis. We have designed an inquiry-based, semester-long laboratory activity amenable to majors or nonmajors and to introductory or advanced biology students. This activity utilizes *Drosophila melanogaster*, the fruit fly, as a model organism that allows students to investigate how different rearing media additives affect female fecundity measured as numbers of eggs laid. To explore the feasibility of our activity aimed in helping students learn the processes of science, we assigned the activity independently to three different student populations. These included 1) students in an undergraduate biology laboratory; 2) an independent undergraduate research project; 3) a Distance Education Biology Master's graduate student summer research project. The goal of this laboratory activity is to allow students the opportunity to design a controlled experiment, formulate testable hypotheses, identify variables, make quantitative and qualitative observations, and analyze data using a simple computer spreadsheet program.

864C

Effects of the antioxidant properties of blueberries on morality rates and *INDY* gene expression in *Drosophila*. Kimberly Carlson, Jenna Derr. Biology, University of Nebraska at Kearney, Kearney, NE.

Aging may involve free radical accumulation, which causes cell damage. Foods with antioxidant properties, such as blueberries, may extend the longevity of an organism. In addition to environmental factors, genes also play a role in aging and death. In *Drosophila melanogaster*, genetic mutations to the *INDY* gene (I'm not dead yet) have shown to decrease the rate of aging. Other genes involved in the antioxidant process of aging are *catalase* and *superoxide dismutase*. The objective of this experiment is to determine if blueberries added to instant fly food affects mortality rates and gene expression profiles of *D. melanogaster*. To do this, *D. melanogaster* were reared on media with or without blueberries, mortality curves tallied, and flies collected for RNA analysis of age related genes by quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR). Mortality curve graphs showed that flies reared on blueberries lived longer than those from control medium. This research will provide insight into the genetic and environmental components of the aging process.

865A

The Regulation of Lifespan, Fecundity and Other Phenotypes in *Drosophila* by DILP-producing Median Neurosecretory Cells of the Brain. Susan J Broughton, Cathy Slack, Timothy Bass, Nazif Alic, Jake Jacobson, Tomoatsu Ikeya, Anna Maria Tommasi, Linda Partridge. Department of Biology, University College London, London, GB.

The insulin/IGF-like signalling (IIS) pathway, present in all multicellular organisms, regulates diverse functions including growth, development, fecundity, metabolic homeostasis and lifespan. In the fly, ligands of the IIS pathway, the *Drosophila* Insulin-like peptides (DILPs), regulate growth and hemolymph carbohydrate homeostasis during development, and are expressed in a stage- and tissue-specific manner. We have shown previously that ablation of the median neurosecretory cells (MNCs) in the adult brain that produce a subset of the DILPs leads to an array of phenotypes in adult flies including increased fasting glucose levels in the hemolymph, increased storage of lipid and carbohydrate, reduced fecundity, extension of median and maximal lifespan and increased resistance to oxidative stress and starvation. Here, we report the results of experiments to determine the roles of the individual DILPs produced by the MNCs in the mediation of these phenotypes.

866B

Expression analysis of Cytochrome P450s in Drosophila melanogaster. Henry Chung, Tamar Sztal, Lee Willoughby, Chris Lumb, Mohan Sridar, Philip Batterham, Phillip Daborn. Department of Genetics, University of Melbourne, Melbourne, Victoria, AU.

Cytochrome P450s are enzymes present in most organisms, from single cell prokaryotes to multi-cellular animals. Insects have on average 100 different cytochrome P450 family members, perform various important biochemical reactions. Although some P450s have been implicated in the detoxification of xenobiotics (toxic plant compounds and insecticides), and others in the synthesis of 20-hydroxecdysone from plant sterols, the function of the majority of insect P450s is unknown. We systematically examined the expression of all the P450s in the *Drosophila melanogaster* genome in 3rd instar larvae (feeding and wandering). We showed that most P450s are expressed in the midgut, malpighian tubules and fat bodies, where they might have broad roles in the metabolism of endogenous and exogenous compounds. We also showed that other P450s are expressed in specialized tissues such as the corpus allatum and oenocytes, where they could play key roles in controlling biochemical pathways in development. Overexpression/knockdown of some of these P450s leads to developmental lethality, suggesting important developmental roles.

867C

Lifespan extension by anti- diabetic drug metformin in Drosophila. Dae-Sung Hwangbo[1], Kyung-Jin Min[2], Ho-Jin Koh[3], Laurie J. Goodyear[3], Marc Tatar[2], Daniel Promislow[1], (* equal contribution). 1) Department of Genetics , University of Georgia , Athens , GA; 2) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI; 3) Research Division, Joslin Diabetes Center and Department of Medicine, Harvard Medical School, Boston, MA.

The biguanide drug family has long been suggested to have the potential to extend life span. Among the members in this group, metformin has received particular attention due to its well-known anti-diabetic effect. Recent gene expression studies have suggested that metformin-treated mice had a gene expression profile similar to mice on dietary restriction (DR). Dietary restriction reduces food or caloric intake without causing malnutrition and is the only non-genetic method that extends lifespan in almost all animals tested so far. Here we show that metformin can significantly extend lifespan in Drosophila without any reduction in fecundity or food intake rate. In addition, we find that metformin increases resistance to environmental stresses such as starvation and oxidative stress. Metformin also alters intrinsic physiological traits such as whole body triglyceride content and hemolymph glucose level. Current studies are exploring the interaction between metformin to DR, and possible molecular genetic mechanisms by which metformin extends lifespan.

