PROGRAM & ABSTRACTS

16th International Conference
on
ARABIDOPSIS RESEARCH

June 15 – June 19, 2005

University of Wisconsin
Madison, WI

Throughout this **PROGRAM**, the numbers next to abstracts refer to abstract numbers, not the page number in the **ABSTRACT** part of this book.

SESSION OVERVIEW

WEDNESDAY, JUNE 15, 2005	
8:00 - 9:30 pm	SESSION A: Cell Biology
THURSDAY, JUNE 16, 2005	
9:00 - 10:30 am	SESSION B: Cell Signaling
11:00 - 12:30 pm	SESSION C: Development 1 - Flower, Fertilization, Fruit, and Seed
2:00 - 3:30 pm	SESSION D: Development 2 - Shoot and Root
4:00 - 5:30 pm	SESSION E: Metabolism
7:00 - 12:00 am	POSTER SESSION I
FRIDAY, JUNE 17, 2005	
9:00 - 10:30 am	SESSION F: 'omics
11:00 - 12:30 pm	SESSION G: Interaction with the Environment 1 - Abiotic
2:00 - 3:30 pm	SESSION H: Interaction with the Environment 2 - Biotic
7:00 - 12:00 am	POSTER SESSION II
SATURDAY, JUNE 18, 2005	
9:00 - 10:30 am	SESSION I: Genetic and Epigenetic Mechanisms
9:00 am - 12:30 pm	AGA SESSION 1: Evolution and Development
11:00 - 12:30 pm	SESSION J: Novel Tools, Techniques and Resources
2:00 - 5:30 pm	SESSION K (Joint with AGA Session 2): Evolutionary Biology
8:00 - 12:00 am	POSTER SESSION III
SUNDAY, JUNE 19, 2005	
9:00 - 10:30 am	SESSION L: 2010
9:00 am - 12:30 pm	AGA SESSION 3: Quantitative Genetics
11:00 - 12:30 pm	SESSION M: NAASC Choices

Meeting Organizers:

Members of the North American Arabidopsis Steering Committee (NAASC) are serving as the program committee for the 2005 meeting.

Philip Benfey, Duke University
Brenda Winkel-Shirley, Virginia Tech
Greg Copenhaver, University of North Carolina, Chapel Hill
Rob McClung, Dartmouth College
Judith Bender, Johns Hopkins University
Xing-Wang Deng, Yale University
Bonnie Bartel, Rice University, Houston, Texas
Eric Richards, Washington University, St. Louis

Isabell Witt, coordinator for NAASC

The 2005 AGA Symposium, Plant Evolution: Genes and Phenotypes is being organized by **John Doebley,** 2005 President of the AGA.

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American Society of Plant Biologists
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NOTES

PROGRAM OVERVIEW

Poster schedule

All posters will remain up for the entire meeting and can be set up Thursday morning beginning at 7 AM. There will be three poster sessions, one Thursday evening, one Friday evening and one Saturday evening. To determine when you should stand next to your poster:

Find your abstract in this book; your poster number is the number it is assigned in this book, NOT the number it was assigned when you originally submitted it.

All posters with EVEN numbers will be presented on Thursday evening.
All posters with ODD numbers will be presented on Friday evening.

Saturday evening's poster session will be a "free-for-all" – plenty of time to look at all posters, or stand by your own if you need more time for discussion,

Wednesday, June 15, 2005

12:00 noon – 7:00 pm	**Registration**	**Main Lounge**
4:00 - 5:30 pm	**WORKSHOPS I** ***Chair: Rob McClung*** *Molecular Analysis of the Circadian Clock Mechanism*	**Union Theater**
5:30 - 7:00 pm	**Dinner**	**Tripp Commons**
7:00 - 8:00 pm	**KEYNOTE ADDRESS** ***Chris Somerville (Carnegie/Stanford)*** *Energizing Arabidopsis*	**Union Theater**
8:00 - 9:30 pm	**SESSION A:** *Cell Biology*	**Union Theater**
8:00 pm	**Kathy Osteryoung, Michigan State University, Session Chair** Composition and analysis of the chloroplast division machinery	
8:25 pm	**Marisa Otegui, University of Wisconsin** Prevacuolar compartments as proteolytic processing stations for storage proteins in *Arabidopsis*	
8:50 pm	**Tomasz Paciorek, ZMBP Tuebingen, Germany** Auxin inhibits endocytosis and promotes its own efflux from cells	
9:10 pm	**Rajagopal Balasubramanian, Clemson University** A Role for the Actin Cytoskeleton in Hexokinase Mediated Glucose Signaling	

Thursday, June 16, 2005

7:00 am – all day — **Poster set-up** — **See Below Locations**

Great Hall/Reception Room (4th floor)
- Posters of Orals (not including Session Chairs) (#1 – 60)
- 2010 (#61 – 74)
- Cell Biology (#75 – 134)
- Cell Signaling (#135 – 203)
- Databases and Community Resources (#204 – 216)
- Development 1: Flower, Fertilization, Fruit, & Seed (#217 – 308)
- Late Submitted Abstracts: (#660 – 672)

Old Madison/Beefeaters Room (3rd floor)
- Development 2: Shoot and Root (#309 – 388)
- Evolution and Development (AGA) (#389 – 397)
- Evolutionary Biology (#398 – 412)

Tripp Commons (2nd floor)
- Genetic and Epigenetic Mechanisms (#413 – 449)
- Interaction with the Environment 1: Abiotic (#450 – 524)
- Interaction with the Environment 2: Biotic (#525 – 564)

Main Lounge (2nd floor)
- Interaction with the Environment 2: Biotic (#565 – 582)
- Metabolism (583 – 618)
- Novel Tools Techniques and Resources (#619 – 645)
- Proteomics (#646 – 652)
- Quantitative Genetics (AGA) (#653 – 659)

7:00 am - 8:00 pm — **Registration Continues** — **Annex Room**

7:45 - 9:00 am — **Breakfast** — **Tripp Commons**

8:50 - 9:00 am — **Welcome and Announcements** — **Union Theater**

9:00 - 10:30 am — **SESSION B:** — **Union Theater**
Cell Signaling

9:00 am — **Sally Assmann, Penn State University, Session Chair**
Signaling by heterotrimeric and extra-large G proteins in *Arabidopsis* ABA responses

9:25 am — **Kiyotaka Okada, Kyoto University, Japan**
Axis -dependent gene expression in the lateral organ formation

9:50 am — **Li-Sen Young, University of Wisconsin-Madison**
A Mutation in the *Arabidopsis* ADK1 Gene Affects Root Gravitropism, Columella Morphogenesis and Lateral Auxin Transport Across the Root Tip

10:03 am — **Heather Shearer, McMaster University, Canada**
A lipid transfer protein-like protein, DIR1, is involved in long distance signaling during the development of systemic acquired resistance

10:16 am — **Lynn Hartweck, University of Minnesota**
Identification of O-GlcNAc modification of proteins in several signaling pathways

10:30 - 11:00 am — **Refreshment Break** — **Union Theater Lobby**

11:00 - 12:30 pm	**SESSION C:** *Development 1 - flower, fertilization, fruit, and seed*	**Union Theater**
11:00 am	**Rick Amasino, University of Wisconsin, Session Chair** Regulation of Flowering Time and the role of Vernalization	
11:25 am	**George Coupland, Max-Planck Institute for Breeding Research, Germany** The control of flowering by day length in *Arabidopsis*	
11:50 am	**Michitaka Notaguchi, Kyoto University, Japan** Studies on the graft-transmissibility of promotion of flowering by FT in *Arabidopsis*	
12:03 pm	**Melinka A. Butenko** INFLORESCENCE DEFICIENT IN ABSCISSION Controls Floral Organ Abscission in *Arabidopsis*	
12:16 pm	**Moritz Nowack, Max-Planck-Institute for Breeding Research, Germany** A novel positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis	
12:30 - 2:00 pm	**Lunch**	**Tripp Commons**
2:00 - 3:30 pm	**SESSION D:** *Development 2 - shoot and root*	**Union Theater**
2:00 pm	**Gerd Jürgens, ZMBP University of Tuebingen, Session Chair** From embryogenesis to the vegetative plant	
2:25 pm	**Doris Wagner, University of Pennsylvania** Two tales of meristems	
2:50 pm	**Masahiko Furutani, Nara Institute of Science and Technology (NAIST), Japan** The MACCHI-BOU genes regulate organogenesis together with PINOID	
3:03 pm	**Dana MacGregor, University of Chicago** Phosphatidylinositol signaling is involved in the regulation of root system architecture	
3:16 pm	**John Chandler, University of Cologne, Germany** DRN and DRN-LIKE of *Arabidopsis* redundantly control early embryonic patterning through interactions with class III HD-ZIP proteins	
3:30 - 4:00 pm	**Refreshment Break**	**Union Theater Lobby**
4:00 - 5:30 pm	**SESSION E:** *Metabolism*	**Union Theater**
4:00 pm	**Alison Smith, John Innes Centre United Kingdom, Session Chair** Shedding light on metabolism in the dark	
4:25 pm	**Jorge Vivanco, Colorado State University** Root Exudation of Antimicrobials Mediates Pathogen Resistance in *Arabidopsis*	
4:50 pm	**Joost Keurentjes, Wageningen University - Dep. of Genetics, The Netherlands** High throughput metabolomics for the construction of regulatory networks for plant metabolism	
5:03 pm	**Peter Eastmond, University of York, United Kingdom** *Arabidopsis* mutants that are defective in seed storage reserve deposition and mobilization: RDM1 encodes the triacylglycerol lipase that catalyses the first step in storage oil breakdown	

5:16 pm	**Indrani Mukherjee, University Of South Carolina** The FRO3 ferric reductase plays a vital role in iron homeostasis in *Arabidopsis*	
5:30 - 7:00 pm	**Dinner**	**Tripp Commons**
7:00 - 12:00 am	**POSTER SESSION I**	**See Below Locations**

Please present (stand by) your poster if your abstract number in this book is EVEN

Great Hall/Reception Room (4th floor)
- Posters of Orals (not including Session Chairs) (#1 – 60)
- 2010 (#61 – 74)
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- Databases and Community Resources (#204 – 216)
- Development 1: Flower, Fertilization, Fruit, & Seed (#217 – 308)
- Late Submitted Abstracts: (#660 – 672)

Old Madison/Beefeaters Room (3rd floor)
- Development 2: Shoot and Root (#309 – 388)
- Evolution and Development (AGA) (#389 – 397)
- Evolutionary Biology (#398 – 412)

Tripp Commons (2nd floor)
- Genetic and Epigenetic Mechanisms (#413 – 449)
- Interaction with the Environment 1: Abiotic (#450 – 524)
- Interaction with the Environment 2: Biotic (#525 – 564)

Main Lounge (2nd floor)
- Interaction with the Environment 2: Biotic (#565 – 582)
- Metabolism (583 – 618)
- Novel Tools Techniques and Resources (#619 – 645)
- Proteomics (#646 – 652)
- Quantitative Genetics (AGA) (#653 – 659)

Friday, June 17, 2005

7:45 - 9:00 am	**Breakfast**	**Tripp Commons**
9:00 - 10:30 am	**SESSION F:** *'omics*	**Union Theater**
9:00 am	**Natasha Raikhel, University of California Riverside, Session Chair** Exploring Chemical Space in the Plant World	
9:30 am	**Sigrun Reumann, Albrecht-von-Haller-Institute for Plant Sciences, Germany** Studying Novel Plant Peroxisomal Functions by Bioinformatics and Proteomics	
9:50 am	**Moshe Reuveni, ARO, Volcani center, Israel** Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases	
10:10 am	**Linda Walling, University of California** LAPs and DAPs: N-terminal Modifying Enzymes of *Arabidopsis thaliana*	

10:30 - 11:00 am	**Refreshment Break**	**Union Theater Lobby**
11:00 - 12:30 pm	**SESSION G:** *Interaction with the Environment 1 - abiotic*	**Union Theater**
11:00 am	**Rob McClung, Dartmouth College, Session Chair** Abiotic Interactions with the Environment	
11:25 am	**Steve Kay, The Scripps Research Institute** Clocks, photoreceptors and photoperiodism	
11:50 am	**David Salt, Purdue University** Mapping the *Arabidopsis* ionome	
12:03 pm	**Triin Kollist, University of Tartu, Estonia** What can we learn by monitoring rapid O3-induced guard cell responses in *Arabidopsis*?	
12:16 pm	**Stefanie Maruhnich, University of Florida** Two Visual Cycle Homologs, Ccd8/Max4 and a Putative Short-Chain Dehydrogenase/Reductase, are Required for Normal Green Light Responses in *Arabidopsis thaliana*	
12:30 - 2:00 pm	**Lunch**	**Tripp Commons**
2:00 - 3:30 pm	**SESSION H:** *Interaction with the Environment 2 - biotic*	**Union Theater**
2:00 pm	**Barbara Ann Halkier, RVAU Denmark, Session Chair** The potential of engineering natural products to improve disease resistance in *Arabidopsis thaliana*	
2:25 pm	**Jane Glazebrook, University of Minnesota** A Functional Genomics Approach to Disease Resistance Signaling	
2:50 pm	**Adam Bahrami, Harvard University** Pseudomonas syringae manipulates systemic plant defenses against pathogens and herbivores	
3:10 pm	**Remco Van Poecke, University of Minnesota** Efficient discovery of regulatory loci in plant defense by exploitation of natural variation	
3:30 - 4:00 pm	**Refreshment Break**	**Union Theater Lobby**
4:00 - 5:30 pm	**WORKSHOPS II (Concurrent)** **(a) Natural Variation and Comparative Genomics** *Tom Mitchell-Olds*	**Union Theater**
	(b) Getting Databases Talking: Mechanisms to Facilitate Data Integration and Data Mining across the *Arabidopsis* Community *Christopher Town and Heiko Schoof*	**3650 Humanities**
5:30 - 7:00 pm	**Dinner**	**Tripp Commons**

7:00 - 8:30 pm	**TAIR Introductory Workshop: including info on stock ordering, registration**	**3650 Humanities**
7:00 - 8:30 pm	**TAIR: Workshop on Microarray Data/Go Annotations**	**1111 Humanities**
7:00 - 12:00 am	**POSTER SESSION II**	**See Below Locations**

Please present (stand by) your poster if your abstract number in this book is ODD

Great Hall/Reception Room (4th floor)

Posters of Orals (not including Session Chairs) (#1 – 60)
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Interaction with the Environment 2: Biotic (#565 – 582)
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Novel Tools Techniques and Resources (#619 – 645)
Proteomics (#646 – 652)
Quantitative Genetics (AGA) (#653 – 659)

Saturday, June 18, 2005

7:00am – 8:00 pm	**AGA Registration**	**Annex Room**
7:45 - 9:00 am	**Breakfast**	**Tripp Commons**
9:00 - 10:30 am	**SESSION I:** *Genetic and epigenetic mechanisms*	**Union Theater**
9:00 am	**Daphne Preuss, University of Chicago, Session Chair** Genetic and Epigenetic Mechanisms	
9:25 am	**Craig Pikaard, University of Washington** Role of RNA polymerase IV in siRNA-mediated DNA methylation and heterochromatin formation	
9:50 am	**Dmitry Belostotsky, State University of New York at Albany** Mutational and TAP tag-assisted proteomic analyses and inducible RNA interference reveal the role of the *Arabidopsis* exosome in embryo/ endosperm identity and imprinting, functional specialization of its subunits, and novel RNA substrates	
10:10 am	**Renate Schmidt, Max-Planck Institute of Molecular Plant Physiology, Germany** Monitoring transgene silencing in *Arabidopsis* - a broadly applicable, non-invasive and sensitive system	

9:00 am - 12:30 pm	**AGA SESSION 1 (concurrent with Sessions I & J):** *Evolution and Development* ***Christopher Day, University of Wisconsin, Session Chair***	**3650 Humanities**
9:00 am	**Vivian Irish, Yale University** Evolution of MADS box gene function in the angiosperms	
9:45 am	**David Baum, University of Madison** The evolution of inflorescence architecture in Brassicaceae	
11:00 am	**Neelima Sinha, University of California Davis** Transcription factors, gene expression and leaf evolution	
11:45 am	**Alexis Maizel, Max-Planck Institute for Developmental Biology, Germany** Molecular Evolution of LEAFY transcription factor in land plants	
12:05 pm	**Ji-Young Lee, Duke University** Evidence of genetic conservation of diverse nectaries within the eudicots	
10:30 - 11:00 am	**Refreshment Break**	**Union Theater Lobby/3650 Humanities**
11:00 - 12:30 pm	**SESSION J:** *Novel Tools, Techniques and Resources*	**Union Theater**
11:00 am	**Gregory Copenhaver, University of NC at Chapel Hill, Session Chair**	
11:10 am	**Albrecht von Arnim, University of Tennessee** In Vivo Protein-Interaction Assays Based on Bioluminescence Resonance Energy Transfer (BRET)	
11:30 am	**Michael Fromm, University of Nebraska** Isolation of TAP-tagged Protein Complexes From Plants	
11:50 am	**Vladimir Shulaev, VBI Virginia-Tech** Using metabolomics and transcriptomics data to study metabolic networks in *Arabidopsis*	
12:10 pm	**Wolfram Weckwerth, Max-Planck Institute of Molecular Plant Physiology, Germany** Metabolomics and proteomics in *Arabidopsis thaliana*– transitions from pattern recognition to biological interpretation	
12:30 - 2:00 pm	**Lunch**	**Tripp Commons**
2:00 - 5:30 pm	**SESSION K (Joint with AGA Session 2):** *Evolutionary Biology*	**Union Theater**
	David Baum, University of Wisconsin, Session Chair	
2:00 pm	**Michael Purugganan, North Carolina State University** Adaptation and variation in *Arabidopsis* flowering	
2:45 pm	**Magnus Nordborg, University of Southern California** Linkage disequilibrium mapping in *Arabidopsis*	
3:30 - 4:00 pm	**Refreshment Break**	**Union Theater Lobby**
4:00 pm	**Tom Mitchell-Olds, Max-Planck Institute for Chemical Ecology, Germany** Evolution of ecologically important traits in relatives of *Arabidopsis*	
4:45 pm	**Shin-Han Shiu, University of Chicago** Pronounced Expansion of Transcription Factor Families in Plants	

5:05 pm	**Jocelyn Hall, Harvard University** Developmental mechanisms underlying fruit diversification in Brassiceae (Brassicaceae)	
5:30 - 7:00 pm	**Dinner**	**Tripp Commons**
7:00 - 8:00 pm	**Wilhelmine E. Key Lecture, joint with AGA** *June Nasrallah, Cornell* Mating system evolution in crucifers	**Union Theater**
7:00 - 8:30 pm	**TAIR: Accessing and analyzing information about biochemical pathways**	**3650 Humanities**
7:00 - 8:30 pm	**TAIR: Use of Ontologies for Annotating Gene Expression and Phenotypes in *Arabidopsis***	**1111 Humanities**
8:00 - 12:00 am	**POSTER SESSION III**	**See Below Locations**

Free-for-All: A time for further discussions

Great Hall/Reception Room (4th floor)

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Novel Tools Techniques and Resources (#619 – 645)
Proteomics (#646 – 652)
Quantitative Genetics (AGA) (#653 – 659)

Sunday, June 19, 2005

7:45 - 9:00 am	**Breakfast**	**Tripp Commons**
9:00 - 10:30 am	**SESSION L:** *2010*	**Union Theater**
9:00 am	**Machi Dilworth, National Science Foundation, Session Chair**	
9:10 am	**Mary Schuler, University of Illinois Urbana** *Arabidopsis* cytochrome P450 monooxygenases	

9:30 am	**Richard Vierstra, University of Wisconsin** Ubiquitin-Protein Ligase (E3) Families	
9:50 am	**Alice Harmon, University of Florida** The CDPK Superfamily	
10:10 am	**David Meinke, Oklahoma State University** Indispensable Genes Required for Seed Development in *Arabidopsis*	
9:00 am - 12:30 pm	**AGA SESSION 3 (concurrent with Sessions L & M):** *Quantitative Genetics* ***Don Waller, University of Wisconsin, Session Chair***	**3650 Humanities**
9:00 am	**Susan McCouch, Cornell University** Discovery and characterization of alleles associated with domestication-related traits in rice	
9:45 am	**Jeff Conner, Michigan State University** The roles of genetic integration and constraint in adaptive evolution: a floral case study	
11:00 am	**Loren Rieseberg, Indiana University** The Nature of Intrinsic Postzygotic Isolation	
11:45 am	**Mark Rausher, Duke University** Identification of a gene causing reproductive isolation in Phlox	
10:30 - 11:00 am	**Refreshment Break**	**Union Theater Lobby**
11:00 - 12:30 pm	**SESSION M:** *NAASC Choices*	**Union Theater**
11:00 am	**Susan J. Lolle, Purdue University, Session Chair** Non-mendelian inheritance of ancestral sequences in *Arabidopsis*.	
11:30 am	**Sean May, NASC Nottingham stock center, United Kingdom** "Asking for Arrays"	
11:50 am	**Cheng Lu, University of Delaware** Deep profiling by massively parallel signature sequencing elucidates the small RNA component of the transcriptome	
12:10 pm	**Pauline Fung, University of Toronto, Canada** The Pyrabactins: small molecule agonists of the abscisic acid signaling pathway	
12:30 - 2:00 pm	**Lunch**	**Tripp Commons**

NOTES

PROGRAM

KEYNOTE SPEAKER

Abstract #1

1 **Energizing *Arabidopsis***
Chris Somerville509

WEDNESDAY, JUNE 15

8:00 - 9:30 p.m. – Union Theater

Session A

Cell Biology

Kathy Osteryoung: Chair

Abstracts #2-5

2 **Composition and analysis of the chloroplast division machinery**
Katherine Osteryoung

3 **Prevacuolar compartments as proteolytic processing stations for storage proteins in *Arabidopsis***
Marisa Otegui, Rachel Herder, L Staehelin

4 **Auxin inhibits endocytosis and promotes its own efflux from cells**
Tomasz Paciorek, Eva Zazimalova, Jan Petrasek, York-Dieter Stierhof, Juergen Kleine-Vehn, David Morris, Gerd Juergens, Niko Geldner, Jiri Friml

5 **A Role for the Actin Cytoskeleton in Hexokinase Mediated Glucose Signaling**
Rajagopal Balasubramanian, Abhijit Karve, Muthugapatti Kandasamy, Richard Meagher, Brandon Moore

THURSDAY, JUNE 16

9:00 - 10:30 a.m. – Union Theater

Session B

Cell Signaling

Sally Assmann: Chair

Abstracts #6-10

6 **Signaling by heterotrimeric and extra-large G proteins in *Arabidopsis* ABA responses**
Sarah Assmann, Lei Ding, Sona Pandey, Liza Wilson, Caroline Gibson

7 **Axis -dependent gene expression in the lateral organ formation**
Kiyotaka Okada, Keiro Watanabe, Seiji Takeda, Shunji Funaki, Yuhei Tsuchida, Taisuke Nishimura, Ryuji Tsugeki, Noritaka Matsumoto

8 **A Mutation in the *Arabidopsis* ADK1 Gene Affects Root Gravitropism, Columella Morphogenesis and Lateral Auxin Transport Across the Root Tip**
Li-Sen Young, Benjamin Harrison, Barabara Moffatt, Patrick Masson

9 **A lipid transfer protein-like protein, DIR1, is involved in long distance signaling during the development of systemic acquired resistance**
Heather Shearer, Asif Mohammad, Melody Neumann, Karen Haines, Zhiying Zhao, Robin Cameron

10 **Identification of O-GlcNAc modification of proteins in several signalling pathways**
Lynn Hartweck, Cheryl Scott, Peter Matusmoto, D Chen, S Juarez, J Alamillo, C Simon-Mateo, J Perez, Juan Garcia, Neil Olszewski

THURSDAY, JUNE 16

11:00 - 12:30 p.m. – Union Theater

Session C

Development 1 - Flower, Fertilization, Fruit, and Seed

Rick Amasino: Chair

Abstracts #11-15

11 **Regulation of Flowering Time and the role of Vernalization**
Richard Amasino

12 **The control of flowering by day length in *Arabidopsis***
George Coupland, Laurent Corbesier, Coral Vincent, Iain Searle, Frederic Cremer, Reka Toth, Antonis Giakountis, Dean Ravenscroft, Franziska Turck, Jose Gentilhomme, Hugo Konijn, Ryosuke Hayama, Seonghoe Jang , Stephan Wenkel

13 **Studies on the graft-transmissibility of promotion of flowering by *FT* in *Arabidopsis***
Michitaka Notaguchi, Yasufumi Daimon, Mitsutomo Abe, Takashi Araki

14 ***INFLORESCENCE DEFICIENT IN ABSCISSION* Controls Floral Organ Abscission in *Arabidopsis***
Melinka A. Butenko, Grethe-Elisabeth Stenvik, Reidunn B. Aalen

15 **A novel positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis**
Moritz Nowack, Paul Grini, Marc Jakoby, Marcel Lafos, Csaba Koncz, Arp Schnittger

THURSDAY, JUNE 16

2:00 - 3:30 p.m. – Union Theater

Session D

Development 2 - Shoot and Root

Gerd Jürgens: Chair

Abstracts #16-20

16 **From embryogenesis to the vegetative plant**
Gerd Juergens

17 **Two tales of meristems**
Chang Seob Kwon, Dilusha William, Louis Saddic, Meina Lu, Yanhui Su

18 **The *MACCHI-BOU* genes regulate organogenesis together with *PINOID***
Masahiko Furutani, Masao Tasaka

19 **Phosphatidylinositol signaling is involved in the regulation of root system architecture**
Dana MacGregor, Paul Brannon, Jee Jung, Karen Deak, Jocelyn Malamy

20 **DRN and DRN-LIKE of *Arabidopsis* redundantly control early embryonic patterning through interactions with class III HD-ZIP proteins**
John Chandler, Melanie Cole, Britta Grewe, Annegret Flier, Wolfgang Werr

THURSDAY, JUNE 16

4:00 - 5:30 p.m. – Union Theater

Session E

Metabolism

Alison Smith: Chair

Abstracts #21-25

21 **Shedding light on metabolism in the dark**
Alison Smith

22 **Root Exudation of Antimicrobials Mediates Pathogen**
Jorge Vivanco

23 **High throughput metabolomics for the construction of regulatory networks for plant metabolism**
Joost Keurentjes, Ric de Vos, Jingyuan Fu, Ritsert Jansen, Dick Vreugdenhil, Maarten Koornneef

24 ***Arabidopsis* mutants that are defective in seed storage reserve deposition and mobilization: *RDM1* encodes the triacylglycerol lipase that catalyses the first step in storage oil breakdown**
Peter Eastmond

25 **The FRO3 ferric reductase plays a vital role in iron homeostasis in *Arabidopsis***
Indrani Mukherjee, Nathan Campbell, Erin Connolly

FRIDAY, JUNE 17
9:00 - 10:30 a.m. – Union Theater
Session F
'omics
Natasha Raikhel: Chair
Abstracts #26-29

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26 **Exploring Chemical Space in the Plant World**
Natasha Raikhel
27 **Studying Novel Plant Peroxisomal Functions by Bioinformatics and Proteomics**
Lavanya Babujee, Franziska Lueder, Changle Ma, Hartmut Kratzin, Virginie Wurtz, Sigrun Reumann
28 **Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases**
Moshe Reuveni, Patricia Kane
29 **LAPs and DAPs: N-terminal Modifying Enzymes of *Arabidopsis thaliana***
SangYoul Park, Ben Dunn, Linda Walling

FRIDAY, JUNE 17
11:00 - 12:30 p.m. – Union Theater
Session G
Interaction with the Environment 1 - Abiotic
Rob McClung: Chair
Abstracts 30-34

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30 **Abiotic Interactions with the Environment**
C. Robertson McClung
31 **Clocks, photoreceptiors and photoperiodism**
Steve A. Kay, Ghislain Breton, Frank G. Harmon, Samuel P. Hazen, Takato Imaizumi, Alessia Para, Jose Pruneda-Paz, Thomas F. Schultz
32 **Mapping the *Arabidopsis* ionome**
David Salt, Ana Rus, Brett Lahner, Elena Yakubov, Ivan Baxter
33 **What can we learn by monitoring rapid O3-induced guard cell responses in *Arabidopsis*?**
Triin Kollist, Heino Moldau, Jaakko Kangasjarvi, Hannes Kollist
34 **Two Visual Cycle Homologs, *Ccd8/Max4* and a Putative Short-Chain Dehydrogenase/Reductase, are Required for Normal Green Light Responses in *Arabidopsis thaliana***
Stefanie Maruhnich, Dawn Bies, Kevin Folta

FRIDAY, JUNE 17
2:00 - 3:30 p.m. – Union Theater
Session H
Interaction with the Environment 2 - Biotic
Barbara Ann Halkier: Chair
Abstracts 35-38

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35 **The potential of engineering natural products to improve disease resistance in *Arabidopsis thaliana*.**
Barbara Ann Halkier, Majse Nafisi, Bjarne Hansen, Gunter Brader, E. Palva
36 **A Functional Genomics Approach to Disease Resistance Signaling**
Jane Glazebrook, Raka Mitra, Ingrid Peterson, Masanao Sato, Lin Wang, Fumiaki Katagiri
37 ***Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores**
Adam Bahrami, Jianping Cui, Elizabeth Pringle, Gustavo Hernandez-Guzman, Carol Bender, Naomi Pierce, Fredrick Ausubel
38 **Efficient discovery of regulatory loci in plant defense by exploitation of natural variation**
Remco Van Poecke, Lisa Lenarz-Wyatt, Fumiaki Katagiri

SATURDAY, JUNE 18

9:00 - 10:30 a.m. – Union Theater

Session I

Genetic and Epigenetic Mechanisms

Daphne Preuss: Chair

Abstracts 39-42

39 Genetic and Epigenetic Mechanisms
Andy Cal, Anne Hall, Sarah Hall, Song Luo, Bonnie Scott, Jay Shrestha, Daphne Preuss

40 Role of RNA polymerase IV in siRNA-mediated DNA methylation and heterochromatin formation
Thomas Ream, Jeremy Haag, Yasuyuki Onodera, Olga Pontes, Pedro Costa Nunes, Craig Pikaard

41 Mutational and TAP tag-assisted proteomic analyses and inducible RNA interference reveal the role of the *Arabidopsis* exosome in embryo/endosperm identity and imprinting, functional specialization of its subunits, and novel RNA substrates
Sergei Reverdatto, Julia Chekanova, Nikolai Skiba, Jose Alonso, Vladimir Brukhin, Joseph Ecker, Ueli Grossniklaus, Dmitry Belostotsky

42 Monitoring transgene silencing in *Arabidopsis* - a broadly applicable, non-invasive and sensitive system
Renate Schmidt, Isabell Witt, Berthold Lechtenberg

SATURDAY, JUNE 18

9:00 - 12:30 p.m. – 3650 Humanities

AGA Session 1 (concurrent with Sessions I & J)

Evolution and Development

Christopher Day: Chair

Abstracts 673-677

673 Comparative Genomics of Angiosperm MADS Box Genes
Vivian Irish

674 The evolution of inflorescence architecture in Brassicaceae
David Baum

675 The role of KNOX genes in shoot morphogenesis and compound leaf development
Neelima Sinha

676 Molecular Evolution of LEAFY transcription factor in land plants
Alexis Maizel

677 Evidence of genetic conservation of diverse nectaries within the eudicots
Ji-Young Lee

SATURDAY, JUNE 18

11:00 - 12:30 p.m. – Union Theater

Session J

Novel Tools, Techniques and Resources

Gregory Copenhaver: Chair

Abstracts 43-46

43 In Vivo Protein-Interaction Assays Based on Bioluminescence Resonance Energy Transfer (BRET)
Chitra Subramanian, Jong-Chan Woo, Fujun Zhou, Xiaodong Xu, Carl Johnson, Albrecht von Arnim