868A

***Drosophila* life span: genetic background, sex specificity, mating and social status.** Konstantin Iliadi, Natalia Iliadi, Gabrielle Boulianne. The Hospital for Sick Children, Toronto,Canada.

Comparative analysis of lifespan in *Drosophila* has often yielded controversial results that can be attributed to differences in experimental design and/or genetic background. To further investigate the relative contributions of these effects we examined the lifespan of two laboratory wild-type strains: *Canton S* (CS) and *Oregon R* (OR). The choice of these strains was motivated by the fact that they usually serve as a source of wild-type background for transgenic lines during outcrossing. The data clearly show significant effects for both genetic background and the method of maintenance. Individually kept flies live relatively longer then those maintained in groups; however they show a higher degree of phenotypic variation either between sexes or due to mating status. Irrespective of the genotype of individually kept flies, mated males lived significantly longer than virgins, whereas female' lifespan was very genotype dependent. Mated CS females also had shorter lifespan than virgins confirming *eo ipso* "a cost of reproduction" model however, a similar effect was not observed for OR females. For flies maintained in groups, the reproductive status had a much less marked effect on survival of either virgin or mated groups regardless of whether they were maintained separately by sex or in sex mixed groups. For OR we observed a very strong sex-dependent effect on lifespan between groups of males and females. In these groups females lived much longer then males. The only combination where we did not observe any significant differences between sexes and genotypes is within mixed groups. The absence of differences in mixed populations may reflect flies' natural environments wherein flies have regular sexual and social contacts. Therefore, this experimental combination may be more appropriate for studies involving lifespan comparisons in *Drosophila* or in screens to search for genes that may be involved in longevity. This may especially be important for those lines in which the genetic background is unknown or in those cases where its very difficult to equilibrate.

869B

Identification of Delayed Aging of Negative Geotaxis Mutants. Melanie Jones, Michael Grotewiel. Dept. of Human Genetics, VCU, Richmond, VA.

Many age-related functional declines that occur in humans, including locomotor senescence, also occur in Drosophila. One of the most commonly evaluated locomotor behaviors assessed in adult flies is negative geotaxis, an innate escape response elicited by banging flies to the bottom of a container. The response to this stimulation is measured as the distance climbed up the container wall. As flies age, the distance climbed becomes smaller. To identify mutants with delayed aging of negative geotaxis (DANG), we performed a forward genetic screen on ~1000 transposon insertion lines. Four mutants that had the most robust delay of locomotor senescence were chosen for further characterization. Phenotype characterization revealed that none of these four DANG transposon insertion lines had an increase in lifespan and all of the mutants were resistant to various stresses including starvation, thermal stress, desiccation, and oxidative stress. One mutant harbors a P element insertion in the PDK1 locus. The PDK1 gene product mediates insulin signaling, a pathway known to influence lifespan in worms, flies, and mice. Revertants of the PDK1 insertion were generated via precise excision. Senescence of negative geotaxis was returned to normal in the revertant lines, indicating the P element insertion in PDK1 causes the delayed aging of locomotor senescence in these flies. These results suggest that the insulin signaling pathway is a key determinant in locomotor senescence in Drosophila. The positions of P elements in other DANG mutants are being determined by inverse PCR. Further study of these DANG mutants will reveal key genes and possible pathways involved in delayed behavioral decline in Drosophila.

870C

The sensory influence on lifespan appears to be conserved in Drosophila. Ivan Ostojic[1], Werner Boll[2], Joy Alcedo[1]. 1) Growth control, Friedrich Miescher Institute, Basel, CH; 2) Institute of Molecular Biology, University of Zurich, Zurich, CH.

The lifespan of an animal is influenced by genetic and environmental factors. These factors interact and this interaction, at least in C. elegans, seems to be mediated by the sensory system (Apfeld and Kenyon, 1999). Surprisingly, only subsets of sensory neurons were shown to influence longevity (Alcedo and Kenyon, 2004). Through laser ablation of specific cells, a specific subset of gustatory neurons was found to inhibit longevity and a different subset of gustatory neurons was found to promote longevity by acting on the worm's insulin signaling pathway (Alcedo and Kenyon, 2004).The influence of the insulin signaling pathway on lifespan is conserved in many organisms (reviewed by Kenyon, 2005). Here we show that the influence of the gustatory system on lifespan also appears to be conserved in Drosophila and that this also seems to be mediated by the insulin signaling pathway.

871A

Evaluation of *foxo* activity during nutritional stress and development. Jennifer D. Slade, Jody-Lynn E. Rotchford, Brian E. Staveley. Biology, Memorial University, St. John's, Newfoundland, CA.

The transcription factor *foxo* is negatively regulated by the insulin signaling pathway and influences the expression of a myriad of targets. The target genes can be grouped into those which lead to cellular death, affect metabolism, and mediate the organismal response to both oxidative and nutritional stress. To quantify *foxo* activity in *Drosophila*, a novel *foxo*-responsive luciferase transgene leads to the production of luciferase as *foxo* is activated that, in the presence of luciferin, emits a measurable light. This reporter gene provides the ability to monitor *foxo* transcriptional activity under a wide range of experimental conditions.

To explore the importance of *foxo* in nutritional stress, adult flies were starved of amino acids and aged. Adults with one functional copy of *foxo* were found to survive as well as those with two, while adults with no *foxo* activity were found to be extremely sensitive to nutritional stress exhibiting significantly reduced lifespans. Luciferase assays were performed to quantify *foxo* activity during starvation in developing mutant larvae. Larvae were collected and starved of amino acids over a period of 4 days. Levels of *foxo* activity were measured at 24 hour intervals. As the time of starvation increases, *foxo* activity also increases until death.

In addition to three conserved akt phosphorylation sites, *foxo* bears a conserved minibrain phosphorylation site. The minibrain (*mnb*) kinase regulates the numbers and types of neuronal cells in the neuroblast proliferation centre of larvae, which leads to the formation of adult optic lobes. We have found that phenotypes dependent upon *foxo* overexpression are enhanced in *mnb* mutants, and suppressed by the directed expression of *mnb*. The *foxo*-responsive luciferase assay can be used to help understand the role of *foxo* in biological phenomena. Support was provided by the NSERC Discovery Grant awarded to BES.

872B

CHANGES IN MALE GERMLINE STEM CELL CYCLE ACTIVITY DURING AGING IN WILD TYPE AND LONG-LIVED FLIES. Matthew Wallenfang[1], Renuka Nayak[2], Karina Rodriguez[1], Steve DiNardo[2]. 1) Dept of Biological Sciences, Barnard College, New York, NY; 2) Dept of Cell and Dev Biology, Univ of Pennsylvania, Philadelphia, PA.

One hypothesis to explain aging-related phenotypes is that changes occur over time in the adult stem cells that maintain most tissues. These cells persist throughout the lifetime of the organism and may divide many more times than non-stem cells. Thus they may be particularly susceptible to factors contributing to aging, and phenotypic changes in these cells would likely have a large influence on the phenotype of tissues. We have described changes that occur in male germline stem cells (GSCs) of Drosophila melanogaster during aging, which have the advantages of being readily identifiable and have available a wealth of genetic tools for further study. We find that although GSCs are lost during aging, a mechanism exists to replace these cells and maintain relatively high numbers of GSCs throughout the lifetime of the fly. As GSCs age, however, the division rate of GSCs slows significantly. Interestingly, this slowing is not observed in long-lived methuselah (mth) mutant flies. Mth encodes a putative 7-pass transmembrane protein, however its cellular function remains unknown. Currently we are further exploring the role of mth in controlling stem cell aging, including determining whether mth functions in GSCs, in associated somatic cells of the testis, or elsewhere.

873C

EFFECTS OF OXIDATIVE DAMAGE ON MALE GERMLINE STEM CELL CYCLE ACTIVITY. Tarnima Ahamed, Khadeejah Bari, Christine Chang, Matthew Wallenfang. Dept of Biological Sciences, Barnard College, New York, NY.

We have previously described changes that occur in male germline stem cells during aging, including a significant slowing of the cell cycle. Accumulation of cellular damage by reactive oxygen species (ROS) is thought to be a major contributing factor to cellular aging. We have begun to investigate to what extent ROS-induced damage contributes to germline stem cell cycle slowing during aging. By exposing flies to sub-lethal concentrations of paraquat we can increase ROS in cells; conversely by ectopic expression of superoxide dismutase (SOD) we can decrease accumulation of ROS, and then measure cell cycle activity using BrdU labeling. We are additionally investigating whether somatic stem cells in the testis are more sensitive to accumulation of aging-related damage than germ cells, which due to their immortality likely contain mechanisms to protect them from such damage.

874A

Morphometric analysis of the loss of CNS neurons, an age -associated neuropathology in Drosophila. Kristopher Beckwith[1], Kebreten Manaye[2], Atanu Duttaroy[3]. 1) Human Genetics, Howard University, Washington, DC; 2) Dept of Physiology and Biophysics; 3) Dept of Biology, Howard University.

Vacuolar organization of the brain is typical to all neurodegeneration mutants of Drosophila. Similar vacuolar organization was also reported in the old fly brains as a function of age presumably due to the loss of CNS neurons. H&E stained sections of the fly brain were analyzed for quantitative morphometric measurement of vacuole volume and their distribution in the brain using dedicated morphometric software Neurolucida version 7.0 (Microbrightfield, USA). We first looked into wild type (CantonS) fly brains with a maximum life span of 67 days and quantitatively established how loss of CNS neurons occurs in the fly brain as a function of age from the initial appearance of increasing vacuolar sizes. Vacuolar volume was measured using the Cavilery Method on 7 μm sections randomly throughout the brain. We are now looking into many long-lived fly lines to correlate the amount of neuronal loss in the brain with their corresponding ages. For example, a fly line with a maximum life span of 85 days showed much greater loss of CNS neurons compared to the wild type flies. Finally, a short lived mutant strain of Drosophila (maximum life span 40 days), which has been shown to undergo accelerated aging also showed a significant number of such vacuoles in their brain. This observation raises an interesting issue; could brain vacuolar organization be used as reliable cellular marker to establish fly aging??