44 Isolation of TAP-tagged Protein Complexes From Plants
Michael Fromm

45 Using metabolomics and transcriptomics data to study metabolic networks in *Arabidopsis*
Vladimir Shulaev

46 Metabolomics and proteomics in *Arabidopsis thaliana*– transitions from pattern recognition to biological interpretation
Wolfram Weckwerth

SATURDAY, JUNE 18

2:00 - 5:30 p.m. – Union Theater

Sessions K (Joint with AGA Session 2)

Evolutionary Biology

David Baum: Chair

Abstracts 47-51

47 Adaptation and variation in *Arabidopsis* flowering
Michael Purugganan

48 Linkage disequilibrium mapping in *Arabidopsis*
Sung Kim, Keyan Zhao, Maria Jose Aranzana, Erica Bakker, Katrin Jakob, Clare Lister, John Molitor, Chikako Shindo, Chunlao Tang, Brian Traw, Honggang Zheng, Fengzhu Sun, Joy Bergelson, Caroline Dean, Paul Marjoram, Magnus Nordborg

49 Evolution of ecologically important traits in relatives of *Arabidopsis*
Thomas Mitchell-Olds, Juergen Kroymann, Maria Clauss, and Andrew Heidel

50 Pronounced Expansion of Transcription Factor Families in Plants
Shin-Han Shiu, Ming-Che Shih, Wen-Hsiung Li

51 Developmental mechanisms underlying fruit diversification in Brassiceae (Brassicaceae)
Jocelyn Hall, Kathleen Donohue, Elena Kramer

SATURDAY, JUNE 18

7:00 - 8:00 p.m. – Union Theater

Wilhelmine E. Key Lecture (Joint with AGA)

Abstract 52

52 Evolution of mating systems in *Arabidopsis*: from outcrossing to selfing and back
June Nasrallah

SUNDAY, JUNE 19

9:00 - 10:30 a.m. – Union Theater

Session L (Concurrent with AGA Session 3)

2010

Machi Dilworth: Chair

Abstracts 53-56

53 *Arabidopsis* cytochrome P450 monooxygenases
Mary Schuler, Hui Duan, Shahjahan Ali, Sanjeewa Rupasinghe, Jyothi Thimmapuram, Natanya Civjan, Mark Band, Matthew Hudson, Daniele Werck-Reichhart, Stephen Sligar

54 FUNCTIONAL ANALYSIS OF THE UBIQUITIN-PROTEIN LIGASE (E3) FAMILIES IN *ARABIDOPSIS*
Richard Vierstra, Judy Callis, Xing-Wang Deng, Mark Estelle, Michael Gribskov

55 Analysis of the Arabidopis CDPK Superfamily
John Cushman, Jeffrey Harper, Estelle Hrabak, Michael Sussman, Alice Harmon

56 Indispensable Genes Required for Seed Development in *Arabidopsis*
David Meinke, Allan Dickerman

SUNDAY, JUNE 19

9:00 - 12:30 p.m. – 3650 Humanities

AGA Session 3 (concurrent with Sessions L & M)

Quantative Genetics

Don Waller: Chair

Abstracts 678-681

678 Discovery and characterization of alleles associated with domestication related traits in rice
Susan McCouch

679 The roles of genetic integration and constraint in adaptive evolution: afloral case study
Jeff Conner

680 The Nature of Intrinsic Postzygotic Isolation
Loren Rieseberg

681 Identification of a gene causing reproductive isolation in Phlox
Mark Rausher

SUNDAY, JUNE 19

11:00 - 12:30 p.m. – Union Theater

Session M (Concurrent with AGA Session 3)

NAASC Choices

Susan J. Lolle: Chair

Abstracts 57-60

57 Non-mendelian inheritance of ancestral sequences in *Arabidopsis*.
Robert Pruitt , Susan Lolle

58 Asking for Arrays.
Sean May

59 Deep profiling by massively parallel signature sequencing elucidates the small RNA component of the transcriptome
Cheng Lu, Shivakundan Tej, Shujun Luo, Christian Haudenschild, Blake Meyers, Pamela Green

60 The Pyrabactins: small molecule agonists of the abscisic acid signaling pathway
Pauline Fung, Freeman Chow, Bennet Deakin, Yang Zhao, Sean Cutler

POSTER ABSTRACTS

Great Hall/Reception Room (4th floor)

Topic: 2010

Abstracts 61-74

61 Role of the ubiquitin E3 ligase SCFAtSKP2 in cell proliferation
Silvia Jurado, Sara Diaz-Trivino, Crisanto Gutierrez, Carlos del Pozo

62 Comprehensive analysis of protein-protein interactions within the cytokinin signaling pathway in *Arabidopsis thaliana*
Hakan Dortay, Nijuscha Mehnert, Lukas Burkle, Thomas Schmulling, Alexander Heyl

63 Molecular definitions of cytochrome P450s in *Arabidopsis thaliana*
Sanjeewa Rupasinghe, Hui Duan, Natanya Civjan, Shahjahan Ali, Mary Schuler

64 Variations in *CYP74B2* (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg ecotypes of *Arabidopsis*
Hui Duan, Meng-Yu Huang, Kathryn Palacio, Mary Schuler

65 Generation & characterization of gene- & enhancer-trap transposon insertion lines in *Arabidopsis thaliana*
Cai-Ping Feng, John Mundy

66 *Arabidopsis* gene networks regulated by interactions between C and N
Rodrigo Gutierrez, Laurence Lejay, Dennis Shasha, Gloria Coruzzi

67 Protein-protein interactions involving *Arabidopsis* polyadenylation factor subunits: Towards an understanding of the architecture of the polyadenylation apparatus and its associations with other processes in plants
Arthur Hunt, Kevin Forbes, Balasubrahmanyam Addepalli, Srinivasa Rao

68 Functional analysis of the *Arabidopsis* NRL (NPH3/RPT2-like) protein family
Sunjoo Joo, Johanna Morrow, Renee Harper, Bethany Stone, Emmanuel Liscum

69 *Arabidopsis* mRNA Polyadenylation: New Poly(A) Signals, and the Role of CPSF in Sexual Development
Quinn Li, Johnny Loke, Ruqiang Xu, Hongwei Zhao

70 Coiled-coil Protein Composition of 22 Proteomes: Differences and Common Themes in Subcellular Infrastructure and Traffic Control
Annkatrin Rose, Shannon Schraegle, Eric Stahlberg, Iris Meier

71 **At the Cutting Edge of Plant Biology Education: Engaging University and High School Biology Students in *Arabidopsis* 2010 Functional Genomics Research**
James Stark, Patricia Springer, Adan Colon-Carmona

72 **Functional Analysis of the RING-type Ubiquitin Ligase Family of *Arabidopsis*: A novel RING domain-containing protein is essential for growth and development**
Sophia Stone , Luis Williams, Judy Callis, Michael Kerber , Edward Kraft

73 **Functional genomics of *Arabidopsis* defense responses against *Pseudomonas syringae***
Lin Wang, Jane Glazebrook

74 **Novel gene discovery in the *Arabidopsis* genome**
Yong-Li Xiao, Beverly Underwood, William Moskal, Hank Wu, Wei Wang, Julia Redman, Erin Monaghan, Christopher Town

POSTER ABSTRACTS
Great Hall/Reception Room (4th floor)
Topic: Cell Biology
Abstracts 75-134

75 ***Arabidopsis* point mutants *aggregates of GFP fluorescence-1* and *bubblebath-1* are defective in vacuole biogenesis and gravitropism**
April Agee, Glenn Hicks, David Carter, Natasha Raikhel

76 **Prolamellar body membrane formation and gene expression in *Arabidopsis***
Gregory Armstrong, Maria del Rosario Barbieri, Mary Mason

77 **Simple C-terminal targeting sequences are statistically over-represented across protein families: identifying known and novel peptide signatures with computational genomics**
Ryan Austin, Nicholas Provart, Sean Cutler

78 **Subcellular location of Xylem Cysteine Proteinase 2 (XCP2) within differentiating tracheary elements of *Arabidopsis thaliana* determined by use of microwave-assisted sample processing for electron microscopy**
Utku Avci, Eric Beers, Candace Haigler

79 **Use of an *Arabidopsis* CAD mutant to determine the function of CADs of *Arabidopsis* and tree origin**
Aymerick Eudes, Richard Sibout, Armand Seguin, Brigitte Pollet, Catherine Lapierre, Lise Jouanin

80 **AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana***
Yan Xiong, Diane Bassham

81 **Genetic dissection of ARP2/3 functions in *Arabidopsis***
Steven Brankle, Jie Le, Taisiya Zakharova, Dan Szymanski

82 **Direct Interaction of a Divergent CaM Isoform and the Transcription Factor, MYB2, Enhances Salt Tolerance in *Arabidopsis***
Woo Sik Chung, Jae Hyuk Yoo

83 **The *mum5*mutant has a mutation in a putative pectin methylesterase**
Michelle Facette, Chris Somerville

84 **The *Arabidopsis* PEX12 gene is required for peroxisome biogenesis and essential for development**
Jilian Fan, Sheng Quan, Travis Orth, Chie Awai, Joanne Chory, Jianping Hu

85 **A novel cross-linking strategy aimed at investigating precursor interactions with the chloroplastic Tic complex and with stromal molecular chaperones**
John Froehlich, Kenneth Keegstra

86 **Characterization of the anti-microtubule drug supersensitive *Arabidopsis* mutant 28-2b**
Charitha Galva, Alex Paredez, John Sedbrook

87 **FZL, an FZO-like protein in plants, links thylakoid morphogenesis to chloroplast division**
Hongbo Gao, Hongbo Gao, Katherine Osteryoung

88 **A structure-function study of COP9 Signalosome subunit 7**
Yair Halimi, Moshe Dessau, Nurit Levanon, Joel Hirsch, Daniel Chamovitz

89 **Formation and function of prevacuolar compartments in the *Arabidopsis* embryo**
Rachel Herder, Marisa Otegui

90 **AtTBPs in *Arabidopsis* encode proteins that bind plant telomeric DNA and induce DNA bending in vitro**
Moo Gak Hwang, Myeon Haeng Cho

91 **AtCYCA2;3, a key regulator in the termination of endoreduplication rounds in *Arabidopsis***
Kumiko Imai, Yohei Ohashi, Tomohiko Tsuge, Takeshi Yoshizumi, Minami Matsui, Atsuhiro Oka, Takashi Aoyama

116 The plant UBX-domain containing (PUX) protein family regulates the function of *Arabidopsis* CDC48, a conserved essential AAA-ATPase
Rebecca Posthuma, David Rancour, Sookhee Park, Barbara Bates, Sebastian Bednarek

117 Interfering with *MOR1* expression causes microtubule disruption and abnormal morphological development
Madeleine Rashbrooke, David Collings, Geoff Wasteneys

118 Identification and Functional Analysis of Two Small Heat-Shock Proteins from Plant Peroxisomes
Changle Ma, Martin Haslbeck, Lavanya Babujee, Sigrun Reumann

119 The RHL1-RHL2-RHL3 protein complex is required for plant endoreduplication
Gethin Roberts, Nicola Stacey, Christian Roberts, Anthony Maxwell, Keith Roberts, Keiko Sugimoto-Shirasu

120 The role of SNAREs and vesicle trafficking in plant cytokinesis and cell-plate formation
Anton Sanderfoot, Burak Ozkosem

121 Genetic Analysis of SECY1 and SECY2 Function
Courtney A. Skalitzky, Jessica A. Hankinson, Gregory R. Heck, Donna E. Fernandez

122 Chemical genomics of the endomembrane system in *Arabidopsis*
Marcela Rojas-Pierce, Eun-Ju (Julie) Sohn, Natasha Raikhel

123 Characterization of SIAMESE, a putative cell cycle regulator involved in endoreplication
Michelle Speckhart, Matt Brown, Viktor Kirik, Martin Hulskamp, Dirk Inze, Lieven De Veylder, Jason Walker, Taylor Gwin, Jason Churchman, John Larkin

124 Analysis of monoubiquitin-dependent protein degradation in *Arabidopsis*
Christoph Spitzer, Aneta Bijelovic, Swen Schellmann, Martin Huelskamp

125 Determining which glutamate receptors mediate depolarization responses to glutamate and glycine in *Arabidopsis* root and hypocotyl cells
Nicholas Stephens, Qi Zhi, Edgar Spalding

126 The Power Of Chemical Genomics To Study the Link Between Endomembrane System Components and the Gravitropic Response: Target Identification Studies With 2-Anilinophenazine
Marci Surpin, Marcela Rojas-Pierce, Jacob Vasquez, Natasha Raikhel

127 Autophagic Nutrient Recycling in Arabdiopsis *thaliana* Directed by the ATG8 and ATG12 Conjugation Pathways
Allison Thompson, Jed Doelling, Anongpat Suttangkakul, Richard Vierstra

128 *PARTING DANCERS*, a gene, requires for the homologous recombination during meiotic prophase I in *Arabidopsis thaliana*
Wei Zhang, Changbin Chen, Hong Ma, Asela Wijeratne, Ljudmilla Timofejeva

129 Expression and function analyses of the AtPLDzeta2 gene
Yukimi Yamamoto, Yohei Ohashi, Atsuhiro Oka, Takashi Aoyama

130 Analysis of Exo70 exocyst subunits family function in plants
Lukas Synek, Marek Elias, Edita Drdova, Michael Quentin, Marie-Theres Hauser, Viktor Zarsky

131 Composition and Function of the *Arabidopsis* WAVE Complex During Epidermal Morphogenesis
Chunhua Zhang, Steven Brankle, Eileen Mallery, Daniel B Szymanski

132 The *Arabidopsis ROCK-N-ROLLERS* gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation
Changbin Chen, Wei Zhang, Ljudmilla Timofejeva, Hong Ma

133 A Novel Genetic Screen to Identify Chloroplast Import Mutants
Rong Zhong, Gayle Lamppa

134 Disparate Roles for the Regulatory A Subunits in *Arabidopsis* PP2A
Hong-Wei Zhou, Tina Simolari, Sylvia Cho, Michael Clarke-Pearson, Alison DeLong

POSTER ABSTRACTS

Great Hall/Reception Room (4th floor)

Topic: Cell Signalling

Abstracts 135-203

135 *Arabidopsis* SERK1 receptor recycling mediated by CDC48A
Jose Aker, Rumyana Karlova, jan-Willem Borst, Sacco de Vries

136 Expression of Response Regulators during reproductive Development in *Arabidopsis*
Monica Alandete-Saez, Stefano Gattolin, Corinna Powell, Erold Naomab, Zinnia Gonzalez-Carranza, Jeremy Roberts

137 SPIKE1, a DOCK-family protein, is a Guanine Nucleotide Exchange Factor for Rho Of Plants (ROP) and positively regulates the WAVE-ARP2/3 pathway
Dipanwita Basu, Eileen Mallery, Daniel Szymanski

138 Functional characterization of OST1 kinase : a key element of ABA signalling pathway in *Arabidopsis* guard cells
Christophe Belin, Pierre-Olivier de Franco, Stephane Chaignepain, Jean-Marie Schmitter, Helene Barbier-Brygoo, Sebastien Thomine

139 Accumulation and biological activity of enzymatically and non-enzymatically formed oxylipins
Susanne Berger, Christoph Grun, Katharina Dueckershoff, Daniel Matthes, Christiane Loeffler, Bianca Buettner, Joerg Durner, Martin Mueller

140 Ethylene Signaling and EIN3 Protein Levels in *Arabidopsis*
Richard Vierstra, Brad Binder, Anthony Bleecker, Jennifer Gagne, Georg Hemmann

141 The Protein Kinase Genes AtMAP3Kepsilon1 and AtMAP3Kepsilon2 are Required for Pollen Development in *Arabidopsis thaliana*
Suraphon Chaiwongsar, Peter Jester, Sean Monson, Patrick Krysan

142 *EER2* encodes a novel factor required for proper modulation of ethylene responses in *Arabidopsis* leaves and etiolated seedlings
Matthew Christians, Paul Larsen

143 Withdrawn

144 Photobiological and Electrophysiological Studies of Glutamate-Like Receptors in Etiolated Hypocotyls of *Arabidopsis*
Tessa Durham , Edgar Spalding

145 Transcriptional and posttranscriptional regulation of RNS1 in response to wounding and ABA stress
Melissa Feile, Nicole LeBrasseur, Pamela Green, Gustavo MacIntosh

146 Identification of Movement Domains in SHORT-ROOT
Kimberly Gallagher, Philip Benfey

147 Protein-protein interaction involving of ethylene receptor of *Arabidopsis*
Zhiyong Gao, Yi-Feng Chen, G. Eric Schaller

148 Genetic and Physiological Analysis of the Brassinosteroid Transcriptional Regulator, BZR1
Joshua Gendron, Nathan Gendron, Catherine Sun, Zhi-Yong Wang

149 The role of light-mediated RNA degradation in the regulation of the *Arabidopsis* circadian clock
Esther Yakir, Rachel Green

150 *Arabidopsis* CSN5A and CSN5B subunits are present in distinct COP9 signalosome complexes that play unequal role on plant development
Giuliana Gusmaroli, Suhua Feng, Xing Wang Deng

151 Combining genomics and reverse genetics to identify and characterize components in sugar-response pathways
Tim Heisel, Chunyao Li, Kat Larson, Sue Gibson

152 Sugars and Phytohormones Regulate Seed Germination and Early Seedling Development via a Complex Signaling Network
Yadong Huang, Donna Pattison, Sue Gibson

153 Multiple Tyrosine Residues Are Critical In Gibberellin-Sensitivity Of DELLA Protein, RGL2
Alamgir Hussain, Dongni Cao, Jinrong Peng

154 Isolation and characterization of a bZIP transcription factor from *Arabidopsis* regulating the endoplasmic reticulum stress response
Yuji Iwata, Nozomu Koizumi

155 Degradation of the Auxin Response Factor, ARF1
Jemma Jowett, Jason Ramos, Judy Callis

156 MALDI-TOF/MS based identification of proteins present in the *Arabidopsis* Somatic Embryogenesis Receptor-like kinase 1 complex
Rumyana Karlova, Sjef Boeren, Eugenia Russinova, Jose Aker, Jacques Vervoort , Sacco de Vries

157 GCR1, GPA1 and prephenate dehydratase are required for blue light-induced production of phenylalanine in etiolated *Arabidopsis*
Katherine Warpeha, Yevgeniya Lapik, Lon Kaufman

158 The GCR1, GPA1, Pirin1, NF-Y-A5/B9 signal chain mediates both blue light and ABA responses in *Arabidopsis*
Katherine Warpeha, Samuel Hawkins, Yevgeniya Lapik, Marybeth Anderson, Jennifer Yeh, Snehqli Upadhyay, Lon Kaufman

159 Cloning and characterization of FB1-resistant (*fbr*) mutants from activation tagging populations
Sadaf Khan, Julie Stone

160 Loss of function mutation of COBRA, which is a determinant of oriented cell expansion, invokes cellular defense mechanism in *Arabidopsis thaliana*
Jae-Heung Ko, Jeong-Hoe Kim, Sastry Jayanty, Gregg Howe, Kyung-Hwan Han

161 CTR1, a MAPKKK that is a negative regulator of the ethylene-signaling pathway, is a target of phosphatidic acid
Paul Larsen, Christa Testerink, Dieuwertje van der Does, John van Himbergen, Teun Munnik

162 WRKY target genes of WRKY53 transcription factor during leaf senescence in *Arabidopsis thaliana*
Thomas Laun, Ying Miao, Ulrike Zentraf

163 Non-cell-autonomous Signaling and Developmental Control Mediated by AtPAPK1
Jung-Youn Lee, Gili Ben-Nissan

164 PINOID acts as a positive regulator for auxin efflux
Sang Ho Lee, Hyung-Taeg Cho

165 Effects of Increased ER-localized Ca2+on Recovery From Osmotic Stress
Sang-Yoon Lee, Heike Winter-Sederoff, Niki Robertson

166 Modulation of Brassinosteroid Signaling by an Atypical Basic Helix-Loop-Helix Protein
Hao Wang, Bin Kang, Shozo Fujioka, Jia-Yang Li, Jianming Li

167 Hormonal cross-talk and signalling to the *Arabidopsis* microtubule cytoskeleton requires the POLARIS peptide
Stuart Casson, Keith Lindsey, Paul Chilley, Petr Tarakowski, Nathan Hawkins, Kevin Wang, Patrick Hussey, Mike Beale, Joe Ecker, Goran Sandberg

168 Plant Responses to Stresses: Transcript Profiling of Stress/Defense-Related Genes of *Arabidopsis thaliana*
Shahina Maqbool, Surabhi Raina, Guru Jagadeeswaran, Biswa Acharya, Inder Singh, Ritu Mukherjee, Heidi Appel, Jack Schultz, Ramesh Raina

169 Auxin and TAC1 regulate telomerase through a novel ubiquitin ligase
Shuxin Ren, Thomas McKnight

170 Identification of genes required for Age-Related Resistance response using a Microarray/Reverse Genetics approach
Asif Mohammad, F. Al-daoud, J. Carviel, H. Shearer, M. Neumann, Robin Cameron

171 Functional differentiation of Aux/IAA proteins, IAA7 and IAA19, involved in auxin responses in *Arabidopsis*
Daisuke Nakamoto, Hideki Muto, Kotaro Yamamoto

172 Defining a Role for Members of the *Arabidopsis* Aux/IAA Gene Family
Sam Nalle, Susan Bush, Julia Adams, Rachel Nelson, Paul Overvoorde

173 brassinosteroids signaling through the multi-heterodimerization between two different families of leucine rich-repeats receptor-like kinases
Yu Jeong Jeong, Hyun Mi Park, Bong Mi Kim, June Seung Lee, Jianming Li, Kyoung Hee Nam

174 Activation-tagged suppressors of *phyB-4* identify points of cross-talk between hormones and photomorphogenesis
Michael Neff, Shozo Fujioka, Girish Murthy, Suguru Takatsuto, Mary Schuler, Yukihisa Shimada, Hideharu Seto, Leeann Thornton, Edward Turk, John Walker, Huachun Wang, Shigeo Yoshida, Jingyu Zhang

175 ginC, a new locus regulating glucose responses
Victoria Lumbreras, Montserrat Pages

176 Structure-Function Studies of *Arabidopsis thaliana* NPH3
Ullas Pedmale, Johanna Harris, Renee Harper, Andrei Motchoulski, Mannie Liscum

177 Tissue specific combinatorial control of the ABA response in seeds revealed by expression analysis of the endosperm during germination
Steven Penfield, Li Yi, Alison Gilday, Stuart Graham, Ian Graham

178 WVD2, a novel microtubule binding protein affecting plant anisotropic cell expansion, morphology and helical handedness
Robyn Perrin, Jessica Will, Yan Wang, Christen Yuen, Patrick Masson

POSTER ABSTRACTS

Great Hall/Reception Room (4th floor)

Topic: Databases and Community Resources

Abstracts 204-216

204 Industry Collaboration and the Novelty and Persistence of *Arabidopsis* Research
James Evans

205 AraCyc and MetaCyc: databases to survey the metabolic network in plants and microorganisms
Hartmut Foerster, Peifen Zhang, Christophe Tissier, Ron Caspi, Carol Fulcher, Becky Hopkinson, Pallavi Kaipa, Markus Krummenacker, Suzanne Paley, John Pick, Peter Karp, Seung Rhee

206 Latest Developments on the Microarray Data Available at TAIR
Margarita Garcia-Hernandez, Nick Moseyko, Neil Miller, Mary Montoya, Iris Xu, Dan Weems, Brandon Zoeckler, Seung Rhee, Eva Huala

207 Controlled Vocabularies For Describing And Comparing Phenotypes And Gene Expression In Angiosperms
Leonore Reiser, Katica Ilic, Felipe Zapata, Peter Stevens, Shulamit Avraham, Anuradha Pujar, Leszek Vincent, Marty Sachs, Pankaj Jaiswal, Doreen Ware, Elizabeth Kellogg, Mary Polacco, Seung Rhee, Susan McCouch, Lincoln Stein

208 Report on resource project in RIKEN BRC
Masatomo Kobayashi, Hiroshi Abe, Satoshi Iuchi, Toshihiro Kobayashi

209 Introducing IGB for *Arabidopsis*: a resource for *Arabidopsis* genome data visualization
Ann Loraine, Gregg Helt, Ed Erwin, Sue Rhee, Eva Huala

210 NASC Germplasm Resources and Ontologies
Sean May

211 Oligogo, A Web Based Primer Design Site for *Arabidopsis* Gene Families
Ronan O'Malley, Govindraj Chandrasek, Sara Patterson, Anthony Bleecker

212 CAPSGEN and CAPSTRACKER: Programs for the Generation, Storage and Validation of in silico generated Molecular Markers
Jonathan Taylor, Kevin Tonon, Sean Cutler, Nicholas Provart

213 Genomics-Related Stocks Distributed by ABRC
Randy Scholl, Emma Knee, Luz Rivero, Deborah Crist, James Mann, Julie Miller, Pamela Vivian, Garret Posey, Zhen Zhang, Ling Zhou

214 The *Arabidopsis* Biological Resource Center – 2004-2005 Resource Acquisitions and Distribution
Randy Scholl, Emma Knee, Deborah Crist, Luz Rivero, Natalie Case, James Mann, Julie Miller, Garret Posey, Pamela Vivian, Zhen Zhang, Ling Zhou

215 The Botany Array Resource: Using Public Microarray Data to perform e-Northerns, Expression Angling, and Promoter Analyses
Kiana Toufighi, Siobhan Brady, Ryan Austin, Eugene Ly, Nicholas Provart

216 High throughput production of full-length ORF clones from low-expressing *Arabidopsis* genes
Beverly Underwood, Yong-Li Xiao, Hank Wu, Wei Wang, William Moskal, Erin Monaghan, Julia Redman, Christopher Town

POSTER ABSTRACTS

Great Hall/Reception Room (4th floor)

Topic: Development 1: Flower, Fertilization, Fruit, and Seed

Abstracts 217-308

217 Functional analysis of flowering-time gene *FD*
Mitsutomo Abe, Sumiko Yamamoto, Yasufumi Daimon, Yoko Ikeda, Michitaka Notaguchi, Takashi Araki

218 The MADS domain factors AGL15 and AGL18 act redundantly to repress flowering in short days
Benjamin J. Adamczyk, Melissa D. Lehti-Shiu, Donna E. Fernandez

219 Identification of small RNAs in fruit crops
Andrew Gleave, Charles A-Dwamena, Bhawana Nain, Ross Crowhurst, Annette Richardson, Daya Dayatilake, Philip Martin, Michael Clearwater, Bart Janssen, Robert Schaffer, Kate Thodey, Rebecca Bishop, Robin MacDiarmid

220 *Arabis alpina* as a model species to study perennialism
Maria Albani, Renhou Wang, Coral Vincent, George Coupland

221 A Tapetal Specific NAC transcription factor involved in pollen development
Veria Alvarado, Terry Thomas

222 Site of action of a floral pathway integrator, FLOWERING LOCUS T
Yasufumi Daimon, Masaki Kobayashi, Koji Goto, Mitsutomo Abe, Takashi Araki

223 TFL1 control of plant architecture: a *cen*tral role?
Kim Baumann, Desmond Bradley

224 Efforts at Generating Apomixis Using Reverse-Genetics
Kelly Biddle, David Stelly

225 Beyond boundaries: LOLLO is required for boundary formation and embryo development
Lorenzo Borghi, Marina Bureau, Rudiger Simon

226 Sphingosine Metabolites Play A Role in Plant Growth and Development
Mannie Liscum, Brandon Celaya, Jason Barr, Steve Alexander

227 Two Receptor-Like-Kinases are essential for the control of tapetum development
Jean Colcombet, Carlos Vera, Julian Schroeder

228 Functional Characterization of FW2.2
Bin Cong, Steven Tanksley

229 Comprehensive interaction map of the *Arabidopsis* MADS box transcription factors
Stefan de Folter, Richard Immink, Marco Busscher, Gerco Angenent

230 Towards Understanding Phenotypic Diversity within Brassicaceae
B Deakin, SR Cutler

231 The nuclear actin-related protein ARP6 is a developmental regulator required for the maintenance of *FLC* expression and repression of flowering in *Arabidopsis*
Richard Meagher, Elizabeth McKinney, Roger Deal, Muthugapatti Kandasamy

232 Leaf polarity and growth factors play important roles in patterning the fruit in *Arabidopsis*
Jose Dinneny, Detlef Weigel, Martin Yanofsky

233 Genetic Analysis of the ubiquitin-specific proteases and ubiquitin conjugating enzymes in *Arabidopsis thaliana*
Jed Doelling, Gulsum Soyler-Ogretim, Jasen Wise, Richard Vierstra

234 Brassinosteroid receptor BRI1 regulates floral timing
Malgorzata Domagalska, Fritz Schomburg, Andrew Millar, Richard Amasino, Richard Vierstra, Ferenc Nagy, Seth Davis

235 Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility
Linda Enns, Masahiro Kanaoka, Keiko Torii, Luca Comai, Kiyotaka Okada, Robert Cleland

236 Linking Polycomb activity to morphogenesis via the *Arabidopsis* formin, *AtFH5*
Jonathan Fitz Gerald, Mathieu Ingouff, Frederic Berger

237 The *quartet1* tetrad pollen phenotype is caused by mutations in a putative pectin methylesterase gene
Kirk Francis, Gregory Copenhaver

238 ATG6/VPS30, a component of phosphatidylinositol 3-kinase, plays essential roles in pollen germination in *Arabidopsis*
Yuki Fujiki, Kohki Yoshimoto, Yoshinori Ohsumi

239 Mechanisms of INO function in ovules of *Arabidopsis thaliana*
Thomas Gallagher, Charles Gasser

240 Genetic analysis of gametophytic parental effect mutants in *Arabidopsis*
Paul E. Grini, Nirma Skrbo, Gerd Jurgens, Moritz Nowack, Martin Hulskamp, Arp Schnittger, Reidunn Aalen

241 A molecular basis for TFL1 and FT evolution
Yoshie Hanzawa, Tracy Money, Antonio Serrano-Mislata, Francisco Madueno, Desmond Bradley

242 *BAM1* and *BAM2* are members of the LRR-RLK family that are essential for proper anther development in *Arabidopsis thaliana*
Carey Hendrix-Hord, Brody DeYoung, Steven Clark, Hong Ma

243 BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in *Arabidopsis*
Shelley Hepworth, Yuelin Zhang, Sarah McKim, Xin Li, George Haughn

244 Identification and Characterization of Proteins that Interact with AGAMOUS-Like 15 (AGL15), a MADS-Domain Transcription Factor that Preferentially Accumulates in the Plant Embryo
Kristine Hill, Sharyn Perry

245 Characterisation of an *Arabidopsis* flowering time mutant
Stephen Jackson, James Holmes, Karl Morris, Lesley Codrai, Alison Huttly, Isabelle Carre

246 *Arabidopsis* endosperm development in WT, *acc1*, and *cac1A* mutant ovules
Hilal Ilarslan, Xu Li, Joel Schmidt, Basil Nikolau, Eve Syrkin Wurtele

270 Generation of transgenic tomato with *Arabidopsis* early flowering *LEAFY (LFY)* and *APETALA1 (AP1)* genes
Bushra Mirza, Abida Yasmeen

271 The *Arabidopsis* SeedGenes Project
Rosanna Muralla, Colleen Sweeney, Sandrine Casanova, Penny Hlubek, Ryan Jenlink, Allan Dickerman, David Meinke

272 Molecular and Genetic Characterization of *B-CLASS MODIFIER* (*BCM*) genes
Anwesha Nag, Nan Xin, Jerome Liu, Yingzhen Yang, Miya Dunets, Thomas Jack