875B

Maternal protection against oxidative damage is offered through mitochondrial superoxide dismutase(MnSOD). Amy Belton, Renee Forde, Atanu Duttaroy. Dept Biol, Howard Univ, Washington, DC.

The enzyme Manganese Superoxide Dismutase (MnSOD) is responsible for the continual neutralization of mitochondrial superoxide radicals, which are byproducts of aerobic oxygen metabolism. Sod2n283 is a bona fide null mutant for MnSOD which lives a maximum lifespan of 24 hours. However, no distortion was noticed in the eclosion ratios between homo and heterozygote Sod2n283 flies which ecloses at the appropriate 1:2 ratio. Immunohistochemical studies show that homozygous Sod2n283 embryos (10-14 hour old) still carries the MnSOD protein, suggesting thereby that the protein was maternally contributed. GFP selection was employed to sort out the homozygous null larvae and pupae where no MnSOD protein was found by western blotting. We therefore conclude that the maternal MnSOD protein lasts until the early larval stages but is exhausted by third instar. However, absence of MnSOD is not associated with larval or pupal lethality. Efforts are underway to understand the requirement of MnSOD during early embryonic development.

876C

Functional Study of *Drosophila melanogaster* Omega-class Glutathione S-Transferase (DmGSTO). Kiyoung Kim, Jaekwang Kim, Hyunsuk Suh, Songhee Kim, Jeongbin Yim*. Laboratory of Biochemistry, School of Biological Sciences, Seoul National University, Seoul, Korea.

Glutathione S-transferases are a family of multifunctional enzymes, which play important roles in cellular detoxification. Biological function of omega-class GSTs (GSTOs), however, remained to be elucidated. In our previous study, five DmGSTOs, CG6781, CG6673-A, CG6673-B, CG6776 and CG6662, were cloned and characterized from *Drosophila melanogaster*. All exhibited high thiol transferase and dehydroascorbate reductase (DHAR) activities, characteristics of GSTO. CG6781 is found to be the structural gene for *sepia* which encoded PDA synthase. Especially, Recombinant proteins of CG6673A and B had much higher DHAR activity compared with other DmGSTOs. K_m values for dehydroascorbate (DHA) of the CG6673A and B were 1.03 ± 0.99 mM and 0.39 ± 0.04 mM , respectively. K_m values for glutathione (GSH) of the CG6673A and B were 4.23 ± 0.40 mM and 4.33 ± 0.23 mM, respectively. DHAR which converts DHA to ascorbate (AsA), is a key enzyme for recycling AsA. To investigate *in vivo* function of CG6673, loss-of-function mutants of CG6673, *DmGSTO*^{DB58}, were generated. In the mutants of CG6673, DHAR activity was dramatically decreased to about 10% of wild type. They were sensitive to paraquat, oxidative damage inducer and had slightly reduced life span compared to wild type.

877A

Neuronal expression of *jafrac1*, a *Drosophila* homolog of *hPrxII*, extends lifespan and rescues oxidative stress induced lethality. Kyu-Sun Lee[1], Dong-Seok Lee[2], Sung-Kyu Ju[1], Kweon Yu[1]. 1) Development/Differentitation, KRIBB, Daejeon, KR; 2) Division of Animal Science, Kaangwon University, Chuncheon, KR.

Oxidative stress has been widely implicated as an important factor in aging process and neurodegenerative diseases. Antioxidant defense systems neutralize oxidative cytotoxic by-product reactive oxygen species (ROS). Peroxiredoxin (Prx) is an antioxidant enzyme which protects organisms against the oxidative damage by scavenging peroxides. Here, we present functional analysis of *Drosophila jafrac1* gene which is the *Drosophila* 2-Cys *Prx* homolog of human *PrxII* (*hPrxII*) gene. *Jafrac1* was expressed during all developmental stages. When *jafrac1* or *hPrxII* was over-expressed using the pan-neural *elav-Gal4* driver, the lifespan of *Drosophila* was significantly extended compared to the wild-type control. But, when *jafrac1-RNAi* was expressed using the *elav-Gal4* driver, the lifespan was significantly reduced. Paraquat, a ROS inducing chemical reagent, treatment generated oxidative stress induced lethality in the wild-type flies. However, *elav>jafrac1* and *elav>hPrxII* flies significantly rescued this lethality. Interestingly, over-expression of *jafrac1* using the sensory neuron driver *MJ94-Gal4* and cholinergic neuron driver *Cha-Gal4*, not glial cell driver *Repo-Gal4*, rescued the lethality. In addition, *elav>jafrac1* and *Cha>jafrac1* flies suppressed paraquat induced JNK activation. These results suggest that neuronal oxidative damage is an important determinant of lifespan and indicate that *Drosophila jafrac1* and its human homolog *hPrxII* play a protective role in neuronal cells against oxidative damage.

878B

Graded Reduction of Manganese Superoxide Dismutase Causes a Proportional Acceleration of Functional Aging. Ian Martin[1], Michael Grotewiel[1], Atanu Duttaroy[2]. 1) Dept Human Genetics, VCU, Richmond, VA; 2) Biology Department, Howard University, Washington DC.