273 Gene trap identification of genes involved in *Arabidopsis* petal and stamen differentiation
Naomi Nakayama, Juana Arroyo, Joseph Simorowski, Bruce May, Robert Martienssen, Vivian Irish

274 Lipid Transfer Proteins enhance cell wall extension. A new function for an old protein
Jeroen Nieuwland, Richard Feron, Bastiaan Huisman, Annalisa Fasolino, Cornelis Hilbers, Jan Derksen, Titti Mariani

275 In-vivo analysis of MADS-box transcription factors involved in ovule development
Isabella Nougalli Tonaco, Richard Immink, Jan Willem Borst, Sacco de Vries, Gerco Angenent

276 TWO-IN-ONE, a new player required for conventional and non-conventional modes of cytokinesis in *Arabidopsis*
Sung-aeong Oh, Andrew Johnson, Daisy Rahman, Soon-ki Park, David Twell

277 Identification of FLOWERING LOCUS H (FLH) Candidate Genes that Respond to Vernalisation in *Arabidopsis* using DNA Microarray
Eng Ong, Gary Glonek, Terence Speed, Anthony Gendall

278 Guidance signals that direct pollen tube entry into micropyle are highly variable across species and species-specific and developmentally regulated
Daphne Preuss, Ravi Palanivelu

279 Functional Analysis of the CCAAT Binding Transcription Complex Containing *Arabidopsis* LEAFY COTYLEDON1, a Central Regulator of Embryogenesis
Soomin Park, Hyeseung Lee, Liana Chan, Robert Fischer, Robert Goldberg, John Harada

280 Isolation of AP3/PI protein complexes from floral extracts
Eileen Piwarzyk, Thomas Jack

281 Constructing a regulatory network for the *Arabidopsis* female gametophyte
Jayson Punwani, Josh Steffen, Michael Portereiko, Ryushiro Kasahara, Gary Drews

282 Molecular Analysis of Self-compatibility in *Arabidopsis* allotetraploids
Sheetal Rao, Jeff Chen

283 Finding targets for a Subtilisin-like Serine Protease in *Arabidopsis thaliana*: a proteomic approach
Carsten Rautengarten, Berit Ebert, Sophie Haebel, Thomas Altmann

284 Restoration of replum development in the *Arabidopsis* fruit by suppressors of *replumless fruitfull*
Adrienne Roeder, Martin Yanofsky

285 *Arabidopsis thaliana* methyl-CpG-binding domain (MBD) proteins are involved in plant development – including the regulation of flowering time
Ellen Maryann Rosenhave, Anita Berg, Silja S. Amundsen, Kim Andresen, Mirela Mahic, Reidunn B. Aalen

286 *PDX2* a *de novo* vitamin B6 biosynthetic pathway gene, is essential for seed development in *Arabidopsis*
Beth Rueschhoff, Margaret Daub

287 *FLC PROMOTER 1* is Required for the Winter-Annual Habit in *Arabidopsis thaliana*
Robert Schmitz, Lewis Hong, Richard Amasino

288 Putative lipid/sterol binding domains in homeodomain transcription factors of *Arabidopsis*
Kathrin Schrick, Marguerite Leeds, Bhylahalli Srinivas, Martin Hulskamp, Herbert Sauro

289 Characterization of flowering time QTL in a new RIL population
Christopher Schwartz, Suresh Balasubramanian, Norman Warthmann, Tsegaye Dabi, Julin Maloof, Justin Borevitz, Joanne Chory, Detlef Weigel

290 Natural variation in *Arabidopsis thaliana* for the vernalization response
Christopher schwartz, Joel Basken, Riamsalio Phetchareun, Richard Amasino

291 Comparative Analysis of CONSTANS-like B-box Zinc Finger Protein Family of *Arabidopsis*
Su Young Shin, Hye Jin Kim, Young Hun Song, Ju Hwoan Kang, Hyo Sic Kim, Na Young Song, Geon Hui Son, Jong Chan Hong

292 Analysis of *IDA-like* (*AtIDL*) genes in *Arabidopsis thaliana*
Grethe-Elisabeth Stenvik, Melinka A. Butenko, Nora M. Tandstad, Asbjorn Holmgren, Reidunn B. Aalen

293 TOPLESS is involved in floral organ identity
Heidi Szemenyei, Kendra Hogan, Jeff Long

POSTER ABSTRACTS

Old Madison/Beefeaters Room (3rd floor)

Topic: Development 2: Shoot and Root

Abstracts 308-388

314 RAP2.6L is involved in a transcription factor network during shoot development in *Arabidopsis*
Ping Che, Sonia Lall, Dan Nettleton, Stephen Howell

315 Functional Interdependence between SHR and SCR in Radial Patterning in *Arabidopsis* Roots
Hongchang Cui, Giovanni Sena, Kim Gallagher, Philip Benfey

316 Osmotic regulation of root system architecture
Karen Deak, Jocelyn Malamy

317 Genetic regulation of gene expression during shoot development in *Arabidopsis*
Rhonda DeCook, Sonia Lall, Dan Nettleton, Stephen Howell

318 *Arabidopsis* homologs of the TOR (target of rapamycin) and RAPTOR proteins regulate plant growth and are essential for embryo development
Dorothee Deprost, Yao Lei, Rodnay Sormani, Maryse Nicolai, Hoai-Nam Truong, Christophe Robaglia, Christian Meyer

319 *Arabidopsis* extra-large G-Proteins (XLGs) are negative regulators of root growth and development in darkness
Lei Ding, Caroline Gibson, Sarah Assmann

320 Tropic growth responses are proceeded by the formation of a spatial gradient of morphogen-dependent transcription
Alex Esmon, Amanda Tinsley, Renee Harper, Karin Ljung, Goran Sandberg, Mannie Liscum

321 Compensatory system: Coordination of cell proliferation and cell expansion during leaf morphogenesis
Ali Ferjani, Ushio Fujikura, Gorou Horiguchi, Hirokazu Tsukaya

322 Genetic analysis of compensation system(s) in leaf development
Ushio Fujikura, Ali Ferjani, Gorou Horiguchi, Hirokazu Tsukaya

323 CKH2/PICKLE negatively regulates a set of cytokinin responses
Kaori Furuta, Minoru Kubo, Yao-Guang Liu, Daisuke Shibata, Tatsuo Kakimoto

324 The Transcription Factor ATMYB2 Regulates Whole Plant Senescence through Cytokinin Anabolic Pathway
Yongfeng Guo, Susheng Gan

325 Identification of downstream targets of *KANADI 1*
Yael Harrar, Sean Chen, Randall Kerstetter

326 Some pathways that regulate flowering also influence the rate of vegetative shoot maturation
Laurie Mentzer, Ed Himelblau

327 Maintenance of apical-basal polarity in *Arabidopsis thaliana* transition stage embryos by TOPLESS and HAG1
Martin Hobe, Jeff Long

328 Characterization of the *BLADE-ON-PETIOLE* genes in *Arabidopsis*
Mattias Holmlund, Mikael Norberg, Ove Nilsson

329 The novel mutant *uni* altered axillary meristem formation in *Arabidopsis*
Sachiko Endo, Ken-ichiro Hibara, Masao Tasaka

330 Role of class 1 *knox* genes in *asymmetric leaves1* mutant in *Arabidopsis thaliana*
Fumiaki Ogasawara, Masaya Ikezaki, Yoshihisa Ueno, Chiyoko Machida, Yasunori Machida

331 Identification and functional analysis of QTL that regulate root system architecture
Melissa Lehti-Shiu, Paul Ingram, Jonathan Fitz Gerald, Theresa Biesiada, Jocelyn Malamy

332 BOBBER encodes a NudC domain protein that affects multiple aspects of *Arabidopsis* development
Nick Kaplinsky, M. Kathryn Barton

333 Isolation and Characterization of *siamese* Phenotypic Modifiers
Remmy Kasili, Jason Walker, John Larkin

334 Functional analysis of GCN proteins in *Arabidopsis*
Tomohiko Kato, Shusei Sato, Satoshi Tabata, Takashi Hibino

335 Translational regulation in the specification of abaxial leaf identity
Tengbo Huang, Jeon Hong, Randall Kerstetter

336 sku11 mutant affects directional cell expansion and trichome development
noha Khalifa, Alex paredez, John Sedbrook

337 Overexpression of COG1 represses photoperiodic flowering irrespectively on circadian clock in *Arabidopsis*
Jeongsik Kim, Donha Park, Yumi Kim, Pyung Ok Lim, Hong Gil Nam

338 Regulatory genes of leaf senescence in *Arabidopsis*
Pyung Ok Lim, Hyo Jung Kim, Jin-Hee Kim, In-Chul Lee, Yun Jeong Lee, Hong Gil Nam

339 Functional Analysis of *bpg1*-D (bushy and pale green) mutant
Youn-Sung Kim, Sun-Young Kim, Min-Sun Lee, Sang-Gyu Kim, Ju Yun, Chung-Mo Park

340 Isolation of genetic targets of *ASYMMETRIC LEAVES 1*
Kirsten Knox, Andrew Hudson

341 Global comparative transcriptome analysis identifies gene network regulating secondary growth in *Arabidopsis thaliana*
Jae-Heung Ko, Eric Beers, Kyung-Hwan Han

342 The *ASYMMETRIC LEAVES2* is involved in the formation of a flat leaf lamina in the presence of the *ASYMMETRIC LEAVES1* gene
Shoko Kojima, Yoshihisa Ueno, Hidekazu Iwakawa, Endang Semiarti, Teppei Soma, Hirokazu Tsukaya, Takaaki Ishikawa, Yasunori Machida, Chiyoko Machida

343 Direct regulation of *CAPRICE* and *GLABRA2* transcription by WEREWOLF protein to determine the cell fate in root epidermis
Yoshihiro Koshino-Kimura, Takuji Wada, Tatsuhiko Tachibana, Kiyotaka Okada

344 Molecular and genetic interactions associated with functions of *BP*, *AS1* and *AS2* in *Arabidopsis*
Kumuda Kushalappa, Eddy Risseeuw, Prakash Venglat, Daoquan Xiang, Vivijan Babic, Robert Martienssen, Gopalan Selvaraj, Raju Datla

345 Positional Signaling Mediated by a Receptor-Like Kinase
Su-Hwan Kwak, Ronglai Shen, John Schiefelbein

346 The LATERAL ORGAN FUSION MYB transcription factors regulate lateral organ separation in *Arabidopsis*
Dong-Keun Lee, Matt Geisler, Patricia Springer

347 Paralogous R2R3 MYB proteins, FLP and MYB88 control *Arabidopsis* stomatal patterning
Eun Kyoung Lee, Jessica Lucas, Lien Lai, Fred Sack

348 Genome-wide survey of cell- or tissue-type enriched transcription factors (TFs) and regulation of their expression in *Arabidopsis* roots
Ji-Young Lee, Juliette Colinas, Jean Wang, Philip Benfey

349 Two *MDR* genes with different effects on auxin transport and the control of root growth in *Arabidopsis*
Daniel Lewis, Guosheng Wu

350 Functional Characterization of *LATERAL ORGAN BOUNDARIES* in *Arabidopsis*
Wan-ching Lin, Bin Shuai, Amanda Mangeon, Barbara Jablonska, Patricia Springer

351 NRT2.1, a high-affinity nitrate transporter, plays a role in determining root architechture independent of its nitrate transport activity
Daniel Little, Hong-Yu Rao, Sabrina Oliva, Francoise Daniel-Vedele, Anne Krapp, Jocelyn Malamy

352 The role of subtilases in shoot development
Jianxiang Liu, Renu Srivastava, Sonia Lall, Ping Che , Stephen Howell

353 Temperature dependent isolation of enhancers and suppressors of topless-1
Kyle Shively, Jeff Long

354 ZBF2, a bZIP Transcription Factor, Plays Differential Regulatory Role in Hypocotyl and Cotyledon Growth during Photomorphogenesis
Chandrashekara Mallappa, Vandana Yadav, Prem Negi, Sudip Chattopadhyay

355 Functional characterization of the *LATERAL ORGAN BOUNDARIES DOMAIN* GENE *LBD25*
Amanda Mangeon, Wan-ching Lin, Patricia Springer

356 Functional diversity and redundancy among members of the XTH gene family: A apecific role of *AtXTH27* in vascular development in rosette leaves of *Arabidopsis*
Akihiro Matsui, Ryusuke Yokoyama, Motoaki Seki, Kazuo Shinozaki, Taku Takahashi, Yoshibumi Komeda, Kazuhiko Nishitani

357 D-type Cyclins link developmental cues to the cell cycle
Spencer Maughan, Jeroen Nieuwland, Walter Dewitte, James Murrray

358 Role of Protein Phosphorylation In Polar Auxin Transport
Marta Michniewicz, Yang Xiong, Dolf Weijers, Remko Offringa, Jiri Friml

359 ZBF1, a bHLH Transcription Factor, Acts as a Repressor of Blue Light Signaling in *Arabidopsis*
Sreeramaiah N Gangappa, Vandana Yadav, Chandrashekara Mallappa, Shikha Bhatia, Sudip Chattopadhyay

360 Inhibition of brassinosteroid biosynthesis rescues *nph4* defects in tropistic responses of hypocotyl
Akimitsu Ikeura, Daisuke Nakamoto, Tadao Asami, Kotaro Yamamoto

361 *TERMINAL FLOWER2*, the *Arabidopsis HP1* homologue, a gene involved in several processes during plant development
Lars Nilsson, Katarina Landberg, Alessia Para, Annika Sundås Larsson

362 Genetic characterization of the *blade-on-petiole* mutants
Mikael Norberg, Mattias Holmlund, Ove Nilsson

363 Genetic Screen to Identify Components of SCARECROW-Controlled Pathways in *Arabidopsis thaliana*
Raja Payyavula, Kun Yuan, John Ross, Joanna Diller

364 Cytokinin Regulated Transcription Factors are Involved in Leaf and Cotyledon Development
Aaron Rashotte, Joseph Kieber

365 Enhancer trap line WG335 identifies a TCP domain protein of *Arabidopsis* that is essential for post-embryonic development
Rashida Patel, Scott Douglas, Daniel Riggs

366 The WEREWOLF MYB protein directly regulates *CAPRICE* transcription during cell fate specification in the *Arabidopsis* root epidermis
Yeon Hee Kang, Kook Hui Ryu, Young-hwan Park, Ildoo Hwang, John Schiefelbein, Myeong Min Lee

367 LMI1 acts as a meristem identity regulator downstream of LEAFY
Louis Saddic, Richard Collum, Doris Wagner

368 TORTIFOLIA1, a plant-specific, microtubule-associated protein gives direction to plant organ expansion
Henrik Buschmann, Monika Hauptmann, Christoph Fabri, Peter Hutzler, Clive Lloyd, Tony Schaeffner

369 Members of the *MIR164* microRNA family are redundantly required for normal meristem function
Patrick Sieber, Elliot Meyerowitz

370 Role of the CAPRICE family of myb genes in root epidermal development
Marissa Simon, John Schiefelbein

371 A Connection Between Apical-Basal and Adaxial-Abaxial Polarity in *Arabidopsis* Embryogenesis
Zachery Smith, Jeff Long

372 Functional analysis of CLE40 in *Arabidopsis* root meristem development
Yvonne Stahl, Andrea Bleckmann, Rudiger Simon

373 Is a CLV-like signaling pathway operating during *Arabidopsis* root development?
Colette ten Hove, Eva Casamitjana-Martinez, Ben Scheres, Renze Heidstra

374 Regulation of LATERAL SUPPRESSOR – a gene involved in the formation of axillary meristems
Andrea Eicker, Klaus Theres

375 Control of organ growth and epidermal patterning by synergistic interactions of receptor-like kinases
Keiko Torii, Shannon Bemis, Jessica McAbee, Lynn Pillitteri, Elena Shpak, Dan Sloan

376 Genetic characterization of the *bps1* root-derived mobile signal
Jaimie M. Van Norman, Leslie E. Sieburth

377 *FLAVODENTATA*: genetic interactions and positional cloning
Irina Vvedenskaya, Randall Kerstetter

378 The UGF protein family: Analysis of gene expression and protein interactions
Vanessa Wahl, Tanja Weinand, Markus Schmid

379 Functional analysis of a laccase-like multicopper oxidase (LMCO) in *Arabidopsis thaliana*
Chieh-Ting Wang, Jeffrey F. D. Dean

380 Signaling cascade controls stomata development and patterning in *Arabidopsis thaliana*
Huachun Wang, Yidong liu, Njabulu Ngwenyama, John Walker, Shuqun Zhang

381 WVD2-Like Proteins Regulate Plant Growth Behavior and Anisotropic Cell Expansion
Yan Wang, Jessica Will, Robyn Perrin, Christen Yuen, Patrick Masson

382 The Dof Transcription Factor, OBP3, Modulates Phytochrome and Cryptochrome Signaling by Altering Hormone Signaling in *Arabidopsis*
Jason Ward, Carie Cufr, Megan Denzel, Sarah Galanti, Michael Neff

383 A hormonally-specified growth checkpoint regulates axillary bud growth in *Arabidopsis thaliana*
Sally Ward, Ottoline Leyser

384 Light-stimulated leaf growth in *Arabidopsis thaliana*
Claire Woodward, Elizabeth Van Volkenburgh

385 Requirement of homeobox gene STIMPY for meristem growth and maintenance
Xuelin Wu, Detlef Weigel

386 DNA-binding properties of a MYB protein needed for stomatal patterning
Zidian Xie, Fred Sack, Erich Grotewold

387 Characterization of *transport inhibitor response (tir2)* mutant in *Arabidopsis*
Masashi Yamada, Philip Jensen, Mark Estelle

388 ***MYR1*** **and** ***MYR2*** **are redundant genes that regulate flowering time, petiole elongation, and lateral shoot outgrowth in** ***Arabidopsis***
Chengsong Zhao, Eric Beers

POSTER ABSTRACTS

Old Madison/Beefeaters Room (3rd floor)

Topic: Evolution and Development (AGA)

Abstracts 389-397

389 **Conservation and divergence of flowering time gene expression in wild and domesticated sunflower**
Benjamin Blackman, Scott Michaels, Loren Rieseberg

390 **TILLinG reveals selection at certain missense changes**
E Greene, C Codomo, C Burtner, B Till, L Comai, S Henikoff

391 **Differential gene expression in the homoploid hybrid species** ***Helianthus deserticola*** **and its progenitors** ***H. annuus*** **and** ***H.petiolaris***
Zhao Lai, Briana Gross, Yi Zou, Justen Andrews, Loren Rieseberg

392 **Evidence of genetic conservation of diverse nectaries within the eudicots**
Ji-Young Lee, Stuart Baum, Sang-Hun Oh, Cai-Zhong Jiang, John Bowman

393 **Molecular Evolution of LEAFY transcription factor in land plants**
Alexis Maizel, Mitsuyasu Hasebe, Takako Tanahashi, Detlef Weigel

394 **Accumulation of male benefit genes and recombination arrest in Silene (white campion): on sexual dimorphism and Y chromosome evolution**
Jitka Zluvova, Sevdalin Georgiev, Bohuslav Janousek, Ioan Negrutiu

395 **Darwinian selection on a selfing locus in** ***Arabidopsis thaliana***
Kentaro Shimizu, Jennifer Cork, Ana Caicedo, Charlotte Mays, Richard Moore, Kenneth Olsen, Stephanie Ruzsa, Graham Coop, Carlos Bustamante, Philip Awadalla, Michael Purugganan

396 **Evolutionary diversification of the** ***LFY – TFL1*** **interaction in the rosette flowering species** ***Leavenworthia crassa***
Marek Sliwinski, Michael White, David Baum

397 **Conservation of YABBY protein functions between** ***Arabidopsis*** **and rice**
Takahiro Yamaguchi, Hiro-Yuki Hirano, Hirokazu Tsukaya

POSTER ABSTRACTS

Old Madison/Beefeaters Room (3rd floor)

Topic: Evolutionary Biology

Abstracts 398-412

398 **Two-locus F1 hybrid incompatibility of two wild** ***Arabidopsis thaliana*** **ecotypes**
Kirsten Bomblies, Janne Lempe, Detlef Weigel

399 **Maternal Phenotypic Effects on Season of Germination Can Explain Life History Variation in** ***Arabidopsis thaliana***
Elizabeth Boyd, Lisa Dorn, Cynthia Weinig, Johanna Schmitt

400 **Evolution under strong balancing selection : polymorphism at the self-incompatibility locus SRK in the genus** ***Arabidopsis***
Vincent Castric, Mikkel Schierup, Jesper Bechsgaard, Sabrina Le Cam, Xavier Vekemans

401 **Genome inventory of sequence polymorphisms for** ***Arabidopsis thaliana***
Richard Clark, Norman Warthmann, Glenn Fu, Kelly Frazer, Detlef Weigel

402 **The ecological genetics of germination timing in** ***Arabidopsis thaliana***
Kathleen Donohue, Shane Heschel, Lisa Dorn

403 **Variable patterns of transferred mitochondrial DNA in** ***Arabidopsis thaliana*** **ecotypes and related species**
William Grayburn

404 **Genetic Basis of sister species divergence in Clarkia (Onagraceae)**
Amanda Henry, Norman Weeden

405 **Ecotype-specific evolution of centromeric satellites in** ***Arabidopsis thaliana***
Hidetaka Ito, Asuka Miura, Kazuya Takashima, Tetsuji Kakutani

406 Global survey of gene expression diversity in *Arabidopsis thaliana*
Rebecca Doerge, Richard Michelmore, Daniel Kliebenstein, Marilyn West, Kyunga Kim, Dina St. Clair, Hans van Leeuwen

407 Epistatic and Seasonal Selection at the Major Flowering Time Gene FRIGIDA
Tonia Korves, Karl Schmid, Ana Caicedo, Charlotte Mays, John Stinchcombe, Michael Purugganan, Johanna Schmitt

408 How to measure a cost of adaptation?
Fabrice Roux, Xavier Reboud

409 Hitchhiking effects associated to selection on the self-incompatibility locus (S-Locus) in *Arabidopsis halleri*
Maria Valeria Ruggiero, Bertrand Jacquemin, Vincent Castric, Xavier Vekemans

410 Independent ancient polyploidy events in the sister families Brassicaceae and Capparaceae
Eric Schranz, Thomas Mitchell-Olds

411 Evolution of Centromere-Binding Proteins and their Interactions with Centromere DNA in the Brassicaceae
Bonnie Scott, Song Luo, Sarah Hall, Daphne Preuss

412 Using synthetic *RPP1* gene cluster to model *R* gene evolution in *Arabidopsis*
Jian Sun, Crystal Gilbert, John Jelesko, John McDowell

POSTER ABSTRACTS

Tripp Commons (2nd floor)

Topic: Genetic and Epigenetic Mechanisms

Abstracts 413-449

413 Altered Inheritance and Genetic Mapping of an *Arabidopsis thaliana* RNase Activity Mutant
Melissa Tricker, Michael Abler

414 On the Role of Introns: Reverse-complement and Exact Sequence Matches of Intron n-mers to Coding Sequences of Neighbouring Genes
Ron Ammar, Nicholas Provart

415 Antiquity of microRNAs and their targets in land plants
Michael Axtell, David Bartel

416 Intra- and Interspecific Hybridizations between Genetically Modified (GM) and Non-genetically Modified (Non-GM) Brassica
Nonnatus Bautista, Tamotsu Shiroyama, Lidia Watrud

417 Using Cabbage Leaf Curl Virus to Dissect Silencing and Defense Pathways in *Arabidopsis thaliana*
Steven Bernacki, James Moyer, Niki Robertson

418 Identification and Characterization of Insulators Protecting Gene Expression in the *Arabidposis* Centromere
Andrew Cal, Song Luo, Daphne Preuss

419 Molecular Mechanism of EMF-mediated Floral Repression
Myriam Calonje, Lingjing Chen, Rosario Sanchez, Rieko Nishimura, Z Renee Sung

420 Methylation in coding region causes strong gene silencing of the *phyA* locus in *Arabidopsis*
Rekha Chawla, Scott Nicholson, Vibha Srivastava

421 Disruption of DNA methylation induces genome-specific changes in gene expression in natural *Arabidopsis* allotetraploids
Meng Chen, Jianlin Chen, LU Tian, Ning Wei, Jeff Chen

422 Role of small interfering RNAs in A. *thaliana* rRNA gene expression patterns
Pedro Costa Nunes, Tom Ream, Sasha Preuss, Wanda Viegas, Craig Pikaard

423 DNA translesion polymerases and plant genomic integrity
Marc Curtis, John Hays

424 Control of Nucleolar Dominance by Histone Deacetylase HDA6
Keith Earley, Rachel Reuther, Rick Lawrence, Olga Pontes, Craig Pikaard

425 Multi-species analysis of centromere regions in the Brassicaceae family
Anne Hall, Gregory Kettler, Josef Jurek, Daphne Preuss

426 Genomic and Molecular Evolution of Centromere Satellites in Brassicaceae Species
Song Luo, Anne Hall, Daphne Preuss, Sarah Hall

427 Meiotic chromosome maintenance in *Arabidopsis*
Karen Kaczorowski, Daphne Preuss

428 Distinct DNase I hypersensitive sites are found in transcriptionally-competent gene promoters in *Arabidopsis*
Yuichi Kodama, Shingo Nagaya, Atsuhiko Shinmyo, Ko Kato

429 Genome Rearrangement at Non-Standard Configuration of Ac/Ds Transposon Ends in *Arabidopsis*
Lakshminarasimhan Krishnaswamy, Jianbo Zhang, Thomas Peterson

430 Natrual variation of a subtelomeric sequence in *Arabidopsis thaliana*
Hui-fen Kuo, Eric Richards

431 Genetic and epigenetic consequences of a paracentric inversion in *Arabidopsis thaliana*
Gabriella M. Linc, Hoda M. Ali, Jannie Peters, Jannie Wennekes, Hans de Jong, Maarten Koornneef, Tom Gerats, Ingo Schubert, Paul Fransz

432 Plants with altered small RNA pathways respond to abscisic acid differently
Qing-Jun Luo, Srinivas Gampala, Christopher Rock

433 Dynamics of neo-centromere formation in *Arabidopsis*
Kevin Keith, Song Luo, Jay Shrestha, Robert Shurr, Joanna Fitch, Daphne Preuss

434 RdRP-dependent RNA Silencing of Transgenes with Intrinsic Direct Repeats or Leaky Transcription Terminators
Zhenghua Luo, Baofang Fan, Zhixiang Chen

435 Characterization of *Arabidopsis* Topoisomerase IIA binding and cleavage sequences
Irina Makarevitch, David Somers

436 Establishment of the winter-annual habit in *Arabidopsis* requires *EARLY FLOWERING IN SHORT DAYS*, a putative histone H3 lysine 4 methyl transferase
Sang Yeol Kim, Yuehui He, Yannick Jacob, Richard Amasino, Scott Michaels

437 Lipases in Plant Defense and Death
Jessica Morton, Jyoti Shah

438 DNA methylation and DNA methyl-binding domain genes in rRNA transcriptional regulation
Sasha Preuss, Craig Pikaard

439 A link between miRNA and siRNA pathways mediated by ARGONAUTE1 and DICER-LIKE1
Michael Ronemus, Matthew Vaughn, Robert Martienssen

440 Analysis of a suppressor mutant of the FKBP-like twisted dwarf1 (twd1) mutation in *Arabidopsis*
Marcel Lafos, Claudia Moeller, Dierk Wanke, Brian Dilkes, Burkhard Schulz

441 Genetic and Epigenetic Study of de novo Centromere Formation in *Arabidopsis thaliana*
Jay Shrestha, Song Luo, Daphne Preuss

442 Low-light exposure induces disruption of heterochromatic chromocenters in *Arabidopsis*
Martijn van Zanten, Federico Tessadori, Frank Millenaar, Roel van Driel, Rens Voesenek, Paul Fransz, Ton Peeters

443 Large-scale chromatin decondensation accompanies cell de-differentiation during protoplast culture
Federico Tessadori, Marie-Christine Chupeau, Yves Chupeau, Marijn Knip, Roel van Driel, Paul Fransz, Valerie Gaudin

444 The role of microRNAs in non-additive gene regulation in *Arabidopsis* allopolyploids
Lu Tian, Meng Chen, Ning Wei, Jianlin Wang, Sing-Hoi Sze, Z.Jeffrey Chen

445 Multiple hydrophobic clusters in *Arabidopsis* CBF1 redundantly contribute to transcriptional activation
Zhibin Wang, Eric Stockinger

446 Identification of multiple mutations in the microRNA target site of *TCP4* and of a mutation in the miR319a microRNA by suppressor mutagenesis
Heike Wollmann, Javier Palatnik, Detlef Weigel

447 The *Arabidopsis* SKP1-LIKE1 (ASK1) protein is most abundant in leptotene and likely regulates homologous recombination in male meiosis
Yixing Wang, Ming Yang

448 The biogenesis of endogenous trans-acting siRNAs in *Arabidopsis* is initiated by miRNA-directed cleavage
Manabu Yoshikawa, Scott Poethig

449 *Arabidopsis* mutants with altered responses to HDAC inhibitors, including HC-toxin
Hugh Young, Guri Johal

POSTER ABSTRACTS

Tripp Commons (2nd floor)

Topic: Interaction With the Environment 1: Abiotic

Abstracts 450-524

450 MAPKs and stress hormone signaling during ozone exposure
Reetta Ahlfors, Violetta Macioszek, Jason Rudd, Mikael Brosche, Rita Schlichting, Dierk Scheel, Jaakko Kangasjarvi

451 Stress Responses to Polycyclic Aromatic Hydrocarbons Include Generation of Reactive Oxygen Species, Cell Death and Changes in Gene Expression
Merianne Alkio, David Weisman, Tomoko Tabuchi, Adan Colon-Carmona

452 Differential regulation of salt tolerance genes between *Arabidopsis thaliana* and the halophytic *Arabidopsis* Relative Model System, *Thellungiella halophila*
Pragya Verma, Surya Kant, Simon Barak

453 Functional Characterization of the Nicotianamine Synthase (*NAS*) Gene Family in *Arabidopsis thaliana*
Jennifer Barwick, Judy Krueger, Brett Lahner, David Salt, Erin Connolly

454 Transcriptional regulation of *Arabidopsis thaliana* P450 genes in response to phenobarbital
Metin Bilgin, Hui Duan, Yurdagul Ferhatoglu, Shahjahan Ali, Su Min Park, Mary Schuler

455 Screening of a 35S-cDNA *Arabidopsis* lines library for ABA-insensitive dominant mutants shows negative regulation by a *PP2C* in ABA signaling
Aurelien Boisson-Dernier, Josef Kuhn, Mohammad Maktabi, Marie Beverley Dizon, Julian Schroeder

456 PIK1 (PHYTOCHROME INTERACTOR WITH KELCH REPEATS 1) is a positive acting component of light signaling
Brian Burger, Meng Chen, Joanne Chory

457 Genome-wide identification of transcription factors involved in the initial phase of salt stress in rice
Camila Caldana, Slobodan Ruzicic, Diego Riano-Pachon, Bernd Muller-Rober

458 The role of temperature in redundant auxin biosynthesis pathway utilization
Jessica Calio, Hussein Ibrahim, John Celenza, Jennifer Normanly

459 RFI2, a RING-domain Zinc-finger protein, integrates phytochrome and circadian signaling with photoperiodic flowering control
Mingjie Chen, Min Ni

460 Circadian regulation of auxin signaling in *Arabidopsis*
Michael Covington, Julin Maloof, Marty Straume, Steve Kay, Stacey Harmer

461 *Arabidopsis ONSET OF LEAF DEATH* genes regulate leaf senescence by modifying Age-Related Changes
Marcel Sturre, Jacques Hille, Jos Schippers, Hai-Chun Jing, Paul Dijkwel

462 Identification and characterization of transposon activation tagged mutants for Abiotic stress tolerance
Shital Dixit, Asaph Aharoni, Andy Pereira

463 UV induced Programmed cell death
Georgina Drury, Anna Gordon, Riu He, Patrick Gallois

464 FRD3 is Required for Efficient Root to Shoot Iron Translocation
Timothy Durrett, Elizabeth Rogers

465 Cellular Model for Oxidative Stress Tolerance Activated by Glycine Betaine
John Einset

466 The Role of the Heme-Oxygenase Gene Family in Photomorphogenesis
Thomas Emborg, Richard Vierstra

467 Nitrogen biosensing in *Arabidopsis thaliana*
Cawas Engineer, Robert Kranz

468 Overlapping and Distinct Roles of PRR7 and PRR9 in the *Arabidopsis* Circadian Clock
Eva Farre, Steve Kay, Stacey Harmer, Frank Harmon, Marcelo Yanovsky

469 The Beta Subunit of the Heterotrimeric G-Protein is Required for a Plastidic Response to a Pulse of Green Light
Kevin Folta, Dawn Bies, Stefanie Maruhnich, Amit Dhingra, Alan Jones

470 RFK1, an F-Box Kelch Domain Protein required for Blue-Light Destabilization of *Lhcb* Transcripts
Kevin Folta, Thelma Madzima, Lon Kaufman

471 A Novel Plant-Specific Protein Family Involved in Abscisic Acid Response
Emily Garcia, Tim Lynch, Julian Peeters, Ruth Finkelstein

472 Dissection of ZTL functional domains in the regulation of circadian clock and plant development
Ruishuang Geng, David Somers

473 *Arabidopsis thaliana* diacylglycerol kinase 2 (AtDGK2) exhibits cold-, wound- and mannitol inducible gene expression
Fernando Gómez-Merino, Libia Trejo-Tellez, Aleksandra Skirycz, Bernd Mueller-Roeber

474 Phytohormones regulate various aspects of the circadian systems
Shigeru Hanano, Malgorzata Domagalska, Sevgi Oden, Els Prinsen, Seth Davis

475 Integrated metabolite and transcript profiling of nine *Arabidopsis* accessions to define processes important for acclimated and non-acclimated freezing tolerance
Matthew Hannah, Dana Wiese, Susanne Freund, Oliver Fiehn, Arnd Heyer, Dirk Hincha

476 Coupling of the circadian clock and the 26S proteasome to modulate light signaling pathways in .*Arabidopsis*
Frank Harmon, Steve Kay

477 Investigating the Function of ARG1 and ARL2 in the Root Gravitropic Response of *Arabidopsis*
Benjamin Harrison, Patrick Masson

478 *LUX ARRHYTHMO* Encodes a Novel Myb Transcription Factor Essential for Circadian Rhythms
Samuel Hazen, Thomas Schultz, Jose Pruneda-Paz, Justin Borevitz, Joseph Ecker, Steve Kay

479 HY5 interacting B-box proteins regulate plant growth and development
Chamari Hettiarachchi, Sourav Datta, Mintu Desai, Xing-Wang Deng, Magnus Holm

480 Dissecting ABA and Stress-related Signaling Networks Using ABA Analogs
Daiqing Huang, Suzanne Abrams, Adrian Cutler

481 *Arabidopsis* photomorphogenic regulatory network discovery *in silico* and *in vivo*
Matthew Hudson

482 Exploring a new detergent-inducible promoter active in higher plants and its potential biotechnological application
Gretel Hunzicker, Elmar Weiler

483 FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*
Takato Imaizumi, Thomas Schultz, Frank Harmon, Lindsey Ho, Steve Kay

484 An *Arabidopsis HOS6* Locus Confers Both ABA Hypersensitivity And Tolerance To Dehydration Stress
Gunsu Inan, Paul Hasegawa, Ray Bressan

485 An *Arabidopsis* Cyclin –Like Protein, CLP, is involved in plant development
Yinhua Jin, Jingbo Jin, Dae-Jin Yun, Paul Hasegawa, Ray Bressan

486 Hormonal interactions in plant abiotic stress responses
Reetta Ahlfors, Mikael Brosche, Hannes Kollist, Pinja Jaspers, Tiina Kuusela, Airi Lamminmaki, Dirk Inze, Tapio Palva, Jaakko Kangasjarvi

487 Sugar-mediated Regulation of Phosphate Starvation Induced Response in *Arabidopsis*
Athikkattuvalasu Karthikeyan, Deepa Varadarajan, Ajay Jain, Michael Held, Nicholas Carpita , Kashchandra Raghothama

488 Identification of amino acid residues critical for post-translational regulation of the IRTI metal transporter by iron in *Arabidopsis*
Loubna Kerkeb, Indrani Mukherjee, Josh Ash, Brett Lahner, David Salt, Erin Connolly

489 Functional Profiling of Genes Involved In Early Phytochrome Signaling
Rajnish Khanna, Yu Shen, Peter Quail

490 Genetic and physiologic interactions between ZTL and ELF3
Woe-Yeon Kim, David Somers

491 Transcriptome analysis of class B-HSF double knockout mutant in *Arabidopsis*
Mukesh Kumar, Wolfgang Busch, Friedrich Schoeffl

492 A rice hydroxyproline-rich glycoprotein family gene shows high similarity to yeast decapping enzyme gene, dcp1
Seong-Kon Lee, Beom-Gi Kim, Jin-Ohk Lee, Tack-Ryoun Kwon, Mi-Jeong Jeong , Myung-Ok Byun, Soo-Chul Park

493 Genetic analyses of *shot1* and *2*, suppressor of *hot1-4*, involved in thermotolerance of *Arabidopsis*
Ung Lee, Chris Wie, Elizabeth Vierling

494 The circadian clock provides memory of prior light exposure during hypocotyl growth
Kazunari Nozue, Stacey Harmer, Julin Maloof

495 *Arabidopsis* Metallothioneins: Roles of Seed-Specific Type 4 MTs and Regulation of MT2a Expression in Response to Copper and Oxidative Stress
Metha Meetam, Peter Goldsbrough

496 Ambient temperature cycles or photoperiod diurnally regulate more than half of the *Arabidopsis* transcriptome
Todd Michael, Todd Mockler, Amanda Byer, Fangxin Hong, Samuel Hazen, Marcelo Yanovsky, Steve Kay, Joanne Chory

497 *Arabidopsis* SUMO E3 ligase controls *CBF*-dependent low-temperature signaling and freezing tolerance
Kenji Miura, Jing Bo Jin, Chan Yul Yoo, Vicky Stirm, Ji Young Lee, Dongwon Baek, Yoon Duck Koo, Dae-Jin Yun, Ray Bressan, Paul Hasegawa

498 *short vegetative phase* (*svp*) mutation suppressed late flowering phenotype of *lhy cca1* under continuous light condition
Tsuyoshi Mizoguchi, Atsushi Oda, Sumire Fujiwara, George Coupland, Mayu Nakagawa, Hiroshi Kamada

499 Analysis of the light-induced inhibition of gravitropism in hypocotyl; the relationship between the random hypocotyl-bending by light and the auxin distribution
Akitomo Nagashima, Genki Suzuki, Kensuke Saji, Kiyotaka Okada, Tatsuya Sakai

500 Characterization of a family of remorin proteins in *Arabidopsis thaliana*
David Nelson, Kathryn Raiford, Clark Nelson

501 HRB1, a ZZ-type zinc finger protein, regulates phyB-mediated red and cry-mediated blue light responses
Xiaojun Kang, Jason Chong, Min Ni

502 LIR1, an *Arabidopsis* protein containing a SPX domain and an EXS domain, regulates cry-mediated blue light signaling
Xiaojun Kang, Yun Zhou, Min Ni

503 The *Arabidopsis* WAVY GROWTH 3 protein harboring a RING finger motif regulates the gravitropic response of roots negatively
Susumu Mochizuki, Akane Suzuki, Akiko Harada, Takuji Wada, Sumie Ishiguro, Kiyotaka Okada, Tatsuya Sakai

504 *Arabidopsis RCI2* genes have been structurally conserved during evolution but play different roles in response to abiotic stresses
Julio Salinas, Maria Luisa Ballesteros, Joaquin Medina

505 The Role of Inositol-1,4,5 Triphosphate in Transcriptional Regulation of Gravitopism
Raul Salinas-Mondragon, Jeffery Kimbrough, Imara Perera, Wendy Boss, Christopher Brown, Heike Sederoff

506 Interactions of temperature and the circadian clock
Patrice Salomé, C Robertson McClung

507 *FCL3/MAF3* and *FCL4/MAF2* belong to an *FLC*-independent vernalization pathway
Ying Pan, Michael Schlappi

508 Cold activation of *EARLI1* is light dependent and involves sugar signaling
Michael Schlappi, Jason Bubier

509 Genetic analysis of the circadian clock in *Arabidopsis*
Thomas Schultz, Samuel Hazen, Steve Kay

510 The *Arabidopsis SPR1* gene encodes a novel plant specific microtubule plus-end interacting protein involved in directional cell expansion
Wolf-Rudiger Scheible, John Sedbrook, David Ehrhardt, Sarah Fisher, Chris Somerville

511 PIF1 is degraded by light through ubiquitin-mediated proteasome pathway
Hui Shen, Jennifer Moon, Enamul Huq

512 The *LI*ght response *D*efective *3* mutant accumulates reduced anthocyanin content in response to sucrose- and phytochrome A-inductive conditions
Bridgit Goldman, Timothy Short

513 Modulation of photosynthetic inhibitor herbicide activity by salicylate and other SAR agents
Paul Silverman, Peter Petracek, Daniel Heiman, Prem Warrior

514 Genetic analysis of tocopherol functions in *Arabidopsis* at low temperature
Wan Song, Hiroshi Maeda, Dean DellaPenna

515 Identification of a Novel Arsenic Tolerant Mutant and a Role for *PHYA* in Providing Tolerance
Dong Yul Sung, David Lee, Andrea Raab, Joerg Feldmann, Andrew Meharg, Elizabeth Komives, Hugh Harris, Julian Schroeder

516 Proline biosynthesis in *Arabidopsis*: a model for stress responses
Laszlo Szabados, Gyongyi Szekely, Edit Abraham, Gabor Rigo, Jolan Csiszar, Csaba Koncz

517 Expression analysis of zinc excess and deficiency response of *Arabidopsis* and the related metal hyperaccumulator Thlaspi caerulescens
Judith van de Mortel, Laia Almar Villanueva, Henk Schat, Jeroen Kwekkeboom, Sean Coughlan, Maarten Koornneef, Mark Aarts

518 **The *anthocyanin-impaired-response-1 (air-1)* mutant in the *sos3-1* background of *Arabidopsis thaliana* is deficient in the accumulation of anthocyanins in response to salt stress**
Michael Van Oosten, Ray Bressan

519 **Targeted degradation of *Arabidopsis* HFR1 desensitizes light signaling**
Jianping Yang, Rongcheng Lin, Ute Hoecker, James Sullivan, Xing Wang Deng, Haiyang Wang

520 **Temperature sensing and signaling in the non-extreme range**
Yi Wang, Jian Hua

521 **Identification of the temperature signaling components in *Arabidopsis thaliana***
Yi Wang, Jian Hua

522 **post-translational regulation of ACS5**
Shouling Xu, Joesph Kieber

523 **The *Arabidopsis* SUMO E3 ligase, AtSIZ1 is involved high temperature tolerance independent of heat shock proteins**
Chan Yul Yoo, Kenji Miura, Jing Bo Jin, Jiyoung Lee, Dae-Jin Yun, Ray Bressan, Paul Hasegawa

524 **Characterizing the relationship between magnesium deficiency and aluminum toxicity in plants**
S Robison, R Gardner

POSTER ABSTRACTS

Tripp Commons & Main Lounge (2nd floor)

Topic: Interaction With the Environment 2: Biotic

Abstracts 525-582

525 **RSTK1, an *Arabidopsis* Receptor-like Ser/Thr Kinase, Positively Regulates Cell Death and Resistance against Pathogens**
Biswa Acharya, Ramesh Raina

526 **Identification of regulatory elements involved in R/avr-mediated plant defense signaling**
Lori Adams-Phillips, Jinrong Wan, Xiaoping Tan, Richard Michelmore, Andrew Bent

527 **Indirect activation of the *Arabidopsis* disease resistance protein RPS5 by the Pseudomonas effector AvrPphB**
Roger Innes, Jules Ade

528 **Gene Profiling of Geminivirus-Infected *Arabidopsis thaliana***
Ross Wolfinger, Trino Ascencio-Ibanez, Tzu-Ming Chu, Linda Hanley-Bowdoin

529 **Withdrawn**
Yun-shu Chen

530 **Functional evaluation of plant defence signalling against fusarium ear blight disease in *Arabidopsis***
Alayne Cuzick, Sarah Lee, Martin Urban, Kim Hammond-Kosack

531 **Identification of *Arabidopsis thaliana* Genes Potentially Involved in Mediating AvrRpt2 Virulence Activity**
Agnes Demianski, Zhongying Chen, Barbara Kunkel

532 **Hypersensitivity to ABA and reduced resistance to Botrytis cineria in *Arabidopsis* mutant of a zinc finger transcription factor protein with ankryn repeat domains**
Rahul Dhawan, Xi Chen, Robert Dietrich, Tesfaye Mengiste

533 **RESISTANCE TO FUSARIUM OXYSPORUM 1, a Dominant *Arabidopsis* Disease Resistance Gene, is not Race Specific**
Andrew Diener, Frederick Ausubel

534 **Dissecting regulatory mechanisms controlling the plant defense transcriptome by functional genomics, proteomics and chemical genetics**
Alexandre Evrard, Colleen Knoth, Linda Saetern, Thomas Eulgem

535 **Electrophysiological characterization of bacterial *avrRpt2* gene-specific HR in *Arabidopsis* in the absence of other bacterial signals**
Sharon Pike, Walter Gassmann

536 **Functional characterization of the *Arabidopsis RPS4* disease resistance gene**
Xue-Cheng Zhang, Walter Gassmann

537 **Characterization of *srfr1* and *srfr3* suppressor mutants that reactivate *avrRps4*-induced resistance in *Arabidopsis***
Soon Il Kwon, Jessica Koczan, Walter Gassmann

538 Novel *Arabidopsis* proteins required for full RPS2-mediated resistance to Pseudomonas syringae pv. tomato
Andrew Bent, Ruth Genger, Betania Quirino

539 Inference of comprehensive network neighborhoods associated with phenotypes: a case study using *hin1*/*ndr1* gene family
Suresh Gopalan

540 A Novel Mutant Screen for Activators of Programmed Cell Death in *Arabidopsis*
Anna Gordon, Sergio Ulises, Katica Ilic, Ian Moore, Thomas Berleth, Celia Baroux, Patrick Gallois

541 Functional genomics to define mechanisms of defense against flea beetles in *Brassica napus* and *Arabidopsis*
Margaret Gruber, Limin Wu, Andy Sharpe, Dwayne Hegedus

542 A putative glutamate receptor protein involved in cell wall integrity signalling
Thorsten Hamann, Chris Somerville

543 Negative Regulation of Cell Death and Defense Responses by *BON/CPN* and *BAP* Genes
Jian Hua, Huijun Yang, Shuhua Yang

544 Characterization of a Methionine Sulfoxide Reductase B Gene of *Arabidopsis* Involved in Pathogen Defense
Guru Jagadeeswaran, Ramesh Raina

545 *Pseudomonas viridiflava* induces the jasmonic acid defense pathway in *Arabidopsis thaliana*
Katrin Jakob, Joel Kniskern, Joy Bergelson

546 Differential gene expression in response to infection by *Plasmodiophora brassicae* in a partial resistant ecotype of *Arabidopsis* using CATMA analysis
Melanie Jubault, Christine Lariagon, Jean-Pierre Renou, Ludivine Taconnat, Regine Delourme, Maria Manzanares-Dauleux

547 Transcription factor NtWRKY1 is phosphorylated by the MAP kinase SIPK and mediates HR-like cell death in tobacco
Hong-Gu Kang, Frank Menke, Zhixiang Chen, Jeong Mee Park, Dhirendra Kumar, Daniel Klessig

548 Over expressing a fatty acid amide hydrolase compromises innate immunity in *Arabidopsis*
Li Kang, Yuh-Shuh Wang, Elison Blancaflor, Kent Chapman, Kirankumar Mysore

549 Transcriptome analysis of *Bemisia argentifolli* adult and instar feeding on *Arabidopsis thaliana*
Louisa Kempema, Xinping Cui, Linda Walling

550 Systems Analysis of Natural Variation in *Arabidopsis*/Botrytis Interactions
Heather Rowe, Katherine Denby, Daniel Kliebenstein

551 Investigating the role of lipids in systemic acquired resistance
Kartikeya Krothapalli, Ashis Nandi, Ruth Welti, Jyoti Shah

552 The *Pseudomonas syringae* type III effector protein AvrRpt2 modulates host auxin physiology
Barbara Kunkel, Jennifer Agnew, Jerry Cohen, Zhongying Chen

553 JIN1/AtMYC2-dependent jasmonate signaling is required for suppression of salicylic acid-dependent defenses during infection by Pseudomonas syringae
Neva Laurie-Berry, Vinita Joardar, Ian Street, Barbara Kunkel

554 Identifying natural modifier(s) of a copine mutant bon1bon3
Yongqing Li, Shuhua Yang, Jian Hua

555 Study of biochemical activity of *BON1* and *BAP1* in regulating plant growth and defense response
Jian Hua, Yongqing Li

556 Innate Immune Signaling Pathways Controlling the Response to General Elicitors
Nicole Mammarella, Cristian Danna, Donatello Vairo, Giulia De Lorenzo, Frederick Ausubel

557 Auxin repression in *Arabidopsis* by a flea beetle damage-induced *Brassica napus* gene.
Margaret Gruber, Limin Wu, Cathy Coutu, Andy Sharpe, Dwayne Hegedus

558 Below-Ground Influence of *Arabidopsis* on Its Rhizobacterial Community Structure Varies with Plant Ecotype
Shirley Micallef, Michael Shiaris, Adan Colon-Carmona

559 Green Leaf Volatile Action in *Arabidopsis*
Rossana Mirabella, Piet van Egmomd, Michel Haring, Robert Scuurink

560 The *Arabidopsis thaliana* response to the bacterial pathogen *Pseudomonas syringae*: the search for novel plant defense signaling pathways
Raka Mitra, Jane Glazebrook

561 local adaptation in natural population of *Arabidopsis thaliana*
A. Mosleh Arany, T.J.de Jong & E.van der Meijden

562 Molecular and Genetic Analysis of the Transcription Factor ETHYLENE INSENSITIVE6
Ramlah Nehring, Joseph Ecker

563 HSC70s interact directly with the *Arabidopsis* SGT1s cochaperones *in planta* and are required for *R*-gene-mediated and basal resistance
Laurent Noel, Giuseppe Cagna, Shigeyuki Betsuyaku, Lennart Wirthmueller, Johannes Stuttmann, Jane Parker, Claus-Peter Witte

564 The pathogenic bacteria *Ralstonia solanacearum* directly targets the plant ubiquitin-proteasome pathway by injecting bacterial encoded F-box proteins into the plant cells
Nemo Peeters, Aurelie Angot, Patrick Barberis, Sebastien Cunnac, Esther Lechner, Pascal Genschik, Stephane Genin, Christian Boucher

565 Identification and characterization of *Arabidopsis thaliana* ecotypes with contrasting responses to inoculation with a Brazilian isolate of Xanthomonas campestris pv. campestris
Lilian do Carmo, Alice Quezado-Soares, Eduardo Leonardecz Neto, Carlos Lopes, Betania Quirino

566 Evaluation of *Arabidopsis thaliana* response to infection by the tospoviruses Tomato spotted wilt virus and Groundnut ringspot virus
Betania Quirino, Elizabete Candido, Lilian do Carmo, Pollyanna Campos, Barbara Santana, Antonio Carlos de Avila

567 Genome-wide analysis of gene expression reveals function of AtMYB30 in lipid signalling for the control of disease resistance and hypersensitive cell death
Sylvain Raffaele, Fabienne Vailleau, Susana Rivas, Otto Miersch, Elizabeth Blee, Dominique Roby

568 Identification of genes contributing to nonhost resistance of *Arabidopsis thaliana* against Phytophthora infestans
Lore Westphal, Jorn Landtag, Volker Lipka, Jan Dittgen, Paul Schulze-Lefert, Sabine Rosahl, Dierk Scheel

569 The gene RPB1 confers resistance of *Arabidopsis thaliana* to the obligate biotrophic root parasite *Plasmodiophora brassicae*
Frank Rehn, Andrea Arbeiter, Nadine Galfe, Andrea Zieris, Silvana Gutsch, Johannes Siemens

570 The role of the GH3-gene family during clubroot infection of *A. thaliana*
Cornelia Horn, Jutta Ludwig-Muller, Johannes Siemens

571 Transcription factor Dof15 as a candidate gene regulating glucosinolate metabolism in *Arabidopsis*
Aleksandra Skirycz, Michael Reichelt, Claudia Birkemeyer, Jonathan Gershenzon, Joachim Kopka, Bernd Mueller-Roeber, Isabell Witt

572 Recognition specificity in the FLS2/flagellin interaction
Wenxian Sun, Francis Dunning, Christine Pfund, Adedayo Fashoyin, Andrew Bent

573 Regulation of Plant Defense Responses in *Arabidopsis* by EDR2, a PH and START domain containing Protein
Dingzhong Tang, Jules Ade, Catherine Frye, Roger Innes

574 Indirect defense of *Arabidopsis* against herbivorous insects
Remco Van Poecke, Marcel Dicke

575 Functional Analysis of Pathogen-induced WRKY3 and WRKY4 Transcription Factors in Plant Defense Responses
KurumathurMadam Vinod, Baofang Fan, Zhixiang Chen

576 Analysis of *pmr6* - why is this mutant resistant to powdery mildew?
Sonja Vorwerk, Shauna Somerville, Chris Somerville

577 Combining transcript profiling and reverse genetics to find novel regulators of the defense response against Pseudomonas syringae
Natalie Weaver, Dong Wang, Jun Lu, Thomas Kepler, Xinnian Dong

578 Dissecting the jasmonate signaling pathway in *Arabidopsis*
Yu-Hung Linda Wei, Katherine Denby, Thomas Eulgem

579 Pathogen induced AtWRKY48 functions as a negative regulator in plant defense response
Denghui Xing, Zuyu Zheng, Kurumathurmadam Vinod, Baofang Fan, Zhixiang Chen

580 Spatial and temporal analysis of host gene expression in viral disease development
Chunling Yang, Rong Guo, Dan Nettleton, Jiqing Peng, Steve Whitham

581 Roles of Structurally Related WRKY 20, WRKY25, WRKY26 and WRKY33 Transcription Factors in Plant Defense Responses
Zuyu Zheng, Baofang Fan, Zhixiang Chen

582 An Anti-Insect Protein from *Arabidopsis*
Keyan Zhu-Salzman, Yilin Liu, JiEun Ahn, Jaewoong Moon, Sumana Datta, Ron Salzman, Beatrice Huyghues-Despointes, Barry Pittendrigh, Larry Murdock, Hisashi Koiwa

POSTER ABSTRACTS
Main Lounge (2nd floor)
Topic: Metabolism
Abstracts 583-618

583 Flavonol synthases in *Arabidopsis*: isoform-specific responses to developmental and biotic signals
Anne Alerding, Daniel Owens, James Westwood, Brenda Winkel

584 The Role of Prenyltransferase Stimulating Proteins (PSP) in *Arabidopsis thaliana*
Linda Walling, Virginia Alonzo

585 Transcriptional control of flavonoid biosynthesis: importance of the regulatory feedback loop controlling TT8 expression
Bertrand Dubreucq, Antoine Baudry, Michel Caboche, Loic Lepiniec

586 A novel isoform of glucan water dikinase is required for the breakdown of starch
Lone Baunsgaard, Henrik Lutken, Rene Mikkelsen, Mikkel Glaring, Andreas Blennow

587 WRINKLED1 of *Arabidopsis* Regulates Sugar Metabolism in the Seed and Seedling
Alex Cernac, Carl Andre, Christoph Benning

588 Metabolite profiling of PHB-producing *Arabidopsis* grown in three different illumination regimes
Suh-Yeon Choi, Lauralynn Kourtz, Kristi Snell, Basil Nikolau, Eve Wurtele

589 Flavonoid metabolism in *Arabidopsis* seeds
Lucille Pourcel, Antoine Baudry, Isabelle Debeaujon, Jean-Marc Routaboul, Thomas Goujon, Damaris Grain, Michel Caboche, Loic Lepiniec

590 Genetic, Molecular and Biochemical Studies of 3-Methylcrotonyl-CoA Carboxylase in *Arabidopsis thaliana*
Geng Ding, Ping Che, Eve Wurtele, Basil Nikolau

591 The plastidic glucose 6-phosphate/phosphate translocator GPT1 is crucial for gametophyte development in *Arabidopsis*
Anja Schneider, Patrycja Niewiadomski, Silke Knappe, Karsten Fischer, Ulf-Ingo Fluegge

592 A Biochemical and Modeling Approach to Understand Inositol Phosphate Metabolism in Plants
Javad Torabinejad, Bhadra Gunesekera, Mustafa Ercetin, Glenda Gillaspy

593 Interactions between SHMT and GOGAT in photorespiration
Aziz Jamai, Patrice Salomé, Lars Voll, Andreas Weber, C. Robertson McClung

594 Plastidic lipases similar to DAD1 in *Arabidopsis thaliana*
Elmar Weiler, Christine Boettcher, Stephan Pollmann, Melanie Juenger

595 *kidari1-1*, a Dominant mutation in *Arabidopsis*, confers hyper-Auxin phenotypes
Jeong Im Kim, Altanbadralt Shakhun, Dae-Jin Yun, Pinghua Li, Dong Won Baek, Jae Cheol Jeong, Hans Bohnert, Paul Hasegawa, Ray Bressan

596 The QTL Epithiospecifier modifier 1 (ESM1) represses nitrile formation in combination with ESP during glucosinolate hydrolysis
Zhiyong Zhang, Daniel Kliebenstein

597 α-Tocopherol oxidation products and metabolic analyses during high light stress in *Arabidopsis thaliana*
Naoko Kobayashi, Dean DellaPenna

598 A new type of peroxisomal acyl-coenzyme A synthetase from *Arabidopsis* has the catalytic capacity to activate biosynthetic precursors of jasmonic acid
Erich Kombrink, Katja Schneider, Lucie Kienow, Elmon Schmelzer, Thomas Colby, Michael Bartsch, Otto Miersch, Claus Wasternack, Hans-Peter Stuible

599 The nucleoside diphosphate kinases, a small gene family involved in nucleotide metabolism
Claudia Kopka, Rita Zrenner

600 Functional and Expresssion Analysis of Heteromeric Acetyl-CoA Carboxylase Subunit Genes in *Arabidopsis*
Ling Li, Xu Li, Hilal Ilarslan, Eve Wurtele, Basil Nikolau

601 Phloem Transportation of Maltose in Maltose-excess Mutants
Yan Lu, Jackson Gehan, Thomas Sharkey

602 Functional Importance of Diverse Hydroxyacyl-CoA Hydrolase Genes in A. *thaliana*
Kerry Lucas, John Hawes

603 The physiological role of glutathionylation of plastidic fructose-1,6-bisphosphate aldolase in *Arabidopsis thaliana*
Matsumoto Masayoshi, Ogawa Ken'ichi

604 What is the role of the PII (AtGlnB1) protein in *Arabidopsis*?
Sylvie Ferrario-Mery, Melanie Bouvet, Olivier Leleu, Gil Savino, Michael Hodges, Christian Meyer

605 Metabolomic analysis for understanding of disrupted nuclear-encoded chloroplast protein genes by FT-ICRMS in *Arabidopsis thaliana*
Reiko Motohashi, Masakazu Satou, Fumiyoshi Myouga, Daisaku Ohta, Akira Oikawa, Kazuo Shinozaki

606 Regulation of indolic glucosinolate and indole-3-acetic acid homeostasis by the *Arabidopsis* ATR1 Myb transcription factor
John Celenza, Juan Quiel, Gromoslaw Smolen, Houra Merrikh, Angela Silvestro, Mike Pieck, Jennifer Normanly, Judith Bender

607 The TRANSPARENT TESTA 10 Gene Encodes a laccase-like protein Involved in Oxidative Browning of the *Arabidopsis* Testa
Lucille Pourcel, Jean-Marc Routaboul, Michel Caboche, Loic Lepiniec, Isabelle Debeaujon

608 Flavonol 3-*O*-glycosyltransferases in *Arabidopsis thaliana*
Burkhard Messner, Patrick Jones, Yasutaka Nishiyama, Birgit Geist, Susanna Holzinger, Kazuki Saito, Tony Schaeffner

609 Trehalose-6-phosphate in *Arabidopsis*
Henriette Schluepmann, Mahnaz Aghdasi, Anja van Dijken, Matthew Paul, Sjef Smeekens

610 Starch content and starch-modifying-enzyme activity variations in α- and β-amylase insertional mutants of *Arabidopsis thaliana*
Tracie Bierwagen, Kevin Stokes, Martha James, Alan Myers

611 Light and carbon gene networks defined by genomic analysis of cli186: a carbon and light insensitive mutant.
Karen Thum, Michael Shin, Rodrigo Gutierrez, Manpreet Katari, Gloria Coruzzi

612 The circadian clock gates sensitivity of *GIGANTEA* gene expression to light
Reka Toth, Frederic Cremer, George Coupland

613 Differing roles for hydrolytic and phosphorolytic starch break down
Sean Weise, Thomas Sharkey

614 The *Arabidopsis thaliana* transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis
Ralf Stracke, Frank Mehrtens, Harald Kranz, Pawel Bednarek, Bernd Weisshaar

615 Regulation of Metabolic Networks in *Arabidopsis*
Eve Syrkin Wurtele, Wiesia Mentzen, Nick Ransom, Basil Nikolau, Jing Ding, Dianne Cook, Daniel Berleant

616 Control of Nuclear Genes Encoding Enzymes of Lipid Biosynthesis in Chloroplasts by a Protein Released from Mitochondrial Membranes
Jilian Fan, Changcheng Xu, Christoph Benning

617 High-throughput enzymatic tests coupled with structural modeling identify an *Arabidopsis* farnesoic acid methyltransferase
Yue Yang, Jeannine Ross, Joseph Noel, Feng Chen, Joshua Yuan, Eran Pichersky

618 Genetic analysis demonstrates functional overlap between starch synthases II and III in *Arabidopsis thaliana*
Xiaoli Zhang, Martha James, Alan Myers

POSTER ABSTRACTS

Main Lounge (2nd floor)

Topic: Novel Tools, Techniques, and Resources

Abstracts 619-645

619 GARNet
Ruth Bastow

620 Development of a Gal4-mediated tri-reporter expression system in *Arabidopsis*
Karen Fitzsimmons, Cawas Engineer, Melissa Curran, Robert Kranz

621 A Rapid Transformation System for Octoploid Strawberry
Leighan Howard, Philip Stewart, Amit Dhingra, Craig Chandler, Kevin Folta

622 ResurfP: A response surface aided parametric test for identifying differentials in experiments involving multiple independent measurements of a parameter
Suresh Gopalan

623 MapPlants: A Gene-Mapping Activity Optimized for the Undergraduate Laboratory
Ed Himelblau, Laurie Mentzer

624 The *Arabidopsis* Information Resource (TAIR) – New developments
Eva Huala, Margarita Garcia-Hernandez, Leonore Reiser, Katica Ilic, Hartmut Foerster, Douglas Becker, Tanya Berardini, Rachael Huntley, Aleksey Kleytman, Suparna Mundodi, Neil Miller, Mary Montoya, Nick Moseyko, Jon Slenk, Julie Tacklind, Christophe Tissier, Dan Weems, Christopher Wilks, Iris Xy, Thomas Yan, Daniel Yoo, Peifen Zhang, Brandon Zoeckler, Seung Rhee

625 Analyzing and sorting *Arabidopsis* seedlings using the COPAS PLUS instrument
Bo Wang, Julia Thompson, Patrick Sieber, Elliot Meyerowitz, Zack Nimchuk, Rock Pulak, John Humphrey

626 Development of an Automated Petri Plate Pourer
Jennifer Koerber, Jeanne Layton, Joette Hellebusch, Nigel Malterer, Steve Dulle, Susan Norris

627 Novel plant tissue digestion methodology stream lines plant genomic DNA purification
Michelle Mandrekar, Susan Koller, Rex Bitner, Hemanth Shenoi

628 Uncovering novel cell wall-related genes in *Arabidopsis thaliana*
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1 Energizing Arabidopsis

Chris Somerville

Carnegie Institution and Department of Biological Sciences, Stanford University

During the past 25 years, plant biologists have developed Arabidopsis into a very powerful tool for basic research. However, total federal funding for plant biology is less than 1% of the NIH budget. This ridiculous situation is not improved by having special interest groups within the plant biology community fighting for crumbs. I have long-believed that the heart of the problem is that plant biology is viewed as agriculture. Legislators view the big problem in agriculture as overproduction and do not see the point of further investments in basic research that might cause more overproduction. Additionally colleagues who work on crops species are likely to be first at the federal trough when the arguments are based on improving agriculture. I suggest that a more relevant social context for basic research in plant biology is energy. Although there is enough fossil fuel to meet our energy needs for three to four hundred years, there is broad consensus that continuing to burn fossil carbon will have unpleasant irreversible environmental consequences. World energy demand is approximately 11 TW. Approximately 100,000 TW arrives at the earth from the sun each year. Unfortunately, the energy density is too low to support large-scale use of photovoltaic cells. By contrast, recent studies by DOE indicate that plant biomass can make a significant contribution. However, the species that are suited for biomass production are undomesticated and have not been adapted for this purpose as yet. I will outline some aspects of basic research in plant biology that could have a significant impact on the rate of progress toward greater dependence on renewable sources of energy.