The oxidative damage theory of aging posits that normal aging results from free radical-mediated damage to biological systems. A number of studies in a variety of species support a link between oxidative damage and life span determination. To investigate the role of oxidative damage in age-related declines in physiological function, we assessed the senescence of locomotor and olfactory behaviors in a series of mutants with graded reduction of the key mitochondrial antioxidant, manganese superoxide dismutase (SOD2). Reduction of SOD2 activity by ~50% had little effect on either measures of aging. An ~75% reduction in SOD2 activity, however, lead to accelerated decline of olfactory function without effecting locomotor senescence whereas flies with even greater SOD2 reduction had accelerated declines of both aging parameters. Finally, flies with a complete loss of SOD2 showed a dramatic decline in both behaviors within several hours after eclosion. This study demonstrates that increasing the susceptibility of flies to oxidative damage results in an accelerated senescence phenotype. Furthermore, the degree of acceleration was proportional to the extent of SOD2 reduction. This underscores the important role of SOD2 in protecting organisms from oxidative damage-mediated aging and also suggests that oxidative damage, which accumulates even under normal SOD2 levels, may contribute to age-related functional declines observed in flies.

879C

Modifying metabolism of lipid peroxidation in Drosophila muscle by transgenic expression of mGSTA4-4 with Mhc-Gal4 driver extends life span. Ashis K. Mondal, Sharda P. Singh, Kumar Chandra-Kuntal, Chhanda Mondal Ghosh, John J. Thaden, Robert J. Shmookler Reis, Ludwika Zimniak, Helen Beneš, Piotr Zimniak. Univ. of Arkansas for Med Sciences & VA Hospital., Little Rock, AR.

Accumulation of oxidative damage contributes to organismal aging. One of the consequences of oxidative stress is a chain reaction which leads to the peroxidation of polyunsaturated fatty acids. The resulting lipid hydroperoxides can be converted to α,β-unsaturated aldehydes such as 4-hydroxynon-2-enal (4-HNE), a strong electrophile capable of modifying proteins and altering their function. To test whether 4-HNE-mediated cellular damage affects life span, we expressed in adult Drosophila melanogaster mGSTA4-4, a murine glutathione transferase with high catalytic efficiency for 4-HNE conjugation. The UAS/GAL4 system was used with the following driver lines: Actin-5C-Gal4 (ubiquitous expression), 24B-Gal4 (mesodermal), Mhc-Gal4 (muscle), elav-Gal4 (pan-neuronal), and D42-Gal4 (motorneuronal). Life span determinations were carried out at 25 and 29°C. Ubiquitous or neuronal expression of mGSTA4-4 had little effect on longevity, and mesodermal expression yielded inconsistent results in replicate experiments, indicating an unidentified gene-by-environment interaction. However, muscle-specific expression (Mhc-Gal4 driver) resulted in a robust extension of median lifespan of approximately 20% at both temperatures. Flies expressing mGSTA4-4 in muscle had the same rate of CO2 production as control animals. Thus, because of their longer life span, the transgenic flies had an increased life-long energy metabolism. We interpret the results in terms of a beneficial effect on life span of electrophile detoxification in the indirect flight muscle, a tissue with high rates of both aerobic metabolism and lipid peroxidation. Electrophilic stress by 4-HNE and/or chemically similar lipid peroxidation products is likely to be a contributor to the accumulation of macromolecular damage that leads to organismal aging.

880A

The octopamine receptor OAMB is required in the oviduct epithelium for ovulation of *Drosophila melanogaster*. Hyun-Gwan Lee, Kyung-An Han. Department of Biology and Intercollege Graduate Degree Program in Genetics, Pennsylvania State University, University Park, PA 16802.

The monoamine octopamine is a major neurotransmitter, neuromodulator and neurohormone in invertebrates. Octopamine plays important roles in *Drosophila* female fertility, but the underlying mechanisms are less understood. We identified two octopamine receptors, OAMB-K3 and OAMB-AS, which are produced by alternative splicing of the *oamb* gene and have distinct capacities to activate cAMP and intracellular calcium. Both receptors are expressed in the brain, thoracico-abdominal ganglion and female reproductive system. We generated null and various hypomorphic *oamb* mutants by P-element mediated dysgenesis. Remarkably, the *oamb* null mutant females are impaired in ovulation and hold mature eggs in ovaries. To identify tissue type(s) that require OAMB for ovulation, we employed the GAL4/UAS system. The transgenic oamb females with ubiquitous expression of either OAMB-K3 or -AS only in the adult stage were fecund, indicating that OAMB plays a physiological, as opposed to developmental, role in ovulation. When OAMB expression was targeted in the central or peripheral nervous system, the transgenic *oamb* females remained infertile, indicating that neural OAMB expression is not sufficient or is not required for ovulation. Thus, we generated the enhancer GAL4 lines (RS-GAL4) using the *oamb* genomic region containing a potential enhancer element for ovulation. RS-GAL4 was rather specifically expressed in the reproduction system, especially at the high level in the epithelial cells of the oviduct. Notably, the transgenic oamb females with OAMB-K3 or -AS expression driven by RS-GAL4 were fully fertile, suggesting that OAMB is required in the epithelial cells of the oviduct for ovulation. We are currently conducting a genetic suppressor screen to unravel the cellular mechanism. This work is supported by NIH/NICHD grant.