2 Composition and analysis of the chloroplast division machinery

Katherine Osteryoung

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The division of double-membraned chloroplasts in plant cells is orchestrated by a complex macromolecular machine with components positioned on both the inner and outer surfaces of the organelle and in the intermembrane space. The components of the chloroplast division apparatus must be properly assembled and their biochemical activities coordinated across the two envelope membranes to achieve chloroplast division. The long-term goal of our research is to understand the molecular events driving the constriction of the organelle and its separation into the two daughter plastids. Towards this end, we are using a combination of systems and approaches to identify the components of the chloroplast division complex and establish their biochemical functions. Consistent with the cyanobacterial origin of chloroplasts, most of the plastid division proteins we and others have identified thus far are evolutionarily related to cell division proteins found in prokaryotes, and are localized inside the organelle. These include, among others, the tubulin-like FtsZ1 and FtsZ2 proteins, and the J-domain-like protein ARC6, all of which localize to mid-plastid rings in the chloroplast stroma. Recently, we have uncovered several new cyanobacterial cell division genes that may facilitate identification of additional plastid division genes and proteins. We have also identified one plastid division protein, ARC5, which is a member of the dynamin family of GTPases and is localized on the cytosolic surface of the outer envelope membrane. This protein has no immediate counterparts in bacteria. Together, these data indicate that the chloroplast division apparatus is an evolutionary hybrid, comprising components derived from both the endosymbiotic ancestor of chloroplasts and its eukaryotic host.

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3 Prevacuolar compartments as proteolytic processing stations for storage proteins in Arabidopsis

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The *Arabidopsis* embryo accumulates two types of storage proteins, the 2S albumins and the 12S globulins, inside protein storage vacuoles (PSV). Both 2S albumins and 12S globulins are synthesized as precursors in the ER, exported to the Golgi apparatus, and accumulated in PSVs after processing of the proproteins. The processing of the storage proteins, which involves the cysteine-proteases VPE (vacuolar protein enzyme) and at least one aspartic protease, has been assumed to occur inside the PSVs. Multivesicular prevacuolar compartments carrying storage proteins have been reported in legume embryos but their exact role in PSV assembly is not clear. We have studied Golgi stacks, Golgi-derived vesicles, and prevacuolar compartments during PSV formation in *Arabidopsis* by means of electron tomography of high-pressure frozen/freeze substituted samples and by immunolabeling techniques in *Arabidopsis*, and by subcellular fractionation techniques in *Brassica napus*. Golgi staks produce two distinct types of vesicles: 130 nm vesicles carrying the 2S and 12 storage protein precursors and 30-40 nm vesicles carrying the precursors of the processing proteases. Subcellular fractionation studies confirm this distribution pattern. Interestingly, the prevacuolar compartments contain both storage proteins and processing enzymes. To assess if the storage proteins contained inside the prevacuolar compartment were the precursors or the mature forms, we raised peptide antibodies specific for propeptide that are removed from the storage protein precursors during the proteolytic processing. The simultaneous immunolabeling of mature forms and propeptides demonstrated that the storage proteins are mostly processed inside the prevacuolar compartments, before reaching the PSV. We propose that the observed compartmentalization in the proteolytic processing is related to the different luminal pH values detected inside prevacuolar compartments and developing PSVs, which would affect the solubility of the storage protein and the activity of the processing proteases. Supported by an Antorchas Foundation grant to M.S.O. and NIH grant GM61306 to L.A.S.

4 Auxin inhibits endocytosis and promotes its own efflux from cells

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The plant hormone auxin is a major regulator of plant development implicated in a variety of developmental processes such as organ initiation, directional growth, meristem activity and apical dominance. Auxin is distributed between cells by a polar transport system, which requires asymmetrically localized auxin transport facilitators from the PIN family. Using an inhibitor of subcellular vesicle trafficking - brefeldine A - it was shown that PIN proteins repeatedly internalize and recycle to and from the plasma membrane. In animals, similar subcellular dynamics, termed constitutive cycling, serve as a mechanism to control the subcellular location and thus the activity of proteins. Also signaling molecules including hormones can modulate constitutive cycling, however no such mechanism of hormone action has been demonstrated in plants. Here we show that auxin specifically inhibits the endocytosis of many plasma membrane localized proteins, without visibly affecting other vesicle trafficking pathways in cell. This effect is specific to biologically active auxins and requires activity of the Callosin-like protein BIG. By inhibiting the internalization step of PIN constitutive cycling, auxin increases the levels of PINs at the plasma membrane. Concomitantly, auxin promotes its own efflux from cells by a vesicle trafficking-dependent mechanism. Furthermore, asymmetric auxin translocation during root gravitropism correlates with reduced levels of PIN internalization. Our data imply a novel mode of plant hormone action: by modulating PIN protein trafficking, auxin regulates PIN abundance and activity at the cell surface providing a mechanism for the feedback regulation of cellular auxin transport.

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5 A Role for the Actin Cytoskeleton in Hexokinase Mediated Glucose Signaling

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Glucose has a hormone function in addition to its metabolic role in plant growth. The best understood glucose sensor/transducer is Arabidopsis hexokinase1 (AtHXK1) in which the glucose signaling activity can be uncoupled from glucose phosphorylation. However, the cellular and molecular processes by which AtHXK1 transduces a glucose signal remain poorly defined. Here we show that AtHXK1 is localized to mitochondria and that the actin cytoskeleton has an important role in glucose signal transduction. Localization of HXK1 was shown using different approaches: bio-imaging of transiently or stably expressed AtHXK1:GFP showed continuous colocalization with mitochondria under different treatment conditions; and, leaf organelle purification on Percoll gradients followed by western blotting also demonstrated that HXK1 is localized only to mitochondria. Previous studies have shown that plant mitochondria traffic on actin filaments. Disruption of actin filaments by latrunculin-B or cytochalasin-D altered mitochondrial cellular distribution and blocked HXK1 mediated glucose signaling in protoplast transient expresson assays. In contrast, protoplast treatment with oryzalin to disrupt microtubules did not affect glucose signaling. Furthermore, two null, vegetative actin mutants, act2-1 and act7-4, were shown to be tolerant to 6% glucose (which arrests wild type seedling development) and also were unable to carry out HXK1-dependent glucose signaling. Arabidopsis seedlings expressing actin binding domains of hTalin fused to GFP (A. Hardham, ANU) were used to visualize F-actin under different conditions. hTalin:GFP seedlings showed developmental arrest on 6% glucose, with a loss of filament formation. hTalin:GFP seedlings on 0.5% sucrose had normal growth, but when treated with 0.1 M glucose (but not mannitol) showed actin filament disruption within 60 min. This indicates that F-actin modification is an early response to glucose treatment. Moreover, glucose signaling paradoxically can either require a 'normal' actin cytoskeleton (e.g. responses of act2-1) or can be associated with a loss of F-actin (hTalin:GFP seedling response). Interestingly, AtHXK1-CFP transfected into hTalin:GFP leaf protoplasts was localized predominantly to F-actin, rather than mitochondria. One possibility is that an unidentified actin binding protein or even G-actin is involved in HXK1-dependent glucose signaling.

6 Signaling by heterotrimeric and extra-large G proteins in Arabidopsis ABA responses

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In mammals, heterotrimeric G proteins, composed of alpha, beta, and gamma subunits, couple ligand perception by G-protein-coupled receptors (GPCRs) with numerous signaling cascades. Humans have over 20 different Galpha subunits, 5 Gbeta subunits, and over 10 Ggamma subunits (1), leading to great combinatorial diversity in G-protein signaling. By contrast, the Arabidopsis genome has only single canonical Galpha and Gbeta subunit genes (*GPA1* and *AGB1*, respectively) and two identified Ggamma subunit genes (*AGG1* and *AGG2*). In addition to GPA1, the Arabidopsis genome has three genes encoding "extra-large" GTP-binding proteins (XLGs) (2, 3). The carboxy-termini of conceptually translated XLGs are homologous to GPA1, while the amino-terminal region of each XLG contains a putative nuclear localization signal and a cysteine-rich region. We are investigating the roles of these four GTP-binding proteins with regard to Arabidopsis ABA responses. Through the use of a knockout mutant approach, organ and cell-type specific roles of GPA1 in ABA signaling have been demonstrated (4), suggesting that coupling of GPA1 to diverse cell-specific effectors may functionally compensate for the lack of additional Galpha proteins in plants (5). We are also evaluating the alternative hypothesis that XLG proteins function as additional Galpha subunits in Arabidopsis.

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7 Axis -dependent gene expression in the lateral organ formation

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In order to unveil the signaling cascade which lead region-specific gene expression responsible for axis-dependent organ formation, we are examining expression pattern of three genes working in the early stages of lateral organ development. Lateral organs, such as leaves, young floral buds and floral organs develop from primordia formed at the fixed position in the peripheral region of shoot apical meristem, and are considered to follow three axes; basal-apical, central-marginal, and adaxial-abaxial. The direction of each axis could be fixed in relation to the position of the meristem. FILAMENTOUS FLOWER (FIL), a member of YABBY family, is responsible to the formation of abaxial side tissue and is expressed in the abaxial side of lateral organ primordia, Promoter analysis of FIL showed that the region-specific expression is based on two discrete cis elements: one is responsible to promote expression at both abaxial and adaxial sides, and the other is to repress at adaxial side. On the contrary, PHABULOSA (PHB), a member of homeobox family, is required for formation of the adaxial side tissue, and is expressed at the adaxial side. Recent researches propose that the region-specific expression of PHB is controlled by microRNA which is expressed at the abaxial side. These observations suggest that the two sides in lateral organ primordia mutually control specific gene expression. However, double-staining analysis of FIL- and PHB-expressing regions showed the two regions are partially overlapped, not supporting the simple mutual-repression model. The third gene, PRESSED FLOWER (PRS), a member of homeobox/WOX family, is expressed at the marginal region of lateral organ primordia. But the FIL-PHB overlapping region covers the PRS expressing region. Based on these observations and other ongoing experiments, we are trying to present a model of axes-dependent gene expression and lateral organ development.

8 A Mutation in the Arabidopsis ADK1 Gene Affects Root Gravitropism, Columella Morphogenesis and Lateral Auxin Transport Across the Root Tip

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Gravi-stimulation (GS) in plant organs is perceived by specific statocytes that translate the physical stimulus to a productive signal transduction event that ultimately results in the curvature response of the organ with respect to the gravity vector. Few proteins have thus far been shown to function in the early phases of gravity signal transduction in plant roots. A comparative proteomic approach identified adenosine kinase (ADK) to be differentially represented early in response to gravi-stimulation (GS). The fluctuation of ADK protein spot intensity was accompanied by ADK relocalization into the nuclear fraction after GS. Reverse genetics showed that mutation in one of the two Arabidopsis ADK paralogs, ADK1, results in reduced root sensitivity to GS, altered kinetics of root gravitropic curvature and distorted root cap morphology. Interestingly, the putative auxin efflux facilitator PIN3 was expressed in only a subset of columella cells in *adk1-1* mutant roots, and did not relocalize to the new physical bottom of these cells upon GS, as it does in wild type roots. Also, the auxin-responsive DR5-GUS reporter did not reveal the development of a lateral auxin gradient across *adk1-1* root caps upon GS. Together, the data suggest that ADK1 contributes to the control of early phases of gravitropic signal transduction in the root tip, in addition to contributing to root cap morphogenesis.

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9 A lipid transfer protein-like protein, DIR1, is involved in long distance signaling during the development of systemic acquired resistance

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Defense mechanisms in plants, including systemic acquired resistance (SAR), are induced by various biotic stresses. SAR is elicited in response to local necrotizing infections, which induce the production of an unknown long distance signaling molecule. The signal is perceived in distant tissues, resulting in resistance to normally virulent pathogens. A SAR-deficient *Arabidopsis* mutant, termed defective in induced resistance *(dir1-1)*, can receive and respond to this signal, but either does not produce or does not transmit it. The mutation affects a lipid transfer protein (LTP), which may participate in chaperoning a hydrophobic SAR mobile signal to distant tissues. LTPs have a hydrophobic pocket which can accommodate a hydrophobic molecule such as a fatty acid, lysophospholipid, or, speculatively, a hydrophobic peptide. Leaves of transgenic plants with the *DIR1* promoter driving GUS expression displayed strong staining in vascular tissues, with less staining also observed in leaf mesophyll cells. Furthermore, when these plants were infected by *Pseudomonas syringae* pv. *tomato*, the activity of the *DIR1* promoter was decreased, as demonstrated by reduced GUS staining throughout the leaves. Detection of DIR1/LTP in intercellular washing fluids of wild type, but not *dir1-1* plants strongly suggests that the putative N-terminal signal sequence localizes DIR1 to the extracellular space, as its sequence predicts. DIR1/LTP was detected in petiole exudates (enriched for phloem sap) collected from SAR-induced wild type, but not in *dir1-1* plants, supporting the involvement of DIR1/LTP in long distance SAR signaling. Furthermore, in preliminary experiments using transgenic *dir1-1* plants expressing a DIR1/LTP:GUS fusion protein under the control of the *DIR1* promoter, the SAR response was restored, and GUS activity was detected in exudates from SAR-induced plants. Based on these results, we hypothesize that DIR1/LTP is involved in long distance signaling as either the signal molecule or as the chaperone of the long distance signal.

10 Identification of O-GlcNAc modification of proteins in several signalling pathways

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Mutant studies indicate that O-GlcNAc modification of plant proteins is involved in circadian regulation and viral pathogenesis, and responses to gibberellin (GA), cytokinin and light. Deficiency of the two arabidopsis O-GlcNAc transferases (OGTs), SEC and SPY leads to gamete and embryo lethality indicating that O-GlcNAc modification is also required for development. To understand more about the role of this modification in plant development and signaling pathways we are investigating the role of O-GlcNAc modification of three proteins. O-GlcNAc modification and DELLA proteins both negatively regulate of GA responses. Deletion analysis of a rice DELLA has identified a serine/threonine (S/T) rich region that regulates its activity. Studies with arabidopsis OGT and DELLA mutants suggest that O-GlcNAc modification of DELLA proteins or a protein downstream of the DELLA proteins is required for suppression of GA responses. *E. coli*-expressed SEC modifies the S/T rich region of a DELLA protein. Genetic studies indicate that OGTs and GIGANTEA (GI) act in pathways involved in circadian regulation and light responses. GI is modified by *E. coli*-expressed SEC. The Plum pox virus capsid protein (PPV-CP) is O-GlcNAc modified. While PPV infects wild type, spy and sec plants, the infection spread is slower and the virion titer is lower in sec plants. In addition, PPV-CP from sec plants is not O-GlcNAc modified indicating that SEC modifies the capsid. These experiments suggest that O-GlcNAc modification of PPV-CP, GI and DELLA proteins affects the functioning of these proteins. To investigate the effect of O-GlcNAc modification on the activity of these proteins, we are mapping the modified sites, making non-modifiable mutant versions of them and testing the functionality of the mutant proteins.

11 Regulation of Flowering Time and the role of Vernalization

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Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure during winter to initiate flowering the following spring. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. Our studies of vernalization in Arabidopsis have revealed that meristem competence is a function of the expression level of certain MADS-box genes such as FLOWERING LOCUS C (FLC) that act as repressors of flowering. Exposure to prolonged cold causes an epigenetic switch of these MADS box genes to an unexpressed state, thus rendering the shoot apical meristem competent to flower. This epigenetic switch is caused by covalent modifications to histones of the chromatin of the flowering repressors.

12 The control of flowering by day length in Arabidopsis

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Max Planck Institute for Plant Breeding Research

In most plants the transition to flowering is controlled by seasonal cues such as changing day length. In Arabidopsis a circadian-clock regulated pathway that promotes flowering specifically in response to the longer day lengths of spring and early summer has been described. This pathway includes the GIGANTEA (GI), CONSTANS (CO) and FT proteins, which act in the vascular tissue of the leaves to promote synthesis or transport of a systemic signal that triggers flower development at the shoot meristem. We have studied how circadian-clock regulation and acute responses to light combine to activate this pathway in response to long days. These processes regulate expression of both *GI* and *CO*, and involve both transcriptional and post-transcriptional regulation. Accumulation of CO, a protein containing two B-box zinc fingers, in the nucleus under long days activates *FT* transcription, and thus early flowering. The spatial regulation of this pathway, and the molecular mechanisms that confer a flowering response to day length will be discussed.

13 Studies on the graft-transmissibility of promotion of flowering by *FT* in *Arabidopsis*

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Photoperiodic induction of flowering requires light perception in leaves, followed by transmission of mobile signal from leaves to the apex, where floral meristems are initiated. However, the nature of the mobile signal has remained unknown.

In *Arabidopsis*, floral pathway integrator *FT* acts mainly in the photoperiod pathway through transcriptional regulation by CONSTANS. *FT* encodes a 20kD protein of the PEBP/RKIP family and is expressed in vasculature of cotyledon and leaf where light is perceived. *FT* transcription is immediately induced in these tissues upon transfer from short-day to inductive long-day photoperiod. Promotion of flowering by *FT* requires the activity of another flowering-time gene *FD* which encodes a bZIP transcription factor preferentially expressed in the shoot apex.

To examine whether the effect of *FT* to promote flowering is transmissible across long distance, we performed micrografting experiments. Using 4-day-old seedlings, two-shoot, 'Y-shaped' grafts were assembled on hypocotyls of *ft-1* recipient. There was no difference in flowering time between *ft-1* self-grafts (*ft-1* grafted onto *ft-1*) and intact *ft-1* control, indicating that graft treatment *per se* does not affect the flowering time of recipient plants. First, we grafted *FT* overexpressor (*35S::FT*) as the scion to the *ft-1* stocks. Flowering time of *ft-1* was dramatically accelerated relative to *ft-1* self-grafts or intact controls. This suggests that effect of *35S::FT* to promote flowering was transmitted to recipient *ft-1* plants. We then grafted transgenic plants which express *FT* by tissue specific promoters to the *ft-1* stock. *SULTR2;1::FT* (specific in phloem) is much more effective than *PDF1::FT* (specific in L1 of shoot apex) in promoting flowering of the *ft-1* stock, although these two transgenic plants themselves had similar precocious-flowering phenotype. Finally we grafted WT onto *ft-1* and observed small but significant promotion of flowering. Results of these and other ongoing experiments will be presented.

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14 *INFLORESCENCE DEFICIENT IN ABSCISSION* Controls Floral Organ Abscission in *Arabidopsis*

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Abscission is an active process that enables plants to shed unwanted organs. Because the purpose of the flower is to facilitate pollination, it often is abscised after fertilization. We have identified a floral organ abscission mutant in *Arabidopsis*, *inflorescence deficient in abscission* (*ida*). *ida* is the first floral abscission mutant characterized which shows a complete lack of floral organ abscission. The *ida* gene encodes a small protein with an N-terminal signal peptide, suggesting that the IDA protein is a ligand of an unknown receptor involved in the developmental control of floral abscission (Butenko et al, 2003 Plant Cell 15: 2296-2307). IDA::GUS reporter lines showed the wild-type *IDA* gene to be expressed specifically in the floral abscission zone during the time of abscission. A single copy IDA::GUS line has been crossed to the ethylene insensitive mutant *etr1-1* to investigate the IDA expression in the *etr1-1* background. In addition we have used molecular markers for chitinase and cellulase to track the abscission process in the ida background. Transient GFP expression showed IDA to be localized in the apoplastic space of onion cells. Stable GFP transformants are currently being investigated to look at GFP-expression in planta. In addition we are working on identifying the receptor, and other genes that are components in the same developmental pathway; by doing both yeast two hybrid and activation tagging. Over expression lines will hopefully give us an indication to whether IDA is involved in regulating the last step of the floral abscission, or inhibiting a repair process during abscission.

Hopefully the identification of proteins interacting with IDA will give us further insight into the regulation of the floral abscission process in Arabidopsis.

15 A novel positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis

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Double fertilization of the egg cell and the central cell by one sperm cell each produces the diploid embryo and the triploid endosperm and is one of the defining characteristics of flowering plants (angiosperms). Endosperm and embryo develop in parallel to form the mature seed. However, not much is known about the coordination between these two organisms. Here we present the characterization of a mutant of the Arabidopsis Cdc2 homolog CDKA;1, which confers a paternal effect. In cdka;1 mutant pollen only one, instead of two, sperm cells is produced. Mutant pollen is viable but can fertilize only one cell in the embryo sac, thus allowing a genetic dissection of the double fertilization process. We observed exclusive fertilization of the egg cell by *cdka;1* sperm cells. Moreover, we show that unfertilized endosperm develops, revealing a previously unrecognized positive signal from the fertilization of the egg cell initiating proliferation of the central cell. A similar positive signal can already be found in certain species of lower seed plants (gymnosperms), and thus, might reflect an ancient signal in seed development with implications for the evolution of modern flowering plants.

16 From embryogenesis to the vegetative plant

Gerd Juergens

University of Tuebingen, ZMBP, Developmental Genetics

This overview presents recent advances in vegetative development. Embryogenesis establishes the multicellular organisation of the plant body by a process called pattern formation. Cells adopt specific fates according to their relative position. Pattern formation proceeds along two body axes. The apical-basal axis is partitioned into a series of embryonic structures such as cotyledons, hypocotyl and root, which separate the primary meristems of shoot and root located at opposite ends of the axis. Pattern formation along the radial axis generates the main tissue types such as epidermis, ground tissue (cortex, endodermis) and vascular tissue (xylem and phloem surrounded by pericycle). Following seed germination, the self-maintaining meristems of the seedling produce new shoot (leaves, sideshoots) and root structures. In addition, lateral roots are formed from pericycle cells. During organ development, tissue layers are formed and specific cell types are generated. Towards the end of my talk I discuss new findings from our work addressing cell fate specification in early embryogenesis.

17 Two tales of meristems

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We are interested the developmental role of chromatin remodeling ATPases in *Arabidopsis*. In one approach we investigated maintenance of the stem cell population of the shoot apical meristem. The SNF2-subgroup ATPase SYD is important for stem cell pool maintenance during adult development. We demonstrate using genetic and molecular analyses that SYD primarily acts on the stem cell promoting WUS pathway. In this pathway SYD acts upstream of WUS. Furthermore SYD is specifically recruited to the *WUS* promoter *in vivo* suggesting that SYD directly regulates expression of this homeodomain transcription factor.

In a second approach we investigated the molecular basis of the meristem identity transition downstream of LFY. During the meristem identity switch, cells in the peripheral zone stop producing secondary inflorescences subtended by bracts and instead give rise to flowers. We have identified several direct LFY target genes at this stage in development. The five best characterized LFY targets encode transcription factors and putative signal transduction pathway components. I will discuss our current understanding of the biological role of these new meristem identity regulators.

18 The *MACCHI-BOU* genes regulate organogenesis together with *PINOID*

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The primordia of shoot organs such as leaves arise from the peripheral zone of the shoot apical meristem (SAM). Similarly, cotyledon primordia are formed in the peripheral region of the embryo apex. Both in the SAM and embryo apex, organ primordia emerge in regions where auxin is highly concentrated. The asymmetrical auxin distribution is established by PINOID (PID), a Ser/Thr kinase, through the regulation of cellular localization of PIN-FORMED1 (PIN1), an auxin efflux facilitator. To identify additional genes involved in this process, we carried out a screen for *pid* enhancers, named *macchi-bou* (*mab*). By screening mutations that caused seedlings without cotyledon development, we identified four loci, *MAB1~4*. All *mab pid* double mutants exhibited defects not only in cotyledon development but also in postembryonic organogenesis, suggesting that the *MAB* genes are involved in organogenesis together with *PID* both in embryonic and postembryonic development. A fraction of all *mab* single mutant seedlings displayed defects in cotyledon number, position and separation. In addition to defects of cotyledon development, *mab1* single mutants displayed defects in root development, *mab2* single mutants exhibited the sterility and the aberrant shoot architecture as bracts develop on the inflorescences, *mab3* single mutants were defective in shoot organ formation, and *mab4* single mutants displayed defects in floral organ development. *mab3* was a novel *pin1* allele. The *MAB1* gene was isolated by map-based cloning and it encodes a mitochondrial pyruvate dehydrogenase E1 beta subunit, which converts pyruvate to acetyl-coenzyme A. Our results indicate that a metabolic pathway involving *MAB1* is engaged in organogenesis. A possible role of the *MAB1* gene for organogenesis will be discussed.

19 Phosphatidylinositol signaling is involved in the regulation of root system architecture

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Environmental conditions exert a profound effect on root system architecture. In *Arabidopsis thaliana* ecotype Columbia, the formation of lateral roots from lateral root primordia is repressed or significantly delayed under conditions of mild water stress. Using this repressed phenotype, we screened for mutants with aberrant root architectures and isolated *Lateral Root Development* mutants from a population of 10,000 EMS mutagenized seeds. One of these mutants, *lrd2*, exhibits a dramatic increase in its total lateral root length (TOT) both on water stress and water plentiful conditions. Moreover, the root systems of *lrd2* plants are significantly less affected by the water stress conditions compared with wild type. Further characterization has shown the increase in TOT is due to both an increased initiation of lateral root primordia and a significantly higher percent of primordia that develop into lateral roots compared to wild type. We have cloned the *LRD2* gene and it is predicted to encode a type III α1 phosphatidylinositol 4-kinase (PI4Kα1). Our progress in defining the function of PI4Kα1 in this system will be reported. The identification of *LRD2* as a PI4K reveals a novel role for phosphatidylinositol signaling in the regulation of root system architecture.

20 DRN and DRN-LIKE of Arabidopsis redundantly control early embryonic patterning through interactions with class III HD-ZIP proteins

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Embryo development and the establishment of a SAM to inititate organogenensis are amongst the most fundamental processes of plant development. *DORNROESCHEN/ENHANCER OF SHOOT REGENERATION1 (DRN/ESR1)*, encodes an AP2 domain-containing protein of the ERF (Ethylene Response Factor) type and is involved in shoot apical meristem development and lateral organ formation. A paralogous gene, *DRN-LIKE* exists and is closely linked to *DRN* on chromosome 1. Insertion mutants for both *DRN* and *DRN-LIKE* genes show fused cotyledon phenotypes at low penetrance, similar to those of the *pin1* and *pinoid* mutants. *DRN* also provides a link with the hormone control of gene regulation in the SAM, being positively regulated by auxin and overexpression causes cytokinin-independent effects. This positive auxin activation is consistent with promoter deletion studies and the location of putative auxin response elements. Combining mutant *drn* and *drn-like* mutant alleles shows differential and unequal contributions in different heterozygote/homozygote combinations, with additional embryo-specific phenotypes, confirming a partially redundant function for both genes in embryo patterning.

To understand further DRN/ESR1 and DRN-LIKE function, a yeast two hybrid screen revealed that DRN interacts with the classIII HD-ZIP protein PHAVOLUTA (PHV) and a basic helix-loop-helix protein (bHLH). Experiments also showed DRN-LIKE interacts with these proteins as well as with other proteins from the HD-ZIP III class also comprised of PHABULOSA, REVOLUTA, CORONA and ATHB8. These interactions involves a novel C-terminal domain specific for this sub-class of proteins and we have evidence of ternary protein complexes involving DRN/ESR1, PHAVOLUTA and bHLH. ClassIII HD-ZIP proteins act redundantly to control aspects of leaf development as well as embryo development. We have confirmed all protein-protein interactions in planta using bimolecular fluorescence complementation and biochemically via coimmunoprecipitation. The functional significance of these interactions has been confirmed by in situ hybridisations showing that DRN/ESR1 and DRN-LIKE are expressed in globular and heart stage developing embryos and have partly overlapping expression domains together with those of PHV, PHB and bHLH. These molecular and genetic data clearly imply that embryo patterning is controlled by the redundant function of at least three classes of transcription factors in different combinations and via novel domains.

21 Shedding light on metabolism in the dark

Alison Smith

John Innes Centre

Plants are the source of most of the organic carbon on earth, and are able to create a vast wealth of different components from carbon dioxide and inorganic nutrients. Demand for the products of plant metabolism will expand and diversify in an unprecedented way in the next few decades in response to population increase, climate change, scarcity of fossil fuels and problems of waste disposal. Although the outlines of many metabolic processes in plants are known, many others remain to be discovered. Even for the processes we know about, we have relatively little understanding of either the mechanisms that integrate metabolism with development and with responses to the environment, or the basis of the enormous variations between organs and species in the way in which carbon is allocated to metabolic pathways. Post-genomic developments are at last allowing us to address these broad questions. Genome sequences reveal the metabolic capacity of the plant, transcriptomics, proteomics and high-throughput assays help us to visualise where and when that capacity is expressed, combinations of forward and reverse genetics allow us to probe function and flux control in unprecedented detail and sensitivity, and the developing science of metabolomics gives us a broad view of the whole metabolic network, rather than the narrow snapshots available before. I will use work from my lab and those of our collaborators to illustrate how these new resources have brought about a radical revision of our understanding of a core metabolic pathway, the conversion of starch to sucrose in leaves in the dark.