881B

Investigation of potential tyrosine transporters in the Malpighian tubule. William F. Mueller, Edward M. Blumenthal. Dept. Biol. Sci., Marquette University, Milwaukee, WI.

We have previously reported that the biogenic amine tyramine acts as a diuretic factor in isolated *Drosophila* Malpighian tubules by increasing transepithelial chloride conductance. Furthermore, the tubule is able to synthesize tyramine from tyrosine through the action of the enzyme tyrosine decarboxylase, most likely in the principal cells of the tubule. Because the physiological response of tubules to pulses of tyrosine shows a rapid onset and termination, we believe that bulk cytosolic tyrosine is not used as a substrate for tyramine synthesis and a distinct pool of tyrosine exists for this purpose. The aim of the current study is the identification of the tyrosine transporter in the principal cells of the tubule. Most amino acid transporters utilize the transmembrane sodium gradient to drive transport; however, removal of sodium from the peritubular bath did not alter the depolarizing response to tyrosine. For this reason, we have chosen to focus on the IAAT/KAAT subfamily of SLC6 amino acid transporters, as these proteins can cotransport either sodium or potassium. The *Drosophila* genome contains six IAAT genes. Real-time RT-PCR shows that four of them, *CG15088*, *CG15279*, *CG1698*, and *CG3252*, are highly expressed in the tubule, consistent with previous microarray data (Wang et al., 2004). No mutant lines are available for any of these four genes. In situ hybridization with probes directed against the first three genes shows that they are all expressed in the principal cells of the tubule main segment, as would be expected of a tyrosine transporter. Because the mosquito ortholog of *CG15279* has recently been shown to transport tyrosine (Meleshkevitch et al., 2006), we have begun constructing an inducible RNAi transgene against that gene to study the effects of knocking down its expression in the principal cells. In addition, we have found that flies trans-heterozygous for two overlapping deficiencies that remove *CG15088* are viable; physiological studies are now underway to test the tyrosine sensitivity of tubules from these flies. *Supported by Marquette University.*.

882C

Cardiac functional decline with age is dependent upon regulation of 4EBP activity in the myocardium. Robert Wessells[1], Michael Hayes[1], Rolf Bodmer[2]. 1) Dept Intnl Med/Geriatrics, Univ Michigan, Ann Arbor, MI; 2) The Burnham Institute La Jolla, CA.

Multiple parameters of cardiac function have been observed to decline in an age-related manner, including resting heart rate, rhythmicity and stress tolerance. This cardiac functional decline correlates well with lifespan under most circumstances, as both genetic mutations and dietary interventions that extend lifespan also extend youthful cardiac performance. Significantly, however, the rate of cardiac decline with age is dependent on activation of both the insulin receptor and TOR signaling pathways within the myocardium itself, and the rate at which this decline occurs is separable from lifespan by cardiac-specific genetic manipulation of insulin or TOR signaling. Forced activation of either signaling pathway in the adult myocardium leads to a hastening of cardiac decline, while blocking signaling activity from either pathway largely prevents decline from occurring up to eight weeks of age. Since both insulin/Foxo and Tor signaling are known to affect the activity of the eif4e-binding protein 4EBP, we tested the effects of 4EBP expression in the myocardium. Overexpression of 4EBP prevented cardiac decline to the same extent as overexpression of Foxo or the TOR antagonist TSC. Conversely, overexpression of eif-4e leads to a hastened decline of myocardial function similar to that caused by overexpressing either the insulin receptor or TOR. When TOR and 4EBP are co-overexpressed in cardiac tissue, the positive effect of 4EBP fully predominates, while the negative effect of eif4e overexpression fully predominates over the the positive effect of co-expressed Foxo. These results suggest a model whereby both insulin receptor and TOR activity act to regulate 4EBP activity in the myocardium. The level of 4EBP activity controls the initiation of translation and growth in the cardiomyocytes. The extent to which this growth program is active in the cardiomyocyte population then controls the rate at which organ functional decline occurs.

883A

Genome wide RNAi screen identifies genes related to the insulin pathway as regulators of the transcriptional response to hypoxia. Andres Dekanty, Lazaro Centanin, Pablo Wappner. Fundación Instituto Leloir, Buenos Aires, ARGENTINA.