22 Root Exudation of Antimicrobials Mediates Pathogen

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Using a root infection assay, we found that from a collection of eight *Pseudomonas syringae* strains only one pathovar, *P. syringae pv. tomato* DC3000 (*Pst* DC3000), caused root disease in *Arabidopsis thaliana*. The ability of Pst DC3000 to infect Arabidopsis roots was correlated with the ability of the bacteria to resist the antimicrobial compounds present in the root exudates of Arabidopsis, as well as its ability to block the synthesis/exudation of these compounds. The concentrations of antimicrobials in the root exudates were far higher during infection with non-pathogenic strains than in uninfected plants or in plants infected with the pathogenic strain. Purified antimicrobial compounds were bacteriostatic against the non-pathogenic strains *in vitro* at the same concentrations as those exuded by the roots. Mutants of *Pst* DC3000 in the type three secretion system (TTSS) genes *hrcC* (hypersensitive response) and *hrpL* (hypersensitive response and pathogenesis) elicited the same concentrations of antimicrobials from roots as the non-pathogenic strains. Additionally, we will show that the secretion of compounds by Arabidopsis roots is partially controlled by ABC transporters and that impaired phytochemical secretion in some ABC transporter mutants may account for enhanced susceptibility to *Pst* DC3000. Finally, we will show recent data that suggest that *P. syringae* strains are able to communicate with the roots using volatile and diffusible chemical compounds, and that these bacterial chemicals induce root growth inhibition in Arabidopsis. Understanding the chemical and molecular mechanisms by which Arabidopsis roots communicate with microbes in the soil will improve our understanding of rhizospheric processes.

23 High throughput metabolomics for the construction of regulatory networks for plant metabolism

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In the plant kingdom metabolites play a crucial role in many aspects of plant performance. It has been estimated that over 100.000 metabolites can be involved in diverse processes of which many are common for most plant species. This large variety exceeds that of other eukaryotic organisms and it is thought that it would offer plants an alternative to withstand environmental and biotic threats being unable to spatially escape from it.

However often vitally important, for many metabolites quantitative variation is observed between and within plant species. This indicates that at least part of this variation is genetically regulated. Although numerous metabolites have been identified to play crucial roles in a wide variety of biological processes, little is known about their regulation.

Quantitative trait locus analysis makes use of the natural variation present in segregating mapping populations to identify loci regulating the observed variation. In recent years a few studies have demonstrated the genetic regulation of a limited number of metabolites using targeted detection methods. Until now however, large-scale QTL analyses using undirected metabolomics was hampered due to the high number of masses detected and the difficulty in accurately aligning large numbers of chromatograms.

We show the successful detection and alignment of >5700 masses in 400 samples, which were used for genetic analysis of the *Arabidopsis thaliana* L*er*/Cvi recombinant inbred line population consisting of 160 lines. Extracts of seedlings were subjected to LC-MS and the obtained chromatograms were aligned using Metalign. Mean values of the >5700 detected masses were used in QTL analysis identifying significant QTL for >2700 masses. The QTL profiles of these genetically regulated masses could be correlated in many cases, revealing co-regulated masses. Genetic regulatory networks could be constructed where nodes, representing metabolites, are connected if they are commonly regulated. Co-regulation of metabolites implies them to be involved in the same biological pathway or process. We validated our approach with evidence from literature and indicate candidate genes for the regulation of common metabolic pathways.

Joost Keurentjes is supported by NWO Genomics and CBSG.

24 Arabidopsis mutants that are defective in seed storage reserve deposition and mobilization: *RDM1* encodes the triacylglycerol lipase that catalyses the first step in storage oil breakdown

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Germinating seeds rely on their storage reserves to support growth until they achieve photosynthetic competence. Mutants that fail to deposit these reserves during seed maturation, or are unable to mobilize them after germination, exhibit arrested seedling growth. Providing an alternative source of carbon such as sucrose can often rescue their phenotype. This selection strategy was used to isolate over a hundred arabidopsis *rdm* (reserve deposition and mobilization) mutant lines. The mutants fall into seventeen complementation groups. Eight of these groups appear to be new (*rdm1* to *8*). The remaining lines are allelic to characterized mutants (*wri1*, *pxa1*, *ped1*, *ped2*, *pex5*, *chy1*, *icl*, *mls* and *pck1*).

In arabidopsis oil is the major seed storage reserve, making up ~35% of the seed dry weight. *RDM1*, *2*, *3* and *4* are genes required for storage oil breakdown and when the mutants are grown on sucrose medium for five days they retain more than 90% of their oil. Surprisingly when *rdm1* is sown on soil in the glasshouse it germinates normally and many seedlings become established. In contrast *rdm2*, *3* and *4* behave similarly to existing mutants that are blocked in oil breakdown such as *pxa1* and *ped1*. They have reduced germination frequencies and arrest early in post-germinative growth. *RDM1* has been positionally cloned and the gene product characterized. It encodes the triacylglycerol lipase, which catalyses the first step in storage oil breakdown in germinating seeds.

25 The FRO3 ferric reductase plays a vital role in iron homeostasis in Arabidopsis

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The Arabidopsis *FRO2* gene encodes the iron-deficiency inducible Fe(III) chelate reductase responsible for reduction of iron at the root surface; subsequent transport of iron across the plasma membrane is carried out by a ferrous iron transporter (IRT1). We have identified seven additional FRO family members in the Arabidopsis genome and our current studies are aimed at determining the functions of each FRO gene. After iron is taken up by root cells, it is thought that iron is re-oxidized to the ferric form and transported as Fe(III)-citrate via the xylem to the aerial parts of the plant. Fe(III) chelate reductase activity is required for further iron uptake by leaf cells; presumably one or more FROs function at the leaf plasma membrane to reduce iron. We used real time RT-PCR to examine the expression of each FRO gene in different tissues, in response to iron limitation and in response to light/dark treatment. *FRO3* is expressed at high levels in leaves and *FRO3* expression is induced by iron-deficiency in leaves. Expression of *FRO3* also is elevated in light-treated plants as compared to dark-treated plants. Analysis of a *FRO3-KO* line shows that *FRO3* functions in Fe(III) reduction in leaves. Iron accumulation is altered in the *FRO3-KO* line as compared to wild type as is the expression of a variety of genes involved in iron uptake, localization and storage. Our results demonstrate that *FRO3* functions in reduction of iron in leaves and that *FRO3* is essential for maintenance of iron homeostasis in Arabidopsis

26 Exploring Chemical Space in the Plant World

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Bioactive chemicals have a long history of helping plant physiologists unravel mechanisms, including those involving: inhibitors of GA biosynthesis, inhibitors of ethylene action, inhibitors of auxin transport, cytoskeleton-disrupting drugs, and inhibitors of GDP-GTP exchange proteins, just to name a few. However, this approach has also met with strong criticism due to the complexities associated with understanding the action mode of compounds at the molecular level. This is one reason why drug companies must advertise the side effects of the drugs they sell. What has motivated biologists to revisit their interest in small molecules? While a little more than ten million pure compounds are known in chemical literature, the potential chemical diversity (defined as the number of unique chemical structures) of compounds composed of carbon, hydrogen, nitrogen, oxygen, sulfur, phosphorous, and the halogens (the organic chemists periodic table) of molecular weight <1000 likely exceeds 10^{60}. The compounds that have thus far been tested for effects on plants are therefore only a minute fraction of the structural possibilities. The development of combinatorial and automated techniques for synthesizing novel compounds brought forth significant enhancement in the productivity of chemists and makes the likelihood of synthesizing molecular libraries that are representative of chemical space much greater. These advances allow for the identification of chemicals that specifically disrupt a process or the function of a protein. Once these chemicals are identified, we can combine their use with genetic screens to identify genes involved in the same process. The use of unbiased libraries of diverse small molecules will allow plant biologists to discover numerous new bioactive molecules valuable for studying the function of uncharacterized plant genes. Importantly, when combined with Arabidopsis functional genomics, chemical genomics is powerful for the effective and efficient analysis of regulatory networks underlying a specific process. The chemical genomics approach can address loss-of-function lethality and gene redundancy and allow instantaneous, reversible, tunable, and conditional control of a phenotype. Well-characterized bioactive chemicals and their targets identified in Arabidopsis can be used in non-model species to improve agronomic traits and increase crop value.

27 Studying Novel Plant Peroxisomal Functions by Bioinformatics and Proteomics

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Our knowledge on plant peroxisomal metabolism is limited to the most abundant enzymes that play a role in photorespiration, fatty acid beta-oxidation and ROS metabolism. We applied a bioinformatics approach to specify the peroxisome targeting signals (PTS) for plants by analyzing semi-quantitatively plant ESTs that are homologous to PTS-targeted plant peroxisomal proteins for the nature of their PTS (Reumann, 2004). Specific PTS peptides were defined for higher plants and applied to screen the Arabidopsis genome for unknown peroxisomal matrix proteins. About 220 and 60 proteins were identified that carry a putative PTS1 or PTS2, respectively. About 80% of these proteins are unknown. Novel non-hypothetical proteins include several enzymes involved in alpha-oxidation of unsaturated fatty acids and branched amino acids, 2-hydroxy acid oxidases as well as NADP-dependent dehydrogenases and reductases. Putative regulatory proteins of plant peroxisomes comprise protein kinases, small heat-shock proteins, and proteases. Bioinformatics information for these Arabidopsis proteins has been compiled in the public database "AraPerox" (Reumann et al., 2004).

The predicted targeting of interesting novel proteins is verfied *in vivo* based on YFP and CFP fusion proteins. Indeed, many enzymes have been shown to be targeted to plant peroxisomes, whereas regulatory proteins often fail to be imported, which is most likely due to transiently and highly regulated protein targeting to peroxisomes upon perception of unknown signals. In a complementary approach, we use *Spinacia oleracea* L. and *Arabidopsis thaliana* L. as model organisms for proteome studies of leaf peroxisomes. The contamination by non-peroxisomal proteins is constantly reduced, and many proteins have been identified, including several predicted from our database "AraPerox". Some proteins seem to be modified post-translationally, whereas others are clearly induced by light and other stress conditions. Attempts are currently underway to identify low-abundance and inducible proteins and to map putative phosphorylation sites.

Reumann, S. (2004) Specification of the peroxisome targeting signals type 1 and type 2 of plant peroxisomes by bioinformatics analyses. Plant Physiology 135:783-800.

Reumann, S., Ma, C., Lemke, S. and Babujee, L. (2004) AraPerox. A Database of Putative Arabidopsis Proteins from Plant Peroxisomes. Plant Physiology 136:2587-2608.

28 Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases

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Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases. Moshe Reuveni1 and Patricia M. Kane2. 1Dept of Ornamental Horticulture, ARO Volcani Center, Bet Dagan, Israel; 2Dept. of Biochemistry and Molecular Biology, SUNY Upstate Medical Univ. Syracuse, NY 13210, USA. Vacuolar proton-translocating ATPases (V-ATPases) are ubiquitous proton pumps that acidify multiple organelles in all eukaryotic cells. Organelle acidification is important for protein sorting in the endocytic and biosynthetic pathways, zymogen activation, cytosolic pH and Ca2+ homeostasis, and a number of other fundamental physiological functions. In plant cells, V-ATPases are responsible for acidification of the vacuole and thus critical to the vacuole's ability to accumulate solute and proteins and respond to toxic and osmotic stresses. Vacuolar acidification is linked to some of the most fundamental qualities of different plants, for example, the sour taste of lemon and the color of many flowering plants. V-ATPases are highly conserved multisubunit complexes comprised of the V1 sector, a peripheral membrane complex containing the sites of ATP binding and hydrolysis, attached to an integral membrane complex containing the proton pore, the Vo sector. V-ATPases are composed of at least 14 different subunits, and most eukaryotes encode multiple isoforms of at least one of the V1 subunits in their genomes. These isoforms may help to fine-tune V-ATPase function in different locations or under different physiological conditions. Yeast has emerged as the predominant model system for the study of eukaryotic V-ATPases because of the ease of genetic manipulation of this organism. We are using the available genetic data to replace the yeast (S. cerevisiae) V1 complex with a functional plant V1 complex. Preliminary results suggest that replacement of as many as four of the eight yeast V1 subunits with the corresponding Arabidopsis subunits does not prevent V-ATPase activity. Arabidopsis encodes 3 isoforms for each of 3 different V1 subunits (B, E and G). We are expressing them individually and in combination in the yeast system and characterizing the biochemical properties of the resulting complexes. These experiments will provide unprecedented insight into the basic biochemical properties of plant V-ATPases isoforms as well as the potential regulatory roles of the subunit isoforms in Arabidopsis, in other plants and in other organisms.

29 LAPs and DAPs: N-terminal Modifying Enzymes of *Arabidopsis thaliana*

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The specificity and mechanisms of N-terminal modifications of the Arabidopsis proteome are being investigated. N-terminal modifications occur during synthesis, are required for activation or regulation of activity, and are involved with degradation of proteins/peptides – in essence, during birth, life and death of the protein. There are three basic N-terminal modifications that are being addressed: 1) limited proteolysis to remove one to three amino acids; 2) modification of the a-amino group; and 3) side chain-specific changes. These processes represent important fundamental aspects of protein maturation and turnover and potentially of plant gene regulation. We have identified a diverse set of enzymes including 27 peptidases and 11 transferases that have likely roles in these N-terminal modification reactions in plants. Here we report studies on three leucyl aminopeptidases (LAP1, LAP2, and LAP3) and two aspartyl aminopeptidases (DAP1 and DAP2). Expression programs, phenotypes of *LAP* and *DAP* knock-out mutants, and *LAP* and *DAP* promoter activities as evidenced by *LAP:GUS* and *DAP:GUS* transgenic lines will be presented. LAP and DAP enzymes have been over-expressed in *E. coli* and activities characterized. Combinatorial peptide libraries are being used to determine the substrate specificity of these N-terminal processing enzymes.

30 Abiotic Interactions with the Environment

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Plants interact with both biotic and abiotic factors in their environments. Interestingly, there is a temporal aspect to many of these interactions. It is widely recognized that the ability to perceive and respond to environmental stimuli can vary over developmental time; it is less commonly acknowledged the plant's perception of and response to environmental stimuli may also vary with the time of day. That is, perception and response may be gated by the circadian clock. In this overview I will discuss recent progress in plant responses to abiotic environmental stimuli, including conditions such as light, water, salt and temperature, and I will also describe recent examples where these responses are affected by the circadian clock. I will also discuss recent progress in elucidating the mechanisms by which the plant circadian clock responds to environmental stimuli, emphasizing light and temperature. In particular, I will focus on the roles of the *PSEUDO RESPONSE REGULATOR* (*PRR*) gene family in entrainment of the oscillator by both light and temperature. Mutational analysis indicates that the temperature sensing mechanism that provides input to the clock is distinct from that used by the plant to respond to cold stress. Despite the ability of the plant to sense and to respond to temperature steps or pulses, it is well established that the period of the clock is maintained more or less constant across a broad range of temperatures. This is termed temperature compensation, and recent advances in this area will also be discussed. Work in my laboratory is supported by grants (MCB-0343887 and IBN-0316056) from the National Science Foundation.

31 Clocks, photoreceptiors and photoperiodism

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Our laboratory is undertaking systems level approaches to understanding circadian clock function in Arabidopsis. The long-term goal is to define the circuitry required to generate robust, physiologically relevant rhythms using a combination of forward genetics with whole-genome approaches in an attempt to understand the network of circuits that are required for the core clock, and how the clock exerts its outputs upon the cell. We are beginning to discover that circadian clocks of both plants and animals are composed not of a single autoregulatory loop but rather of multiple positive and negative interlocking feedback loops. The Psuedo Response Regulator proteins PRR7 and PRR9 appear to define one such loop, which interlocks with the previously described feedback loop between CCA1/LHY and TOC1. Forward genetic screens for circadian mutants are not nearly saturated and a recent screen has identified the ELF4 gene and a novel Myb-like DNA binding protein (LUX) as being critical factors for sustaining circadian rhythmicity under constant conditions. The circadian clock is instrumental in photoperiodic time measurement and previous studies have identified FKF1 as an F-box protein that plays a central role in photoperiodic control over time to flowering. We have performed an interaction screen in yeast to identify potential targets for the FKF1 protein and found a novel Dof class transcription factor (Cycling Dof Factor1, CDF1) that appears to function as a key repressor of flowering. FKF1 is involved in the regulation of CDF1 protein turnover, which contributes to photoperiodism. Finally, light plays a crucial role in the response of plants to their environment and in entraining the circadian clock. We identified a clock-regulated F-box protein (AFR) that appears to play a role in regulating light-signal transduction and have identified a potential target of this F-box protein. In summary, the network architecture of the Arabidopsis circadian system is highly complex so as to provide robustness, multiple opportunities for output control, and several pathways for controlling inputs or environmental entrainment of the oscillator(s).

32 Mapping the *Arabidopsis* ionome

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Altering the ability of plants to take up and sequester minerals could have a dramatic impact on both plant and human health. Furthermore, understanding the pathways by which metals accumulate in plants will enable the engineering of plants to either exclude toxic metals or extract them from the soil. We have employed mineral nutrient and trace element profiling, using inductively coupled plasma – mass spectrometry (ICP-MS), as a tool to determine the biological significance of connections between a plant's genome and its elemental profile or "ionome". Our focus is on genes that control uptake and accumulation of solutes, including Ca, K, Mg, P (macronutrients in fertilizer), Co, Cu, Fe, Li, Mn, Mo, Ni, Se, Zn, (micronutrients of significance to plant and human health) and As, Cd, Na and Pb (elements causing agricultural or environmental problems). To date we have analyzed the shoot ionome of approximately 50,000 *Arabidopsis* plants. This includes the completion of a forward genetic screen of over 6000 mutagenized Arabidopsis lines (Lahner et al., 2003 Nat. Biotechnol. 21:1215) and a screen of Arabidopsis ecotypes for variation in the shoot ionome. We have successfully used PCR-based positional cloning, DNA microarray based approaches, and recombinant inbred lines to map over 20 loci that cosegregate with the ionomic traits of interest, and such approaches are allowing the identification of genes involved in regulating the ionome. By varying the concentrations of several nutrients in the soil, we have observed several unexpected alterations in the ionome, including significant differences in the accumulation of macro- and micronutrients in response to changing soil iron levels. In a complementary reverse genetic approach we are also screening the Salk Arabidopsis collection of sequenced T-DNA insertional alleles for genes that affect the shoot ionome. To maximize the value of this ionomics approach, we have developed a searchable online database containing ionomic information on over 22,000 plants [http://hort.agriculture.purdue.edu/Ionomics/database.asp], and this database is being updated regularly.

33 What can we learn by monitoring rapid O3-induced guard cell responses in Arabidopsis?

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The importance of stomata in regulating ozone entry into the leaf interior is widely recognized. Also a number of Arabidopsis O3 sensitive mutants and ecotypes have been isolated. However, comparative data for their stomatal responses to ozone and the role of stomata in modifying their ozone sensitivity are scarce. This is probably due to technical complications in measuring gas exchange of this species. We have constructed an eight-chamber whole-rosette ozone fumigation system which enables to monitore O3, CO2 and H2O exchange in different Arabidopsis mutants/ecotypes simultaneously.

We analysed stomatal conductance (gs) of O3-sensitive mutants rcd1, rcd2, rcd3 and ecotypes Col-0, Ler and WS-2, as well as of abscisic-acid insensitive mutants abi1, abi2. This revelad that the initial values of gs were higher in abi1 and abi2 than in Ler. Similarly gs was higher in rcd1, rcd2, rcd3 than in Col-0. Closer inspection of stomatal behaviour right after the onset of O3 exposure revealed, that there was a rapid transient depression of gs in Col-0, Ler and Ws-2, as well as in mutants rcd1, rcd2. The maximum of given initial depression was reached already within 6-8 min of ozone exposure. Interestingly this depression was absent in rcd3, abi1 and abi-2. The presence or absence of this transient depression in gs is not dependent on O3 concentration as a wide range of different concentrations (75 – 450 ppb) were studied. About two hours after the onset of O3-treatment the stomata started to close ultimately in all cases, the closure rates and injury symptoms were different in different mutants/ecotypes.

The concentration of ABA (measured in rcd1, rcd2, rcd3 and Col-0) was not induced within 3 h of O3 exposure, indicating that initial stomatal closure was induced by ABA. Still after 8 h a ten-fold rise in ABA was detected in wt as well in all rcd mutants. To address mutual relationships between ozone- and ABA-induced stomatal responses and induction of the oxidative burst, we analyzed ROS (reactive oxygen species) production in leaf tissue at the time of the transient depression in gs and also after the reopening in rcd3 and abi2 and corresponding wildtypes. ROS production was visible by confocal microscopy in the stomatas of Col-0, Ler and rcd1 at the time of transient depression in gs due to ozonation. Whereas the stomatas of rcd3 and abi2 did not show ROS production.

34 Two Visual Cycle Homologs, *Ccd8/Max4* and a Putative Short-Chain Dehydrogenase/ Reductase, are Required for Normal Green Light Responses in *Arabidopsis thaliana*

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Light is a critical signal that guides developmental decisions in the dark-grown seedling. Specific quantities and qualities of red, blue and far-red light have well-defined roles in regulating molecular, physiological, and biochemical events during seedling establishment. Effects of green light have been reported and indicate that green light antagonizes aspects of red and blue light mediated development. In our laboratory it has been shown that a short, single pulse of green light opposes the effect of red and/ or blue light regulated stem elongation and plastid transcript accumulation. Genetic studies have shown that the green light signal must be transduced by an unknown photosensory system that requires the beta subunit of the heterotrimeric G-protein. If the sensory system requires a G-protein, it may have other parallels to animal light transduction schemes. Analysis of *Arabidopsis thaliana* genomic sequence revealed the presence of genes homologous to those required for animal vision. These include genes necessary for chromophore synthesis/ modification *Ccd8/Max4* (homologous to RPE65) and a putative short-chain dehydrogenase/reductase with similarity to animal retinal dehydrogenase/reductase (retSDR4). T-DNA insertion mutants of *Ccd8/ Max4*, show defects in known green light responses; possessing short hypocotyls and a failure to down-regulate specific plastid transcripts with pulsed green light. Putative short-chain dehydrogenase/reductase mutants showed an exaggerated advancement of de-etiolation, including short hypocotyls and roots, early hook opening and augmented induction of light-regulated gene expression. The defects in response to green irradiation remarkably parallel the defects observed in the G-beta mutants, suggesting these elements may be intricately related. These studies indicate that plant homologs of three proteins required for rhodopsin-mediated light sensing in animals are required for normal green light sensing in plants.

35 The potential of engineering natural products to improve disease resistance in Arabidopsis thaliana.

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Plants are organic chemists *par excellence*, synthesizing a vast number of small molecules that are used for inter plant and inter species communication such as chemical warfare and as attractants for pollinators. Plants have developed different defence strategies towards their attackers, and pests have similarly designed various pathogenesis strategies for their attacks.

Natural products of the cruciferous plants include the sulphur rich glucosinolates and indole alkaloids. Elucidation of the biosynthetic pathway of glucosinolates has enabled us to explore the potential of engineering specific GS profiles to improve plant disease resistance. We assess disease resistance of the engineered CYP79A2, CYP79A1, and CYP79D2 Arabidopsis plants accumulating high levels of BGS, p-OHBGS or IPGS and MPGS, respectively towards the three plant pathogens *Alternaria brassicicola, Pseudomonas syringae* and *Erwinia carotovora* with distinct pathogenesis strategies. We demonstrate that engineering of specific GS profiles alters disease resistance against specific pathogens either by direct toxic effects or by modulating plant defense signaling.

36 A Functional Genomics Approach to Disease Resistance Signaling

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Plants respond to pathogen attack by activation of a suite of inducible defense responses. Effective defense depends upon rapid activation of these responses. The signal transduction network controlling activation of defense responses is complex. Genetic analysis in Arabidopsis has identified many important components of the signaling network, but it seems likely that many genes with important roles in defense signaling remain to be discovered. Genome-wide expression profiling has been used to identify genes that show expression changes in response to pathogen attack. It is known that several genes that are important in defense show increased expression in response to pathogen attack, so the set of genes that are induced in response to pathogen attack may include some that are important for disease resistance. This idea is being tested using the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Alternaria brassicicola*. Mutants with defects in pathogen-induced genes are isolated from public collections of T-DNA insertion mutants and tested for enhanced susceptibility to these pathogens. Genes that are found to be important for resistance will be studied using a small custom microarray. This array can be used to query a set of pathogen-responsive genes that represents many different patterns of pathogen-responsive gene expression. Characterization of mutants using this array should allow mutants with defects in signaling to be identified. The altered patterns of gene expression in signaling mutants should reveal their roles in the signaling network.

37 *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores

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Many pathogens are virulent because they specifically interfere with host defense responses and therefore can proliferate. Here, we report that virulent strains of the bacterial phytopathogen *Pseudomonas syringae* induce systemic susceptibility to secondary *P. syringae* infection in the host plant *Arabidopsis thaliana*. This systemic induced susceptibility (SIS) is in direct contrast to the well studied avirulence R gene-dependent resistance response known as the hypersensitive response that elicits systemic acquired resistance. We show that *P. syringae*-elicited SIS is caused by the production of coronatine (COR), a pathogen-derived functional and structural mimic of the phytohormone jasmonic acid (JA). These data suggest that SIS may be a consequence of the previously described mutually antagonistic interaction between the salicylic acid and JA signaling pathways. Virulent *P. syringae* also has the potential to induce net systemic susceptibility to herbivory by an insect (*Trichoplusia ni*, cabbage looper), but this susceptibility is not caused by COR. Rather, consistent with its role as a JA mimic, COR induces systemic resistance to *T. ni*. These data highlight the complexity of defense signaling interactions among plants, pathogens, and herbivores.

AB and JC contributed equally to this work.

38 Efficient discovery of regulatory loci in plant defense by exploitation of natural variation

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Plants are exposed to a broad range of biotic attackers, such as microbial pathogens and insect herbivores. To defend themselves against these attackers, plants have evolved many different, often inducible, defense mechanisms. Previously, I've been working with known mutants to get a better understanding of signaling networks underlying induced plant defense. This work focused on mechanistic and ecological aspects of plant defense signaling. In my current project, I focus on the genetic foundation of plant defense signaling networks, more specifically on discovery of new components of these networks. Identification of such components mainly relies on phenotypic characterization of genetic variants. An important source of genetic variation is variation among natural populations (accessions). This resource has so far been underexploited. The main reason for this is the lack of rapid, sensitive methods to survey this variation and isolate the corresponding genes. A major problem is the separation of the different genes affecting the variation between two accessions. Currently, I am working on novel method to efficiently identify new genes involved in plant defense signaling by taking advantage of natural variation among Arabidopsis thaliana accessions. The method aims at rapid separation of the genes underlying this variation using DNA micro-array expression profiles and mapping of the genes on the genome using Affymetrix Genechip. Subsequent cloning of the genes will allow detailed characterization of these genes. Development of this sensitive and efficient method will be a substantial contribution to the elucidation of plant defense signaling networks and to plant systems biology in general.

39 Genetic and Epigenetic Mechanisms

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In this session, I will first summarize recent advances in the understanding of genetic and epigenetic mechanisms in Arabidopsis thaliana. I will also review recent studies from my laboratory that highlight the structure and function of centromeres in Arabidopsis and some of its closest relatives. Centromere functions are highly conserved across all eukaryotes, yet there is a notable lack of centromere DNA sequence homology. The formation of new species can be accompanied by a change in chromosome number, often a consequence of the genome reorganization that follows the hybridization of two species. To provide resources for assembling physical maps of centromeres across the Brassicaceae family, we generated BAC libraries in Arabidopsis arenosa, Capsella rubella, Olimarabidopsis pumila, and Sysimbrium irio (available from Amplicon Express, Pulman, WA). In the Brassicaceae family, chromosome number has changed several times, resulting, in Arabidopsis thaliana, in the creation of some centromeres and the elimination of others. These events were accompanied by the rapid divergence of the population of centromere satellites. By analyzing the satellite content of individual BACs, we showed that the rates of satellite array homogenization across a genome are most rapid over local regions, less rapid within a chromosome, and least efficient across the genome. Even so, plant satellite populations can diverge within 10 million years to forms that entirely lack sequence similarity, a rate of change that is more rapid than that observed previously in primates. We also have compared paracentromeric regions from multiple species, demonstrating that Arabidopsis thaliana, unlike its close relatives, has undergone extraordinary expansions. Despite these dynamic changes, centromeres remain functional. To more readily assess the mechanisms that contribute to centromere activity, we have introduced various centromere DNA fragments into Arabidopsis thaliana, with the goal of forming both dicentric and autonomous chromosomes. Assessment of the function of centromere DNA, derived from a variety of species and introduced into Arabidopsis thaliana, is being assessed. In addition, the interaction of heterologous centromere binding proteins with A. thaliana centromere DNA and with heterologous centromere DNA inserted into the A. thaliana genome is under investigation. These studies will clarify the species-specific requirements for centromere activity and will further define the features required to nucleate centromere function.

40 Role of RNA polymerase IV in siRNA-mediated DNA methylation and heterochromatin formation

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All eukaryotes have three nuclear DNA-dependent RNA polymerases (RNAPs), namely pol I, II and III. Interestingly, plants express genes encoding catalytic subunits for a fourth nuclear polymerase, pol IV, which does not functionally overlap with pol I, II or III and is non-essentiaI. Disruption of the pol IV catalytic subunit genes, RPD1 or RPD2 inhibits heterochromatin association into chromocenters and causes cytosine methylation to be decreased at pericentromeric 5S gene clusters and AtSN1 and AtSN2 retroelements. The loss of CG, CNG and CNN methylation in pol IV mutants implicates a partnership between pol IV and DRM, the methyltransferase responsible for RNA-directed de novo methylation. Consistent with this hypothesis, 5S gene and AtSN1 siRNAs are essentially eliminated in pol IV mutants. Developmental abnormalities that arise in pol IV mutants include delayed flowering and defects in floral organ identity. The data suggest that pol IV helps produce siRNAs that target de novo cytosine methylation events that are required for facultative heterochromatin formation and higher-order heterochromatin associations, thereby directly or indirectly influencing the expression of developmentally regulated genes.

41 Mutational and TAP tag-assisted proteomic analyses and inducible RNA interference reveal the role of the Arabidopsis exosome in embryo/endosperm identity and imprinting, functional specialization of its subunits, and novel RNA substrates

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Exosome is an evolutionarily ancient macromolecular machine composed of multiple hydrolytic and phosphorolytic exoribonucleases as well as additional, auxiliary factors. Yeast exosome was postulated to play a pivotal role in numerous and mechanistically distinct reactions of stable RNA processing, cotranscriptional mRNA proofreading, as well as mRNA degradation. The consequences of exosome depletion have not yet been investigated at the whole-organism level in any of the multicellular species of life.

We have affinity-tagged the Arabidopsis exosome in vivo using the TAP technology and will present its proteomic analysis. We will also present the characterization of several exosome subunit mutants. Remarkably, we find that the loss of the RRP4 subunit leads to an ectopic activation of the endosperm-specific markers in the embryo, loss of parental imprinting, and embryo lethal phenotype. In a stark contrast, the loss of RRP41 results in an arrest of the female gametophyte development. This is the first evidence that the individual subunits of the exosome core have specialized developmental functions.

We have also established a novel genetic depletion system for the RRP4 and RRP41 Arabidopsis exosome subunits using inducible RNA interference (iRNAi). This approach helped reveal the essential requirement for the Arabidopsis exosome at the stages of plant life cycle beyond reproductive development, its role in ribosomal RNA processing, as well as a novel function in processing and/or degradation of the RNAP III transcripts.

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42 Monitoring transgene silencing in Arabidopsis - a broadly applicable, non-invasive and sensitive system

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Pronounced variability of transgene expression and transgene silencing are commonly observed among independent plant lines transformed with the same construct. The comprehensive study of single-copy T-DNA lines harbouring reporter genes of various kind and number under the control of a strong promoter showed that transcript level mediated silencing effectively accounts for the pronounced transgene expression variability seen among transformants (Schubert et al., 2004). These findings are immediately relevant to all over-expression studies in Arabidopsis and to studies that exploit gene fusions, e.g. for localisation. Moreover, the data indicate that a sensitive monitoring system suitable to identify cells exhibiting transgene silencing would allow for an efficient screening of plants showing stable transgene expression. The finding that the silenced state of a transgene is transferred to different transgenes sharing sequence homology in the transcribed region was exploited to develop a non-invasive monitoring system for the detection of transgene silencing in Arabidopsis. The monitoring system is capable to distinguish between transgenic plants showing stable expression from those that display post-transcriptional gene silencing in a highly efficient manner.