The transcriptional response to hypoxia is a highly conserved mechanism mediated by the heterodimeric (α/β) transcription factor HIF (hypoxia inducible factor). We have previously defined a hypoxia-responsive system homologous to HIF in *Drosophila melanogaster*, being the proteins Similar (Sima) and Tango (Tgo) the functional homologues of HIF-α and β subunits, respectively. Sima is regulated by oxygen through the gene *fatiga* (a prolyl hydroxylase) that behaves as an oxygen sensor. To gain insights on the regulation of the transcriptional response to hypoxia, a HIF-Responsive-Element (HRE) luciferase reporter was stably transfected in *Drosophila* S2 cells. Expression of the reporter is strongly induced in hypoxia or upon exposure to Deferoxamine (DFO), in a Sima/Tgo-dependent manner. The HRE-reporter was then used in a genome wide dsRNA-based screen. The screen led to the identification of novel genes presumably required for the transcriptional response to hypoxia, among which we identified components of the insulin-signaling pathway. We have demonstrated both in cell culture and in living embryos, that insulin is a potent activator of Sima-dependent transcription. This effect depends on PI3K and TOR pathways and involves accumulation of Sima protein as well as an increase of its nuclear localisation. It has been shown that PI3K and TOR pathways play a fundamental role in growth regulation. Increased activity promotes growth, while diminished signaling leads to cell and body size reduction. Interestingly, we found that *fatiga* loss-of-function led to body size reduction but *fatiga sima* double mutants display normal size. Consistent with this, Sima flip-out over-expression led to cell size reduction, strongly suggesting that Sima is a cell autonomous negative regulator of growth.

884B

Mating increases starvation resistance and fat reserves in Drosophila melanogaster females. Jadwiga Giebultowicz[1], Brandy Rush[1], Jessica Bruer[1], Robin Roshe[2], Michael Wells[2]. 1) Dept Zoology, Oregon State Univ, Corvallis, OR. 97331, USA; 2) Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ 85721, USA.

Mating stimulates complex physiological changes in females of Drosophila melanogaster. Two long-term effects of mating are increased egg production and shortened life span. Mated females invest substantial nutrients toward progeny production and therefore are predicted to be more susceptible to starvation stress than virgin females. Contrary to this expectation, we found that mated and highly fecund Drosophila females survive significantly longer under starvation than virgin females; the magnitude of these differences depended on age and was highest in 15-day-old females. Higher resistance of mated females to starvation was observed in Canton S, Oregon R and w flies, suggesting that it is a general phenomenon. Increased starvation resistance in mated females correlated negatively with longevity and positively with fecundity in our unselected Canton S cultures of Drosophila. To understand the physiological bases of differences in resistance to starvation, we compared nutrient levels in 15-day-old mated and virgin females. The ability to survive longer under food deprivation is associated with higher food intake in mated females. Despite increased investment in progeny, mated females have significantly higher energy reserves stored in the form of fat compared with virgins. The large amount of stored fat is responsible for superior starvation resistance of mated females. On the other hand, mated females show lower resistance to oxidative stress than virgins. Thus, the resistance to starvation and to oxidative stress changes in opposite directions in reproducing females. *Drosophilamelanogasterw*.

885C

Immune system efficiency is increased in long lived mutants *puc* and *chico* but is unaffected by dietary restriction. Sergiy Libert, Jessica Zwiener, Yufang Chao, Scott Pletcher. Dept CMB, Baylor Col Medicine, Houston, TX.

The immune system is vital for the immediate survival of multicellular organisms by protecting the animal from the damaging effects of toxic molecules, parasites, viruses and bacteria. It is hypothesized that the immune system plays a pivotal role in determining longevity of organism as well. We investigated the efficiency of the innate immune system in flies carrying the longevity extending mutations *puc* (JNK signaling pathway, stress response) and *chico* insulin signaling pathway) as well as animals subjected to dietary restriction (DR), which also extends lifespan. We found that puc heterozygous animals as well as chico homozygous and heterozygous flies have enhanced pathogen resistance. Surprisingly, DR manipulation did not reproducibly change organism pathogen resistance, despite existing data that show that the expression of many immunity related genes are greatly affected by diet. Considering that that chronic or frequent activation of the immune system results in a reduced longevity, we postulate that the longevity extending potential of the above mutations is may be partially obscured by parallel activation of the immune system. Such upregulation is not observed during DR, suggesting the presence of an additional mechanism that suppresses immune response in diet-restricted animals. This hypothesis is being investigated by life-long monitoring of the expression of key immunity-related genes by means of lacZ fusion.

886A

NfκB is a mediator of trade-offs between longevity and pathogen resistance in Drosophila melanogaster. Sergiy Libert, Yufang Chao, Xiaowen Chu, Scott Pletcher. CMB, MHG, Baylor College of Medicine, Houston, TX.

Innate immunity is an ancient system that protects numerous organisms, including humans, from the universe of pathogenic molecules, viruses and microorganisms. Despite its role in promoting pathogen resistance, inappropriate activation and expression of NFκB and other immunity-related effector molecules is a suggested cause of some cancers, cognitive disorders, inflammation, and other diseases of aging. Understanding the mechanisms leading to immune system activation as well as the short- and long-term consequences of such activation on health and lifespan therefore is critical for the development of effective longevity-promoting interventions. Our findings indicate that fat body specific overexpression of a putative pathogen recognition molecule, PGRP-LE, is sufficient for constitutive upregulation of the immune response and for enhanced pathogen resistance to numerous micro-organisms. Primary components of fitness appear to be unaffected by acute activation, but chronic activation leads to an inflammatory state and reduced lifespan. These phenotypes are dependent on NFκB-related transcriptional factor, Relish, and they establish a mechanistic basis for a link between immunity, inflammation, and longevity.

887B

Response to selection for Oxidative Stress using Drosophila melanogaster. Devarati Mukherjee, Yue Wang, Mei-Hui Wang, Wanda Layman, Lawrence Harshman. School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE.

Laboratory selection for survial under oxidative stress was conducted using three different lines derived from an outbred population. Control lines were also started from the same population. Selection for oxidative stress was conducted in a 95% oxygen environment using a glove bag. Selection was conducted for 20 generations. Direct response to selection was measured at generation 15 and 20 for virgin and mated adults. Indirect response to selection such as longevity, fecundity, lipid abundance, soluble protein concentration, response to starvation and desiccation, and development time were also measured.**oxidative stress**.

888C

Role of the coactivator MBF1 in stress and aging. Jan Rynes[1], Marek Jindra[2]. 1) Molecular Biology, University of South Bohemia, Budweis, CZ; 2) Genetics, Biology Center ASCR, South Bohemia, Budweis, CZ.

Multiprotein bridging factor 1 (MBF1) has been characterized as a transcriptional coactivator to the bZIP protein Jun, a nuclear effector and target of the Jun N-terminal Kinase (JNK). Interestingly, MBF1 was shown to mediate Jun-dependent activation specifically in response to oxidative stress (hydrogen peroxide) in human cell lines [Miotto and Struhl 2006, Mol Cell Biol 26:5969-]. We had previously found that *Drosophila* mutants lacking the MBF1 protein were viable but sensitive to hydrogen peroxide and that the MBF1 protein was able to preserve the DNA-binding activity of Drosophila Jun [Jindra et al. 2004, EMBO J 23:3538-]. The effect of JNK signaling on oxidative stress tolerance and lifespan extension [Wang et al. 2003, Dev Cell 5:811-] suggested to us that MBF1 might function to prevent oxidative damage and promote longevity in flies. Our new data show that constitutive overexpression of MBF1 can indeed extend adult lifespan by approximately 20%, while loss of MBF1 leads to short life relative to an isogenic control. Moreover, aging as well as increased temperature cause translocation of the predominantly cytoplasmic MBF1 protein to the nucleus in adult *Drosophila* tissues. These findings, along with recent reports linking MBF1 with enhanced tolerance to heat, drought and bacterial infection in plants [Suzuki et al. 2005, Plant Physiol 139:1313-] suggest a broadly conserved role for MBF1 as a general anti-stress factor.

889A

Deletion of the *Drosophila* homologue of mammalian Herp decreases fly survival in response to ER stress. Nikolaos A. Tountas, Mark E. Fortini. LCDB, NCI-Frederick, Frederick, MD.

The homocysteine-induced endoplasmic reticulum protein (Herp) is an ER-resident membrane protein that contains a ubiquitin-like domain at its N-terminus. The expression of Herp is up-regulated in response to ER stress. Herp has also been shown to interact with the presenilins and enhance the generation of Amyloid β protein in mammalian cell culture experiments. DNA sequence homology searches identified CG14536 as the *Drosophila* homologue of mammalian Herp (dHerp). The dHerp gene maps to the second chromosome and contains 4 exons. We obtained the *Drosophila* line EP(2)2450 from the Szeged collection, which contains a P-element in the 5'UTR of dHerp. Homozygous flies are viable, fertile and produce an mRNA species of the expected size, as detected by Northern blot. We performed P-element excision mutagenesis on EP(2)2450 and generated line Herp29c2 that lacks the 5'UTR, first, and second exons of dHerp. Homozygous Herp29c2 is viable, fertile and does not show any obvious phenotype when maintained at 25°C. Next, we compared the survival rate of line Herp29c2, which presumably lacks dHerp, to that of *Drosophila* line Herp1a1 that was generated by the same P-element excision mutagenesis scheme but retained dHerp. Exposure of Herp1a1 flies to 60μM tunicamycin, an inhibitor of protein N-glycosylation and an ER stress inducer, resulted in 55% survival after 72 hours, while Herp29c2 survival dropped to 27% in the same time period (survival rates are averages of 3 independent experiments). We conclude that deletion of dHerp reduces fly survival in response to ER stress caused by tunicamycin. We intend to test the specificity of this response using different stress inducers and further characterize the function of dHerp. The dHerp-deficient *Drosophila* line that we generated could serve as a model for the study of ER dysfunction as well as diseases with an ER dysfunction component.

This index includes names of speakers for the Opening General Session and for Plenary Sessions I and II and names of all authors of programmed abstracts. The number following an author's name refers to the abstract program number. A, B, or C following a number indicates a poster presentation. The presenting author of an abstract is noted with an asterisk.

C

D

E

F

G

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I

J

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M

N

O

P

Q

R

S

T

The following index is composed of keywords selected by presenting authors from a list in the Conference Call for Abstracts. Abstract program numbers follow each keyword.

■ Cell division and growth control

■ Cytoskeleton and cellular biology

■ Drosophila models of human diseases

■ Evolution and quantitative genetics

■ Gametogenesis and sex determination

■ Genome and chromosome structure

■ Immune system and cell death

■ Neural physiology and behavior

■ Neurogenetics and neural development

■ Organogenesis

■ Pattern formation

■ Physiology and aging

■ Regulation of gene expression

■ Signal transduction

■ Techniques and genomics

This is an index of genes and chromosome aberrations prepared by FlyBase.

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