Schubert, D., Lechtenberg, B., Forsbach, A., Gils, M., Bahadur, S. and Schmidt, R. (2004) Silencing in Arabidopsis T-DNA transformants – the predominant role of a gene-specific RNA sensing mechanism versus position effects. Plant Cell 16, 2561-2572.

43 In Vivo Protein-Interaction Assays Based on Bioluminescence Resonance Energy Transfer (BRET)

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Genome-scale protein interaction maps are now emerging in several species from experimentation based on proteomic and molecular genetic interaction techniques. Interpretation of these data calls for an ability to monitor protein-protein interactions in live cells and in a signal-dependent fashion. Suitable assays have been put into practice using physicochemical concepts such as resonance energy transfer, fluorescence complementation, split-enzyme complementation, fluorescence enhancement of microinjected probes, and others. We have explored the potential of bioluminescence resonance energy transfer between hybrid proteins tagged with Renilla luciferase and yellow fluorescent protein (BRET, Xu et al., PNAS 96, 151-156 [1999]) to visualize protein-interactions in plant cells. A versatile set of traditional and Gateway-based recombination cloning vectors was constructed for the transient and stable expression of BRET-tagged proteins in planta. With the goal of detecting signaling events in the Arabidopsis light signaling network, we have begun to characterize the potential real-time in vivo interactions of a substantial number of light regulatory gene products. For example, using BRET, it was possible to dissect the juxtaposed motifs governing the nuclear exclusion and dimerization of the Arabidopsis repressor of photomorphogenesis, COP1. Progress in tagging Arabidopsis genes under the control of their authentic regulatory elements and in extending BRET from a cuvette-based assay to the imaging level will be presented. The BRET assay has theoretical and practical advantages, as well as limitations, over competing techniques. It is easily implemented and is a user-friendly and robust technique for monitoring the interaction between two specific proteins in a variety of live plant cell types. Funded by NSF MCB-0114653.

44 Isolation of TAP-tagged Protein Complexes From Plants

Michael Fromm
University of Nebraska

Twenty protein kinase genes have been TAP-tagged, transformed into transgenic rice plants and analyzed for interacting proteins in the T1 plants. Protein complexes have been identified for some but not all of the tagged proteins. The key learnings and results of the technology and biology will be presented, as well as suggestions for successfully using this technology.

45 Using metabolomics and transcriptomics data to study metabolic networks in Arabidopsis

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Metabolomics is an emerging technology that along with other genomics platforms (i. e. transcriptomics and proteomics) and integration of experimental results through mathematical modeling is increasingly being used as a systems biology approach to study the topology and dynamics of metabolic and signaling networks in living systems. Our research is focused on developing methods for high throughput metabolite profiling and application of metabolomics to study stress response in plants using Arabidopsis as a model. We employ a combination of gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) for both targeted and non-targeted metabolite profiling of wild type and mutant Arabidopsis plants subjected to various environmental stresses including heat, drought and oxidative stress. We will present metabolite profiling results for several Arabidopsis mutants and discuss how metabolomics data can be used in combination with transcriptomics and bioinformatics tools to study the biochemical networks involved in Arabidopsis response to a particular environmental stress.

46 Metabolomics and proteomics in *Arabidopsis thaliana*– transitions from pattern recognition to biological interpretation

Wolfram Weckwerth

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Metabolomics has proven to be superior for pattern recognition analysis of biological samples than any other post-genome technology. Owing to an amplification of metabolic processes reflected on the level of metabolite concentrations and fluctuations a very rapid sample classification is possible. A cluster of multivariate data mining tools is emerging enabling the biological interpretation of sample patterns (Komponenten-Analyse) and the identification of responsible metabolite markers (Loadings). A step further is the complementation with "orthogonal" protein, phosphoprotein and external (environmental) data. These integrative analyses give a higher level of observation of molecular networking in living systems, and, thus, allow the identification of multiple biomarkers embedded in dynamic correlative networks. The techniques for metabolite, protein and phosphoprotein profiling are presented and their limitations are discussed. Based on recent studies the improvement of systemic analysis in *Arabidopsis thaliana* using integrative metabolite-protein profiling is demonstrated.

47 Adaptation and variation in Arabidopsis flowering

Michael Purugganan

48 Linkage disequilibrium mapping in Arabidopsis

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As part of the NSF 2010 Program, we have carried out a genome-wide survey of polymorphism in *Arabidopsis thaliana* by re-sequencing about 1,500 short fragments in a sample of 96 accessions. The study yielded a very good picture of the pattern of polymorphism in this species. Although *A. thaliana* is a selfing weed, the pattern of polymorphism in general agrees with what is expected for a widely distributed, sexually reproducing species. Linkage disequilibrium (LD) decays rapidly, within 50 kb. Variation is shared world-wide, although population structure and isolation by distance are evident. The data fail to fit standard neutral population genetics models in several ways, rendering the usual "tests of selection" meaningless.

The main motivation for the study was to investigate the feasibility of genome-wide linkage disequilibrium mapping in this species. Because linkage disequilibrium in general decays faster than predicted, within 50 kb, the marker density generated by our re-sequencing study is not sufficient to have truly genome-wide coverage. Furthermore, we find that population structure typically induces very high rates of false positives. Nonetheless, we are able to map several previously known loci involved in variation for flowering time and disease resistance, thus demonstrating the potential utility of the approach.

We argue that future genome-wide association studies must employ higher marker densities, and much larger samples of accessions.

49 Evolution of ecologically important traits in relatives of Arabidopsis

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What evolutionary factors influence natural genetic variation in plant populations? Building upon earlier studies in Arabidopsis thaliana, we examined genetic variation for insect resistance and secondary metabolism in perennial Arabidopsis lyrata. Glucosinolates and trichome density were significantly heritable, as was resistance to some insect herbivores. Within and between populations, significant natural genetic variation for side-chain elongation in aliphatic glucosinolates allow us to target the methylthioalkylmalate synthase (MAM) locus as a candidate for future experiments in "reverse ecology."

In this same genomic region, A. thaliana harbors complex balanced polymorphism influencing growth rate. We fine mapped phenotypic effects segregating within a one centiMorgan chromosome interval for which lines with mapped recombination breakpoints were available, and examined the sequence signature of historical polymorphism. We found two epistatic growth rate QTL within 210 kb, suggesting a massively polygenic architecture of quantitative variation. One QTL was delimited to a single gene, which shows a nucleotide signature of balancing selection, and whose phenotypic effects are reversed depending on genetic background. If this region typifies many complex trait loci, then nonneutral epistatic polymorphism may be an important contributor to genetic variation in complex traits.

50 Pronounced Expansion of Transcription Factor Families in Plants

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Transcription factors (TFs) are central to the regulation of gene expression and are usually members of multigene families. In plants, they are involved in diverse processes ranging from developmental controls to elicitation of defense and stress responses. To investigate differences in expansion patterns of TF gene families between plants and other eukaryotes, we first used Arabidopsis TFs to identify TF DNA binding domains. The Hidden Markov Models of these DNA binding domains were then employed to identify related sequences in rice, Chlamydomonas and 20 other eukaryotic genomes.

Not surprisingly, we found that all Arabidopsis and rice TF families are larger than those in Chlamydomonas. In addition, among 19 families that are shared between animals and plants, more than 14 are larger in plants than in animals. After examining the lineage-specific expansion of TF families in 2 plants, 8 animals, and 2 fungi, we found that plant TF families have undergone much more dramatic expansion compared to those in other eukaryotes. This elevated expansion rate in plants is not simply due to higher duplication rates of plant genomes. Compared with plant genes involved in other functions, TFs have a significantly higher retention rate of duplicate genes. Interestingly, we found that the degree of lineage-specific expansion in Arabidopsis is correlated with that in rice. This pattern of parallel expansion is much more pronounced than the whole genome trend, suggesting the presence of common selection pressure acting on some TFs in both the Arabidopsis and rice lineages.

The high rate of retention among plant TF genes and their propensity for parallel expansion suggest frequent adaptive responses to selection pressure common among higher plants.

51 Developmental mechanisms underlying fruit diversification in Brassiceae (Brassicaceae)

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Great variation in fruit structure is observed within the close phylogenetic vicinity of the model species *Arabidopsis*, particularly in the tribe Brassiceae. Members of this clade exhibit fundamental differences relative to the typical silique of *Arabidopsis*, including indehiscence (meaning that seeds are not released) and the presence of a novel structure known as the joint that fully bisects the fruit laterally into two heteromorphic segments. The joint often represents an abscission zone, which allows the proximal and distal segments to be dispersed as individual subunits. We focus our studies on two closely related taxa from the Brassiceae, *Cakile* and *Erucaria*, that vary in two critical aspects in fruit morphology: (1) dehiscence of the proximal segment and (2) abscission along the joint. We have conducted detailed developmental and histological studies of *Cakile* and *Erucaria* that have contributed to our understanding of the evolution of this phenotype from an *Arabidopsis*-like ancestor. The genetic pathway controlling dehiscence and establishment of the valve boundary has been well characterized in *Arabidopsis*, providing a framework for studying the genetic basis for the morphological modifications of fruits in the Brassiceae. We are focusing our studies all on six genes known to play crucial roles in the patterning of the valve margin layer in *Arabidopsis*, with an initial focus on the *SHATTERPROOF* (*SHP*) genes. Based on current *in situ* hybridization experiments in *Cakile*, it appears that the indehiscence of *Cakile* fruit may be correlated with a lack of *SHP* homolog expression in the valve margin. These results have the potential to provide greater insight into the complex remodeling of fruit morphology that occurred in the Brassiceae.

52 Evolution of mating systems in *Arabidopsis*: from outcrossing to selfing and back

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The mating system adopted by a plant species determines the patterns of genetic variation in and between populations and thus has profound consequences for the rate and mode of evolutionary change. The genus *Arabidopsis* provides ideal material for analysis of the evolution of mating systems. It includes both self-fertilizing species, such as *A. thaliana*, and obligate out-breeders, such as *A. lyrata*. Furthermore, out-crossing in this genus, as in other crucifers, is due to the operation of a well-characterized self-incompatibility (SI) system, which prevents self-fertilization by inhibiting the hydration and germination of self-related pollen at the surface of the stigma epidermal cells. The genetic determinant of SI specificity is the *S*-locus complex, and inhibition of self pollen is effected by allele-specific interactions between two highly polymorphic proteins encoded within this locus: the S-locus receptor kinase SRK, which is localized on the stigma epidermal surface, and its ligand, the S-locus cysteine-rich protein SCR, which is located in the pollen coat. The SRK-SCR interaction triggers a poorly understood signal transduction pathway within the stigma epidermal cell that culminates in the inhibition of self pollen.

The switch from out-crossing to selfing in crucifers is often associated with loss or inactivation of *SRK* and *SCR* genes, demonstrating that these genes are the primary determinants of the out-crossing mating system in this family. The SI trait can be transferred into the self-fertile *A. thaliana*, which lacks functional alleles of these genes, by transformation with an *SRK-SCR* gene pair from *A. lyrata*, but *A. thaliana* ecotypes differ in their ability to express the SI trait. A study of the mechanism and evolution of self-recognition in out-crossers and its breakdown in selfers can therefore draw on two reservoirs of variation provided to us by nature. On the one hand, the extensive polymorphisms of SI genes may be used to identify the specificity-determining residues or domains in the SRK receptor and its SCR ligand, understand how these two proteins co-evolve to maintain their interaction, and possibly explain how the large SI recognition repertoires were generated. On the other hand, the natural variation in expression of SI in transgenic *SRK-SCR* plants belonging to different *A. thaliana* ecotypes may be exploited to identify the loci that were the targets of selection for self-fertility, understand how the S-locus genes and genes of the SI signal transduction pathway were modified in conjunction with the switch to self-fertility, and thereby further elucidate the mechanism of self recognition and evolution of mating systems in crucifers.

53 *Arabidopsis* cytochrome P450 monooxygenases

Mary Schuler[1], Hui Duan[1], Shahjahan Ali[1], Sanjeewa Rupasinghe[1], Jyothi Thimmapuram[2], Natanya Civjan[3], Mark Band[2], Matthew Hudson[4], Daniele Werck-Reichhart[5], Stephen Sligar[3]

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Cytochrome P450 monooxygenase (P450s) function in the biosynthesis of lignins, pigments, defense compounds, fatty acids, hormones and growth regulators as well as in the metabolism of herbicides, insecticides and pollutants. The *Arabidopsis thaliana* genome contains 272 P450 genes and pseudogenes that are classified into 45 families (>40% identity) and 72 subfamilies (>55% identity) according to their primary sequence identities. Mutant analysis and expression in various bacterial, yeast and baculovirus expression systems has now assigned function to 39 of these P450s (http://arabidopsis-P450.biotec.uiuc.edu). Towards understanding expression and regulation of these P450s, microarrays containing gene-specific elements for 265 P450s, 40 biochemical pathway markers and 322 physiological function markers have been analyzed for sets of P450s co-expressed in specific tissues and in response to different signaling molecules (JA, SA), fungal defense activators (BTH) and their combinations, plant growth hormones (ABA, IAA, BR), environmental stresses (ozone, UV-B, hydrogen peroxide), etc. These comparisons have detailed the unique and overlapping responses of P450 and biochemical pathway loci to various cues. Analysis of P450 promoter sequences (existing within 2 kb of ATG) co-regulated by individual or groups of stresses has identified specific as well as common motifs that are over-represented among these promoter sets. Annotation efforts have identified a number of unusually long P450 transcripts spanning two adjacent loci that are spliced to produce dimeric P450 proteins containing two heme binding domains or bicistronic transcripts containing complete ORFs for both phase I (P450) and phase II (OMT) detoxification enzymes. Cloning and expression of individual P450 cDNAs in baculovirus and yeast systems have defined substrate binding profiles for a number of fatty acid hydroxylases and, interestingly, demonstrated that the P450 locus coding for HPL in hexenal signaling has been inactivated in the Col ecotype. Molecular modeling of P450s coupled with high-throughput docking approaches are being used to predict potential substrates for P450s with undefined functions for subsequent binding and activity analyses.

54 FUNCTIONAL ANALYSIS OF THE UBIQUITIN-PROTEIN LIGASE (E3) FAMILIES IN ARABIDOPSIS

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The post-translational conjugation of one or more Ubs to selected intracellular proteins plays an integral role in numerous growth, developmental, and metabolic processes in plants via its ability to modify the function and/or half-life of its targets. Whereas its main function is to commit proteins for degradation by the 26S proteasome, other functions have become apparent more recently, including roles in DNA repair, lysosomal catabolism, intracellular trafficking, and the regulation of transcription. Among the enzymes responsible for Ub conjugation, the Ub-protein ligases (or E3s) are the crucial elements that control both target selectivity and the nature of the Ub linkage. During our current NSF *Arabidopsis* 2010 project, we discovered that *Arabidopsis* may contain over 1,300 E3 components, making this collection one of the largest functional groups in this plant (~5% of the proteome). Surprisingly, this prediction far exceeds those in animals and fungi, suggesting that plants have specifically expanded the pathway. Here we report our progress in annotating the various E3 families, and defining their functions and targets using biochemical assays, protein interaction screens, expression studies, forward and reverse genetics, and proteomic strategies. Recent successes include the identification of large RING, F-Box and BTB protein families that provide the target specificity to E3 complexes, and the discovery that specific isoforms regulate *Arabidopsis* development and the response to light, ethylene and auxin signals. In fact, the biochemical analysis of TIR1 and its relatives demonstrate that a family of E3s actually participates in auxin reception. The results generated by this study will form an essential framework for understanding E3 diversity, help reveal specific functions for each E3 type, and will develop a database of *Arabidopsis* proteins whose abundance and/or functions are affected by Ub addition. All of this work is currently being made available through the web-accessible PlantsUBQ database (http:/plantsubq.genomics.purdue.edu).

55 Analysis of the Arabidopis CDPK Superfamily

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The CDPK superfamily comprises seven types of protein kinases. Calcium-dependent protein kinases (CDPKs or CPKs) contain both a protein kinase catalytic domain and a calcium-binding regulatory domain. There are 34 CDPKs in Arabidopsis, which can be divided into four groups based on sequence similarity. In addition there are eight CDPK-related kinases (CRKs), which have degenerate calcium-binding motifs. The two phosphoenol pyruvate carboxylase kinases (PPKs) have only a catalytic domain and the two PPK-related kinases (PPRKs) contain a C-terminal domain of unknown function. Also related to CDPKs are the three types of SNF1-related kinases (SNRKs), which include 38 enzymes. With the exception of certain SNRK2 and SNRK3 kinases, genetic approaches have not yielded much insight into the physiological functions of individual members of the CDPK superfamily. To better understand the function of the family members we 1) used the yeast two-hybrid system to identify interacting proteins and potential substrates, 2) expressed GFP-tagged kinases to determine their subcellular localization, and 3) used mass spectrometry to identify autophosphorylation sites and protein substrates. In a fourth goal of this project we worked with Santa Fe Community College in Gainesville, FL to develop lessons on DNA technology for community college students. Results from this project are posted on the PlantsP website (http://plantsp.sdsc.edu). Selected results will be described.

56 Indispensable Genes Required for Seed Development in *Arabidopsis*

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The goal of the *Arabidopsis* SeedGenes Project is to present detailed information on indispensable genes with a knockout phenotype in the developing seed. This project was made possible through a T-DNA insertional mutagenesis program initiated at Syngenta (Research Triangle Park, NC) 8 years ago in collaboration with the Meinke laboratory. NSF 2010 funding was obtained in September 2001 to transfer work to the public sector and to make extensive information on *EMB* genes available to the community. The project database and website (www.seedgenes.org) at present include information on 295 genes and 462 mutants. A recent upgrade of the database is described in the poster by Rosanna Muralla *et al*. The frequency of *emb* mutants obtained to date is consistent with the presence of 500 to 1000 total *EMB* genes in *Arabidopsis*. A major challenge for the future is to identify those genes that have so far escaped detection. Forward genetics becomes less efficient with increasing saturation because a greater proportion of mutants identified represent duplicate alleles of known *EMB* genes. The question then becomes which candidate genes represent the most promising targets for reverse genetics. We have identified several classes of genes that merit further analysis, including those that resemble essential genes in other model organisms, those for which a knockout homozygote cannot be identified, and those that share an interacting protein partner, metabolic pathway, or cellular process with a known *EMB* gene. Examples of initial results obtained from these different strategies will be described. The poster by Michael Berg *et al*. provides more detailed information on the shared process approach in relation to tRNA synthetase knockouts.

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57 Non-mendelian inheritance of ancestral sequences in Arabidopsis.

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We have recently demonstrated that *Arabidopsis* plants homozygous for recessive mutant alleles of the *hothead* locus can inherit allele specific DNA sequence information that was not present in the chromosomal genome of their parents. This process appears to occur throughout the nuclear genome and affects coding, as well as, non-coding regions. In addition to changes in single nucleotide polymorphisms, regions containing small deletions and insertions can also be modified lending further support to the idea that this is a template-mediated process. Furthermore, this extra-genomic sequence cache appears to persist for multiple generations. Based on our findings we propose that this process represents a completely novel template-directed mechanism for the epigenetic inheritance of DNA sequence information. These findings contradict the established laws of classical Mendelian genetics where allelic information contained in the nuclear genome is stably inherited and transmitted from one generation to the next in a predictable manner.

58 Asking for Arrays.

Sean May

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Microarrays and other large-scale transcriptomic technologies are now common and powerful research tools for biology and have become accepted within the scientific community as useful stepping-stones to a variety of downstream analyses and discovery processes.

Mechanisms for the generation, storage, distribution and on-line or local analysis of data provided from array experiments have become less of a frontier activity and are becoming increasingly mainstream. A substantial number of sites have appeared within the Arabidopsis community alone offering intersecting sets of array data.

Many of these public sites provide a broad palette of on-line tools or references and links to appropriate tools for analysis of the data or appropriate advice to help the novice, the casual browser and the power-user.

Most current array sites broadly conform to international standards such as MIAME and provide their data in a variety of formats from simple CD/DVD distribution through FTP, database queries and other online meta-tools through to web services such as BioMOBY. The hosts for these sites also normally advocate the onward distribution of data to each other and to repositories such as ArrayExpress and GEO for the general good of the plant community.

This talk will summarise the sources of some of these data and tools along with examples of the mechanisms by which users can find their way through the sometimes overly rich choice of alternatives without sacrificing the option of user choice.

We would also like to include an appeal for producers and users of array data to liaise with the databases described (or their own local informaticians) so that their data may be included, mirrored or otherwise served to the community in a way that takes advantage of the broadest range of available tools.

Example references: NASCarrays - http://affy.arabidopsis.info TAIR – http://arabidopsis.org Genevestigator – https://www.genevestigator.ethz.ch GABIpd - http://gabi.rzpd.de The Botany affymetrix database - http://bbc.botany.utoronto.ca:88

59 Deep profiling by massively parallel signature sequencing elucidates the small RNA component of the transcriptome

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Small RNAs play important regulatory roles in most eukaryotes but only a small proportion of these molecules have been identified. We adapted the method of massively parallel signature sequencing ("MPSS") to sequence more than two million small RNAs from seedlings and the inflorescence of the model plant Arabidopsis thaliana. These data increased the number of distinct small RNA sequences known by more than an order of magnitude. Most of the different sequences represent small-interfering RNAs (siRNAs) that match repetitive sequences, intergenic regions, and genes. Among the most abundant sequences were well-characterized microRNAs (miRNAs) and new miRNA candidates. Interestingly, no bias of small RNAs was observed towards genes with antisense transcription nor was there evidence that miRNAs cause transitivity, i.e. the production of siRNAs that match endogenous miRNA target mRNAs. In contrast, individual or clusters of highly-regulated small RNAs were readily observed. This powerful genome-wide method for identification and measurement of small RNAs extends miRNA prediction capabilities and is applicable to diverse organisms.

60 The Pyrabactins: small molecule agonists of the abscisic acid signaling pathway

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We performed a chemical genetic screen of a 10,000 member small molecule library to identify compounds that disrupt hypocotyl cell expansion in etiolated Arabidopsis seedlings. Six compounds were identified that inhibit germination and ~750 compounds were found to reproducibly inhibit hypocotyl growth by >20%. Two of the germination inhibitors identified are structurally similar and examination of an analog series shows that a pyridine moiety is essential for the activity of these compounds that we have named the Pyrabactins (for *pyr*idine *aba* a*ct*ivatio*n*).

To delineate their mechanism of action, the effects of the Pyrabactins were examined on mutants in the GA and ABA signaling and biosynthetic pathways. These experiments demonstrate that Pyrabactins require a functional ABA signaling, but not biosynthetic, pathway to inhibit germination suggesting that Pyrabactins activate the ABA signaling pathway. To examine this hypothesis further, whole genome transcript profiling was used and these experiments reveal that ABA and Pyrabactin treatments induce strikingly similar expression profiles (Pearson correlation coefficient of 0.89) Collectively our genetic, physiological and transcriptional data suggest that the Pyrabactins inhibit germination by activating the ABA signaling pathway and thus define a new class of synthetic plant growth regulators.

As a first step towards target identification two genetic approaches have been initiated. We have identified mutants resistant to Pyrabactin A, the strongest analog in the series, and these are under investigation. In addition, a screen of 120 ecotypes has uncovered several Arabidopsis isolates that are hypersensitive to the Pyrabactins; genetic analysis of one strain shows that Pyrabactin hypersensitivity segregates as a Mendelian trait that maps to the bottom arm of chromosome 3.

Our discovery of the Pyrabactins illustrates the successful application of forward chemical genetics in identifying new plant growth regulators and illustrates the utility of natural variation for identifying genetic factors that mediate the action of small molecules. Furthermore, the Pyrabactins should be useful tools for dissection and manipulation of the ABA signaling pathway.

61 Role of the ubiquitin E3 ligase SCFAtSKP2 in cell proliferation

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Plant growth and development require a high level of coordination between cell division and cell differentiation. In plants, the majority of the cell cycle regulators have been identified, but very little is known about their function and regulation during plant growth and development. Before cells commit to divide they need to pass the G1/S checkpoint, where the cells sense the external and internal conditions. This G1-S transition is governed by the activity of CDK and by the Retinoblastoma-E2F pathway, which plays a critical role in controlling the expression of genes required for G1-S progression.

In addition, selective ubiquitin-mediated proteolysis through the cell cycle controls the availability, and therefore the activity, of several cell proliferation proteins. Previously, we have found that a member of the E2F family, *AtE2Fc*, is regulated by a balance between gene expression and degradation through the ubiquitin-proteasome pathway, involving the activity of the E3 ubiquitin-ligase SCF(AtSKP2) complex. Now, we have found that AtDPb protein, which *in vivo* forms a heterodimer with AtE2Fc, is also degraded through the SCF(AtSKP2)-ubiquitin pathway. Interestingly, AtDPb is highly unstable in the *axr1* mutant background, suggesting that the lack of RUB1 regulation on CUL1 in *axr1* plants stimulates the degradation of AtDPb or that AtDPb can be degraded through an alternative pathway, different from the SCF.

In Arabidopsis, there are two *SKP2* genes, which are cell cycle regulated, that encode for the AtSKP2A and AtSKP2B proteins. We have demonstrated that these proteins form SCF complexes that have ubiquitin ligase activity. The overexpression of *AtSKP2A* generated plants containing bigger cells in the leaves and increased the number of cells in mitosis in both the shoot and the root meristems as well as in the young developing leaves. In addition, plants that overexpress *AtSKP2A* formed more lateral root (LR) primordia and subsequently developed more lateral roots than the wt plants. A similar increment in lateral root primordia has been found in plants with reduced levels of AtE2Fc, a cell cycle repressor. It is remarkable that AtSKP2A-overexpressing plants accumulated less AtE2Fc protein in the roots, suggesting that the increment in the number of LR might be the consequence of a reduction in the AtE2Fc levels. These and further results will be presented at the meeting.

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62 Comprehensive analysis of protein-protein interactions within the cytokinin signaling pathway in Arabidopsis thaliana

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In *Arabidopsis* the cytokinin signal is transduced via a multistep phospho-relay through a complex two-component system (TCS). More than 30 proteins divided into four major protein families, i.e. sensor histidine kinases, phospho-transmitter, A- and B-type response regulator, are thought to be involved in this process. The succession of the signal along the different modules seems to be clear, but there is only limited knowledge about the abilities of the TCS proteins to interact with each other and how much protein-protein interactions contribute to signal specification.

In order to test the specificity of interactions of the TCS proteins, we cloned most of the genes involved into bait and prey vectors of the yeast two-hybrid system. TCS protein interactions were tested in a matrix fashion, covering in total 500 tested interactions. The putative new interactions were verified by using an *in vitro* affinity co-precipitation method. 26 previously known interactions were verified by this approach and more than 40 new interactions were detected. Of those only four could not be confirmed in the biochemical interaction assay. As expected, most interactions were found between members of the phospho-transmitter protein family and proteins of the other three TCS protein families. However, also some new and unexpected interactions were identified. The implications of these unusual interactions will be discussed.

63 Molecular definitions of cytochrome P450s in *Arabidopsis thaliana*

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Plants contain a significantly larger number of genes in their cytochrome P450 monooxygenase superfamily than most other eukaryotic systems (272 in *Arabidopsis thaliana*, 456 in rice, 56 in human, and 83 in *Drosophila*). Towards defining the functions of these ER-localized proteins, we have heterologously expressed a number of Arabidopsis P450s in baculovirus-infected Sf9 cells, including members of the CYP86A subfamily, CYP94 family, CYP89A subfamily, CYP71B subfamily and CYP96A subfamily. Molecular models have been built for each of these aproproteins and high-throughput virtual screening approaches using plant/insect chemical databases of three dimensional structures have short-listed potential substrates for these and other plant and insect P450s. Experimentally, all *Arabidopsis* CYP86A subfamily proteins and CYP94B1 have been demonstrated to ˜-hydroxylate lauric acid (a prototype short chain fatty acid) and to bind a range of long chain fatty acids. Low-throughput docking approaches with various fatty acid substrates have been used to explore divergences in their substrate recognition sites that have resulted in their different reactivities. Comparisons of the substrate binding models and predicted substrate access channels indicate that significant differences occur among these P450 catalytic sites with some members of the CYP86A and CYP94B subfamilies containing relatively constrained catalytic sites and other members containing larger unconstrained catalytic sites.

64 Variations in *CYP74B2* (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg ecotypes of *Arabidopsis*

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The *CYP74B2* gene in *Arabidopsis* encodes hydroperoxide lyase (HPL), which mediates the breakdown of hydroperoxide (HP) and eventually leads to the production of a group of C6-volatiles. The *CYP74B2* gene in the Columbia ecotype (Col) contains a 10 nt deletion in its first exon that causes it to code for a truncated protein not containing a P450 signature motif and the CYP74B2 gene in the Landsberg ecotype (Ler) codes for a full-length HPL protein. RT-PCR analyses have demonstrated that accumulation of CYP74B2 transcripts in Col is reduced substantially compared to those in Ler. Consistent with the nonfunctional HPL open reading frame in the Col ecotype, in vitro analysis of HPL activity using either 13-hydroperoxylinoleic acid or 13-hydroperoxylinolenic acid as substrates, shows undetectable HPL activity in the Col ecotype and C6-volatile analysis shows substantially reduced amounts of 2(E)-hexenal in the Col ecotype. P450-specific microarrays and full-genome oligoarrays have been used to identify the range of other transcripts expressed at different levels in these two ecotypes potentially as a result of these variations in HPL activity. Correlating with reductions in CYP74B2 transcripts in Col leaves and flowers and the absence of HPL activity, transcripts for enzymes involved in the synthesis of C6-volatiles (LOX2, LOX3), jasmonates (OPR3, AOC) and aliphatic glucosinolates (CYP83A1, CYP79F1, AOP3) are depleted in Col leaves and a subset of these (CYP83A1, AOP3) are also depleted in flowers.

65 Generation & characterization of gene- & enhancer-trap transposon insertion lines in Arabidopsis thaliana

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This study summaries the application of the transposon Ac/Ds system as an efficient insertional mutagenesis for gene-searching or gene knockout engine in the Arabidopsis genome as a partner of the EU FP5 EXOTIC consortium. A collection of some 5000 insertions exhibits the lower ratio (some 4.4%) of visible phenotype morphologically; however, various reporter gene expression patterns are presented in seedlings, roots or flowers, including expression patterns specific to organs, tissues, cell types, or developmental stages. These patterns could be useful for the identification of genes that are expressed in different developmental processes. The diversity of gene expression patterns suggests that the cloning and identification of Arabidopsis genes expressed in any developmental process is feasible using this system. More specifically, GUS expression pattern shows that in both ET and GT lines, plants at flowering stage have higher GUS expression (75.7% & 43.0% in ET lines and in GT lines, respectively) compared to the expression in seedlings (27.8% in ET and 35.3% in GT lines). The average ratio of GUS expression patterns in seedlings and flowering plants are 51.7% in ET lines and 39.2% in GT lines. In seedling plants, root tissues have higher expression than in cotyledon and hypocotyls, which is similar in both of ET and GT lines. But in adult plants of ET lines, flowers have much more expression where GUS patterns are 52.7%. In GT lines, flowers have only 23.7% but rosette leaves have 53.9% that is much higher than in ET lines where it is 9.2% only. Reporter expression patterns in ET and GT lines thus exhibited some similarities but also exhibited differences. Flanking sequences of over 500 independent insertions identified by TAIL-PCR show that the insertions are distributed randomly throughout the Arabidopsis genome; however, several hot spots of Ds insertions occur away from the centromeric regions. Moreover, for any given gene, the insertion was possible at any intragenic position but preferentially located at the 5' end. The complete expression patterns of >5000 lines and the insertion sites of >500 lines have been deposited in public databases and should be of value to researchers using reverse genetics approaches to determine the function of genes of interest.

66 Arabidopsis gene networks regulated by interactions between C and N

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Our long term goal is to have a general understanding of how the assimilation of nitrogen (N) into amino acids is controlled in plants and relates to other aspects of the plant physiology. We have previously shown that signals such as nitrogen (N), carbon (C) interact to affect nitrogen assimilation. We now combine mathematical, genomic and system biology approaches to (1) catalog the nature of carbon (C) and nitrogen (N) interactions, (2) identify the C:N responsive gene networks and (3) understand the underlying biological principles. We used Affymetrix gene chips to explore a matrix of C, N conditions in Arabidopsis. We found that C, N or CN treatments elicit global responses in Arabidopsis roots that are readily distinguishable. We identified many genes that respond with CN only. Thus CN is an important factor that can induce major gene expression adjustments in plants, comparable in number but distinct in profile to those induced by C alone. We found ample evidence for interactions between C and N that extend beyond metabolic pathways. We catalogued these patterns and identified the genes with common regulatory strategies in response to C, N or CN. Functional analysis suggests that CN-responsive genes are involved in protein synthesis and N-responsive genes participate in energy producing metabolic pathways and two-component signal transduction systems. To provide a holistic view of how the cell molecular network is adjusted in response to these external perturbations, we constructed a model of the plant cell metabolic (KEGG, AraCyc) and regulatory network (Transfac). We integrated gene expression data with this model and identified the specific gene networks that respond to C, N or CN inputs. Changes in these gene networks in response to genetic perturbations are being investigated. In addition, efforts are also directed towards exploring the following input combinations: carbon, nitrogen, light and organs. Because testing all combinations of every input dose is not only impractical but impossible, we are using mathematical methods to generate experimental sets that cover many input/dose combinations in an efficient and economical manner. These mathematical methods should allow prediction of gene expression for untested conditions.

67 Protein-protein interactions involving Arabidopsis polyadenylation factor subunits: Towards an understanding of the architecture of the polyadenylation apparatus and its associations with other processes in plants

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Messenger RNA 3' end formation is a central step in the expression of genes in eukaryotes. To gain a better understanding of this process in plants, a comprehensive protein-protein interaction map of the plant polyadenylation apparatus is being assembled. To date, twelve of fifteen clearly-identifiable Arabidopsis polyadenylation factor subunits have been studied. Eight of these twelve subunits interact with more than one additional polyadenylation subunit. Moreover, six of the proteins interact with four or more other subunits. Among this set of proteins is one that has sequence similarity to the eukaryotic polyadenylation factor subunit Fip1. Yeast two-hybrid and in vitro assays indicate that a plant-specific N-terminal domain of the Arabidopsis Fip1 protein interacts with poly(A) polymerase (PAP). Interestingly, this part of the Arabidopsis Fip1 also is involved in interactions with Arabidopsis homologs of CstF77, CPSF30, CFIm-25, and PabN1. Interactions of Fip1 with CstF77 and CPSF30 are seen in yeast and mammals, but the part of the Arabidopsis Fip1 protein that participates in these interactions is not present in other eukaryotic Fip1 proteins. Interactions between Fip1 and CFIm-25 or PabN1 have not been reported in other systems; these may represent plant-specific associations. These results provide conceptual links between PAP and several other putative Arabidopsis polyadenylation factor subunits.

In addition to two-hybrid and copurification assays, a random combinatorial screen for seven amino acid peptides that interact with N-terminal 137 amino acids of the Arabidopsis Fip1 protein has been conducted. Comparison of peptides so identified with the Arabidopsis proteome resulted in the identification of a number of possible Fip1-interacting proteins. Three of these were confirmed by testing for protein-protein interactions in yeast two hybrid tests and by co-purification assays. Additional confirmation was obtained by demonstrating that the respective peptide motif is essential for the three interactions. One of the three proteins is the polyadenylation factor subunit CstF77. The other two are poorly-characterized proteins that implicate Fip1 in transcriptional and/or posttranslational regulation in plants. These results indicate that a random combinatorial approach can be a useful tool with which to identify protein-protein interactions.

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68 Functional analysis of the Arabidopsis NRL (NPH3/RPT2-like) protein family

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Blue light is one of the crucial environmental signals for plant growth and survival. At least five important physiological responses are regulated by blue light: phototropism, stomatal opening, chloroplast movement, leaf expansion and regulation of stem elongation. NPH3 (NONPHOTOTROPIC HYPOCOTYL 3) and RPT2 (ROOT PHOTOTROPISM 2) represent two proteins necessary for normal blue light signaling associated with phototropism (both NPH3 and RPT2) and stomatal opening (RPT2 alone). Both of these proteins have been shown to interact with phot1, a blue light photoreceptor involved in all five of the aforementioned processes. NPH3 and RPT2 are the founding members of a plant-specific protein family that is comprised of 33 members in Arabidopsis, and the goal of the research described here is to define the functional diversification of each of the members of this family. In order to achieve our goal, we are determining their expression profiles and generating both loss- and gain-of-function mutants for each member. Expression analyses were performed using semi-quantitative RT-PCR and reporter expression in transgenic plants carrying translational fusions of NRL proteins (driven by their endogenous promoter) to eGFP. Loss-of-function alleles for each member of the family have been generated by either random transfer-DNA insertion or via RNAi, while gain-of-function alleles have been generated by viral enhancer-driven overexpression of each gene from its native promoter. The various phototropin-mediated blue light responses are currently being assessed in each of the mutant backgrounds to determine if members of the NRL family represent commonly utilized proteins in blue light signaling.

69 Arabidopsis mRNA Polyadenylation: New Poly(A) Signals, and the Role of CPSF in Sexual Development

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Polyadenylation is an essential processing event during mRNA maturation. It begins with the recognition of a set of poly(A) signals by polyadenylation factors, follow by cleavage at the poly(A) site and addition of a poly(A) tail. Using bioinformatic tools, we compiled the polyadenylation signals from two databases of Arabidopsis ESTs and full-length cDNAs (total of 17,000 genes involved). At the genomic level, our results clearly demonstrated the previously defined Near-Upstream Elements (NUE), Far-Upstream-Elements (FUE). There are no highly conserved signals in these elements, and the AAUAAA signal was only found at NUE in about 8-10% of the mRNA. However, a new element was identified around the cleavage/ polyadenylation sites – the cleavage-elements (CE). Furthermore, single nucleotide profiling of the 3' UTRs and the downstream regions revealed a distinct pattern of alternate A and U distributions. This prompted us to examine RNA secondary structures around 3'UTR. Using mFold algorithm, three types of structures were found, each with different configurations around poly(A) sites. Comparing the structures of wild-type and mutant poly(A) signals studied, only those mutants that altered the secondary structures resulted in a significant reduction of the poly(A) site efficiency. This is in agreement with our finding that the secondary structures may play an important role in poly(A) site definition and recognition.

We have also identified a group of Arabidopsis proteins that may play the role of cleavage and polyadenylation specificity (CPSF) factor. Two of the genes (AtCPSF73-I and II) encode the homologues CPSF 73 kD protein. The functionality of the proteins in polyadenylation was demonstrated through their interactions with poly(A) polymerase and other polyadenylation related proteins by yeast two-hybrid, in vitro pull-down and co-immunoprecipitation assays. Both genes are essential because no homozygous mutations were found. However, mutations of AtCPSF73-II specifically interfere the female gamete transmission, while over-expression of AtCPSF73-I by its native promoter resulted in male sterility due to delayed dehisce of the anther. Induced RNAi and over-expression of AtCPSF73-I led to a lethal phenotype in all developmental stages. Our data suggest that the AtCPSF73-I and II genes and their encoding proteins play a critical role in plant development, particularly in male and female gametophyte development and transmission.

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70 Coiled-coil Protein Composition of 22 Proteomes: Differences and Common Themes in Subcellular Infrastructure and Traffic Control

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The coiled-coil motif consists of two or more alpha-helices winding around each other in a supercoil. Long coiled-coil domains form dynamic fibers and scaffolds, allowing proteins to act as molecular "zippers", adapters, spacers, and motors in macro-molecular structures. Using the coiled-coil prediction program MultiCoil, we have previously identified all long coiled-coil proteins from *Arabidopsis thaliana* and established a searchable Arabidopsis coiled-coil protein database, ARABI-COIL [http://www.coiled-coil.org/arabidopsis].

We have now expanded our prediction analysis to identify proteins with long coiled-coil domains from 21 additional fully sequenced genomes. Due to the characteristic sequence repeat pattern of the coiled-coil motif, regions predicted to form coiled-coils often interfere with the statistical determination of significant sequence homologies. To eliminate this problem, we developed a sequence comparison and clustering strategy based on masking the identified coiled-coil domains. Using this method, we compared and grouped all long coiled-coil proteins from the 22 genomes analyzed based on sequence similarities outside their coiled-coil regions.

Long coiled-coils were found underrepresented in most bacterial genomes, however both archaea and eukaryotes contain longer coiled-coils than bacteria despite a lower overall percentage of coiled-coil proteins in the archaeal genomes. The structural maintenance of chromosomes proteins and their relatives stood out as the only long coiled-coil protein family conserved throughout all kingdoms. Motor proteins as well as membrane tethering and vesicle transport proteins are the dominant eukaryotic long coiled-coils with no homologs in prokaryotes. Several clusters of kingdom-specific coiled-coil families emerged, while at the same time a number of plant proteins with unknown function could be grouped with already characterized animal and yeast proteins. The results from this analysis will be used to improve the annotation of Arabidopsis coiled-coil proteins in the ARABI-COIL database.

71 At the Cutting Edge of Plant Biology Education: Engaging University and High School Biology Students in Arabidopsis 2010 Functional Genomics Research

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We are developing a series of inquiry-based laboratory exercises for college and high school students, constructed around an NSF-funded Arabidopsis 2010 Project. The research is focused on elucidating the cellular and molecular roles of each of the 43 members of the recently identified plant-specific Lateral Organ Boundaries Domain (LBD) gene family of Arabidopsis, only two members of which have been partly characterized to date. Functional studies of the remaining LBD family members will employ loss-of-function T-DNA insertional mutants as well as mis-expression and dominant negative-expressing transgenic lines for each LBD gene. Our teaching exercises will utilize the genetic materials generated by the research effort to introduce students to Arabidopsis as a model organism and to the utility of transgenic constructs in studies of gene function, in ways designed to enhance and complement the normal curricula in genetics and molecular biology. Students will then be given the opportunity to directly participate in the research by carrying out phenotypic analysis of the various Arabidopsis lines under experimental conditions that they themselves design and implement. We hope that this approach will enhance both learning and enjoyment in learning on the part of biology students. We will present our progress in this effort.

72 Functional Analysis of the RING-type Ubiquitin Ligase Family of Arabidopsis: A novel RING domain-containing protein is essential for growth and development

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The importance of the ubiquitination/26S proteasome pathway is illustrated by the Arabidopsis genome of which over 1300 genes are predicted to be involved in protein ubiquitination and degradation. A large number of these genes encode for RING-type E3s that contain an octet of cysteine/histidine residues coordinating 2 zinc atoms. Analysis of the Arabidopsis genome identified 469 genes predicted to encode for RING-type E3s. In addition to the canonical E2 binding RING domains, C3H2C3 (RING-H2) and C3HC4 (RING-HC), six types of modified RING domains, RING-C2, RING-HCb, RING-G, RING-D, RING-S/T and RING-v, were identified. The modified RING domains differ in either the spacing between conserved metal ligand residues or had amino acid substitutions at conserved positions. The RING-D domain was found only in the Arabidopsis genome. The RING proteins can also be grouped according to the presence of other domains, such as VWA, Cue, Jumanji, BRCT and WD40, which may facilitate substrate binding. The majority of these subgroups are found in other genomes, however subgroups such as the RING/VWA proteins seem to be plant-specific. Biochemical analysis of over 12% of RING proteins, representing all RING types, demonstrated that the majority of these proteins are capable of catalyzing polyubiquitination in conjunction with members of the *At*UBC8 family of E2s and other specific subgroups of E2s. To determine the biological function of the family of RING-type E3s we are currently analyzing T-DNA insertional lines. Analysis of plants, homozygous for T-DNA insertional mutations in over 100 previously uncharacterized RING protein-encoding genes, has demonstrated that a number of these genes are essential for proper growth and development. For example, a novel RING protein, named KEG, capable of mediating E2-dependent polyubiquitination, was found to be essential for plant growth. In addition to the RING-HCa domain, KEG also contains a kinase domain, ankyrin repeats and a number of previously unidentified repeats. Plants homozygous for mutations within the *KEG* gene do not survive beyond the seedling stage. The *keg* seedlings have both aerial and root defects. The biological role of KEG and a number of other RING proteins are currently being elucidated.

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73 Functional genomics of Arabidopsis defense responses against *Pseudomonas syringae*

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Plant defense against bacterial pathogens is a complex and sophisticated system under the control of numerous genes. Understanding how these genes function and how signals are transduced in the disease signaling network will be potentially beneficial to enhancing disease resistance in crop species. Our lab has developed a high-throughput method for studying functions of genes that are involved in Arabidopsis defense responses against *Pseudomonas syringae*. By using a microarray experiment, genes that were significantly up-regulated after *P. syringae* infection were chosen as our candidates. Homozygous T-DNA insertion mutants of these target genes were then studied to show whether they are capable of causing enhanced disease susceptibility (eds) phenotypes. Once eds phenotypes are confirmed, the corresponding genes will be subjected to further functional studies, which include microarray profiling experiments designed to reveal their roles in disease signaling and studies that lead to understanding of their detailed biological functions. During the conference, I will present the current progress of our project, the methods that we are using, and examples of genes that were found likely to be involved in disease resistance.

74 Novel gene discovery in the Arabidopsis genome

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The Arabidopsis genome contains many genes annotated as "hypothetical" because they are predicted only by computer algorithms with no experimental or sequence support In addition, evidence from whole genome tiling arrays, massively parallel signature sequencing (MPSS) and comparative genomics of Arabidopsis and Brassica indicates existence of many genes that have not yet been annotated. Our research focuses on the confirmation of the expression and structure of hypothetical genes and the discovery novel genes in the Arabidopsis genome by high throughput rapid amplification of cDNA ends (RACE). In addition to confirming many predicted gene structures (~ 2/3 are correct), we found many transcripts with structures different from their predictions. These included many alternatively spliced and multiple polyadenylated transcripts, transcripts arising from both strands, from the strand opposite to that of the prediction, and possible dicistronic transcripts. Moreover, based on Twinscan predictions and non-specific priming events in our RACE process, over 100 new transcripts from intergenic regions were discovered. The high sequence coverage generated by our pipeline reveals that ~ 25% of the genes studied have multiple splicing isoforms. Moreover, based on Twinscan gene predictions as well as non-specific priming events in our RACE process, over 100 new transcripts from intergenic regions were discovered. Full-length cDNA open reading frame (ORF) clones of these novel and hypothetical genes are being generated in a Gateway vector and provided to research community. This research was supported by the National Science Foundation.

75 Arabidopsis point mutants *aggregates of GFP fluorescence-1* and *bubblebath-1* are defective in vacuole biogenesis and gravitropism

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The plant vacuole is an essential organelle that is important for many cellular processes such as protein storage, recycling of cellular contents, cell elongation, and gravitropism. Insertional mutants such as *vacuoleless1*, which is defective in vacuole formation and does not survive embryogenesis (Rojo *et al.*, 2001, *Dev. Cell* 1:303), unequivocally demonstrate the vacuole's essential nature. The lethality of insertion mutations within *VCL1* and other genes encoding endomembrane components limits the value of the insertion approach for dissecting mechanisms of vacuole biogenesis. As an alternative, point mutants with non-lethal defects in vacuole biogenesis may be extremely useful for understanding vacuole biogenesis and endomembrane trafficking. To this end, we used EMS to mutagenize a marker line expressing a fusion of a Tonoplast Intrinsic Protein to GFP (35S::GFP:δ-TIP) and confocal microscopy to screen for seedlings with defects in tonoplast morphology (Avila *et al.*, 2003, *Plant Physiol.* 133(4):1673). Mutants were grouped into four categories based on the appearance of the tonoplast marker: *bubblebath (bub)* mutants accumulate increased numbers of vacuolar vesicles in the cells, *aggregates of GFP fluorescence (agg)* are characterized by large static GFP-fluorescent structures, *trans-vacuolar strands (tvs)* mutants have vacuoles that appear to be transected by thin structures encased by the tonoplast, and a fourth category of mutants have complex phenotypes such as defects in cell shape, or a combination of two or more of the *bub, agg* or *tvs* phenotypes. Interestingly, two *bub* and *agg* mutants have reduced responsiveness to gravity. Quantification of the gravity response indicates that the mutants are incapable of initially orienting vertically in the dark and are unable to reorient in response to a change in the gravity vector. This functional link between the endomembrane system and gravity response has been observed in several other mutants (Kato *et al.*, 2002 *Plant Cell* 14(1):33-46) and as well as for chemicals that affect endomembrane morphology and function (Surpin *et al.*, 2005 *Proc. Natl. Acad. Sci. USA* 102(4):1673). Our point mutants may provide insight into this interactivity of the endomembrane and gravitropic pathways. In addition to phenotypic characterization, we are identifying mutated genes in mutants from each of the four categories. We will present our recent approaches toward the identification of these genes and their roles in vacuole biogenesis and gravitropism.

76 Prolamellar body membrane formation and gene expression in Arabidopsis

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In angiosperms the synthesis of chlorophyll and chloroplast differentiation require light. The cotyledons of angiosperms that germinate in the dark therefore lack chlorophyll, and contain a chloroplast precursor known as the etioplast. This plastid type is characterized by an extensive paracrystalline inner membrane of uncertain function termed the prolamellar body. Upon illumination, dark-grown seedlings begin to green due to the synthesis of chlorophyll made possible by NADPH:protochlorophyllide oxidoreductase—a light-dependent enzyme that is the most abundant protein within the prolamellar body.

The chloroplast proteome consists of approximately 3000 gene products of which more than 95 % are nuclear-encoded and imported into the plastid compartment. Multiple mechanisms have therefore evolved to allow communication between the nucleus and the chloroplast. Several distinct chloroplast-to-nucleus retrograde pathways that regulate primarily, though not exclusively, nuclear genes required for photosynthesis have been identified and partially characterized. In contrast, etioplast differentiation in the absence of light and its impact on nuclear gene expression have remained virtually unexplored.

Our goals in this research area are: (1) to use a whole genome approach to identify Arabidopsis genes whose expression is strongly regulated by etioplast development in dark-grown seedlings, (2) to confirm the link between etioplast differentiation and the regulation of these target nuclear genes using independent assays, (3) to determine target gene expression patterns, and the subcellular localizations and functions of the corresponding protein products in dark-grown and greening seedlings. This research will provide a basis for exploring communication within plant cells between the etioplast and the nucleus.

77 Simple C-terminal targeting sequences are statistically over-represented across protein families: identifying known and novel peptide signatures with computational genomics

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The carboxy terminus is a biologically active and often critical component of many proteins. Short, low-information content peptide signals in the C-terminus are frequently involved in protein activities ranging from post-translational modifications to sub-cellular localisation. Several peptide signals involved in protein sorting and subcellular localisation have been characterised in the literature. These signals include SKL* (peroxisome), KDEL* (ER retention), KKxx* (ER membrane-protein retention) and Caax* (prenylation + membrane association). A prevalent feature of these signals is that they must be anchored at the C-terminus to function properly. We reasoned that due to the action of natural selection, simple sequences like SKL*, KDEL* and potentially novel C-terminal signals, would be statistically over-represented in whole genome datasets. Furthermore, these sequences would be identifiable across protein families, due to the generalised nature of the modifications as independent from any one families' function. A computational investigation of these hypotheses was undertaken using the predicted proteomes derived from 6 eukaryotic genome sequences. Statistical frequencies of tripeptides in the C-terminal region of proteins from each proteome were calculated in a manner that excluded tripeptide prevalence due solely to protein family homology in the C-terminal region. Moreover, tripeptides identified as statistically over-represented in *A.thaliana* were then assessed for the conservation of the sequence between *A.thaliana* proteins and any orthologs present in *O.sativa*. Our analyses show that several known C-terminal targeting sequences (SKL*, KDEL*, KKxx* Caax*) are over-represented across protein families. Moreover, several new sequences were found in multiple datasets; suggesting the existence of previously uncharacterized C-terminal peptide signals conserved throughout eukaryotes. Preliminary work at the bench has focused on C-terminal fusions of novel candidate peptides to green flourescent protein (GFP) and their examination using confocal microscopy. Further bioinformatic results, as well as data from ongoing experimental investigations into the functions of these novel C-terminal sequences will be presented.

78 Subcellular location of Xylem Cysteine Proteinase 2 (XCP2) within differentiating tracheary elements of Arabidopsis thaliana determined by use of microwave-assisted sample processing for electron microscopy

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To better understand the function of a cysteine proteinase, XCP2, in tracheary element differentiation, its subcellular location was determined by immunolabeling and observation with the electron microscope. Proteinases have been implicated in programmed cell death during organ senescence and tracheary element differentiation. Other data indicated that the papain-like cysteine proteinase, XCP2, might be involved in the autolysis of tracheary elements, a process essential to the creation of hollow water-conducting xylem vessels. The presence of XCP2 only in differentiating tracheary elements was supported by analysis in the laser scanning confocal microscope of transgenic Arabidopsis expressing a reporter gene, XCP2 promoter:GUS. Electron microscopic observations showed the immunogold labeling of XCP2 only in differentiating tracheary elements, and they were consistent with the synthesis of XCP2 in the cytoplasmic compartment, its loading into the vacuole, and its release from the vacuole in coordination with programmed cell death. This research was aided by the predictable developmental pattern of tracheary element differentiation near the root tip of germinating Arabidopsis seeds and microwave-assisted fixation and infiltration of whole Arabidopsis tissues. Samples required only 25 minutes processing in the microwave before intact seedling roots were fixed and fully infiltrated in 100% resin, and good preservation of both sub-cellular structure and protein antigenicity were achieved. This research was supported in part by NSF grant, IBM-0131386, to E.P.B. and C.H.H. We thank Earl Petzold for preparation and purification of antibodies.

79 Use of an Arabidopsis CAD mutant to determine the function of CADs of Arabidopsis and tree origin

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In Angiosperms, the lignin biopolymer is composed mainly of guaiacyl (G) and syringyl (S) units. These units are derived from coniferyl and syringyl alcohols, also named monolignols. Cinnamyl alcohol dehydrogenase (CAD), the last enzyme of the monolignol-specific pathway, is responsible for the reduction of hydroxycinnamaldehydes to hyroxycinnamyl alcohols. CAD genes belong to multigene families in plants; For example, nine genes are present in the Arabidopsis genome (Goujon et al, 2003). The expression profile of two of them, AtCAD-C and AtCAD-D, is compatible with a major role in stem lignification (Sibout et al, 2003) but only a double mutant (Atcad cd) affected in these two CAD shows reduced lignin content and a modification of lignin composition with significant levels of cinnamaldehydes incorporated instead of cinnamyl alcohols (Sibout et al, 2005). In order to determine if other CAD proteins possess the ability to reduce cinnamaldehydes, this double mutant has been used for complementation studies. Molecular complementation of this Atcad cd mutant with pine CAD, poplar CAD and SAD as well as with the 9 Arabidopsis CAD genes under the control of the CAD-D promoter suggests different abilities of these genes/proteins in the production of syringyl-lignin.

Goujon et al (2003) Plant Physiol. Biochem. 41 : 677-687.
Sibout et al (2003) Plant Physiol. 132 : 848-860.
Sibout et al (2005) Plant Cell : in press

80 AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana*

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Upon encountering nutrient deficiency, extensive degradation of macromolecules occurs in eukaryotic cells to produce respiratory substrates and raw materials for cell survival. This degradation occurs by vacuolar autophagy, in which a double membrane forms around cytoplasmic components to generate an autophagosome, which is transported to the vacuole. The outer membrane fuses with the vacuole and the inner membrane and its contents are degraded by vacuolar hydrolases. We have identified a small gene family in *Arabidopsis thaliana*, members of which show sequence similarity to the yeast autophagy gene *ATG18*. One member of this family, *AtATG18a*, increases in expression during sucrose and nitrogen starvation and during senescence, conditions in which autophagy is induced. RNA interference was used to generate transgenic lines with reduced *AtATG18a* expression. These lines are hypersensitive to sucrose and nitrogen starvation and show premature senescence, both during natural senescence of leaves and in a detached leaf assay. *AtATG18a* RNAi plants are unable to produce autophagosomes in response to starvation or senescence, and the AtATG18a protein is therefore likely to be required for autophagosome formation. AtATG18a recombinant protein can bind to phospholipids, with a preference for phosphatidylinositol 3 -phosphate, and a GFP fusion protein localizes to punctate structures within the cytoplasm. Further characterization of AtATG18a protein localization and function is now underway.

81 Genetic dissection of ARP2/3 functions in Arabidopsis

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Plant cells use the actin cytoskeleton during morphogenesis and organelle positioning. Little mechanistic data exists regarding the cellular pathways controlling actin filament nucleation or the precise function of particular actin filament arrays in growing plant cells. Trichome or leaf hair morphogenesis has highly constrained requirements for cellular organization and cytoskeletal functions. Our genetic analysis of distorted trichome mutants has identified unambiguously one actin filament nucleation pathway that plant cells utilize. Distorted group genes encode both subunits of the evolutionarily conserved Actin-related proteins (ARP) 2/3 complex and subunits of the WAVE complex. ARP2/3 function is essential for non-plant organisms, but Arabidopsis mutants lacking individual ARP2/3 subunit genes have a normal overall architecture, but display strong trichome distortion. Although we have null alleles of several ARP2/3 subunit genes, we do not know if these mutants reflect the phenotypes that would be observed in the complete absence of ARP2/3 activity. We have taken a combination of forward and reverse genetic approaches to mutate each of the seven ARP2/3 subunit genes in Arabidopsis. The purpose of the work is to determine if each of the seven ARP2/3 subunit homologues are required for normal morphogenesis. Second, we are using the known ARP2/3 complex assembly pathway to construct double mutant combinations that should eliminate ARP2/3 complex activity. We hope also to present data that compares the assembly status of the putative Arabidopsis ARP2/3 complex in wild type and mutant backgrounds.

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82 Direct Interaction of a Divergent CaM Isoform and the Transcription Factor, MYB2, Enhances Salt Tolerance in Arabidopsis

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Calmodulin (CaM), a ubiquitous calcium-binding protein, regulates diverse cellular functions by modulating the activity of a variety of enzymes and proteins. Plants express numerous CaM isoforms that exhibit differential activation and/or inhibition of CaM-dependent enzymes in vitro. However, the specific biological functions of plant CaM are not well known. In this study, we isolated a cDNA encoding a CaM binding transcription factor, MYB2, that regulates the expression of salt- and dehydration-responsive genes in *Arabidopsis*. This was achieved using a salt-inducible CaM isoform (GmCaM4) as a probe from a salt-treated Arabidopsis expression library. Using domain mapping, we identified a Ca^{2+}-dependent CaM binding domain in MYB2. The specific binding of CaM to CaM binding domain was confirmed by site-directed mutagenesis, a gel mobility shift assay, split ubiquitin assay, and a competition assay using a Ca^{2+}/CaM-dependent enzyme. Interestingly, the specific CaM isoform GmCaM4 enhances the DNA binding activity of AtMYB2, whereas this was inhibited by a closely related CaM isoform (GmCaM1). Overexpression of Gm-CaM4 in Arabidopsis up-regulates the transcription rate of AtMYB2-regulated genes, including the proline-synthesizing enzyme P5CS1 (1-pyrroline-5-carboxylate synthetase-1), which confers salt tolerance by facilitating proline accumulation. Therefore, we suggest that a specific CaM isoform mediates salt-induced Ca^{2+} signaling through the activation of an MYB transcriptional activator, thereby resulting in salt tolerance in plants.

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83 The *mum5*mutant has a mutation in a putative pectin methylesterase

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Upon seed imbibition in Arabidopsis, mucilage is extruded from a single layer of cells on the outside of the seed coat. This mucilage is made primarily of the pectic polymer rhamnogalacturonan I. Two previously identified mutants, mucilage modified 3-1 and mum5-1 1, have seed mucilage with aberrant physical properties.

Map-based cloning coupled with a candidate gene approach using the mum5-1 mutant revealed a single basepair change in a putative pectin methylesterase (PME). The pectin methylesterase contains a signal sequence, an extension domain, a PME inhibitor domain, and a PME domain. FTIR spectroscopy suggests mum5-1 has pectin with a lower degree of methyl-esterification. The mum3-1 mutant has an FTIR phenotype identical to mum5-1.

Thus far, no homozygous T-DNA insertions have been obtained in mum5, suggesting a lethal phenotype in the null mutant. Genetic studies to determine the nature of this lethality are underway.

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84 The Arabidopsis PEX12 gene is required for peroxisome biogenesis and essential for development

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Peroxisomes perform diverse and vital functions in eukaryotes and abnormalities in peroxisomal function lead to severe developmental disorders in humans. Peroxisomes are also involved in a wide array of physiological and metabolic functions unique to plants, yet many aspects of this important organelle are poorly understood. In yeast and mammals, various steps in peroxisome biogenesis require the function of PEX proteins (peroxins) among which PEX12 is a RING finger peroxisomal membrane protein involved in the import of matrix proteins. To investigate the role of PEX12 in plants, we identified a T-DNA knockout allele of PEX12 and generated partial loss-of-function pex12 mutants using RNA interference (RNAi). We show that pex12 null mutants are developmentally arrested during early embryogenesis, and that the embryo lethal phenotype can be rescued by overexpression of the PEX12-CFP fusion protein, which targets to the peroxisome. Using virus-induced gene silencing techniques, we demonstrate that peroxisomal number and fluorescence of the YFP-PTS1 (peroxisome targeting signal type 1) protein are greatly reduced when PEX12 is silenced. PEX12 RNAi plants exhibit significantly impaired peroxisome biogenesis, strong inhibition of plant growth, and reduced fertility. Our work provides evidence that the Arabidopsis PEX12 protein is required for peroxisome biogenesis and plays an essential role throughout plant development.

85 A novel cross-linking strategy aimed at investigating precursor interactions with the chloroplastic Tic complex and with stromal molecular chaperones

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The long-term goal of our research program is to understand the molecular details of protein import into chloroplasts. Our immediate objective is to test the hypothesis that a stromal molecular chaperone from the HSP 100 family (Hsp93) forms a complex with other translocation components, i.e., Tic110 and Tic40, to provide the driving force for the transport of precursor proteins into chloroplasts. Despite evidence that Hsp93 is found in translocation complexes, evidence that it interacts directly with precursor proteins during the import process is lacking. To investigate this possibility we have developed a cross-linking strategy using a novel modified precursor protein. The modified precursor proteins used in this investigation are composed of either the bipartite transit peptide of Toc75 that has been His-tagged or fused to GFP-Histag which are designated pToc75 –TPHistag and pToc75 –TP-GFPHistag respectively. In addition, both modified bipartite transit peptides have been mutated so that they cannot be cleaved by the stromal processing protease. Using in vitro import assays, preliminary evidence indicates that our the modified bipartite transit peptide forms a translocation intermediate that appears to span the inner envelope membrane with the uncleaved transit peptide extending into the stroma. A 'trapped' bipartite transit peptide with this topology has not been used in earlier cross-linking studies. We are currently developing procedures to introduce the photoactivatable trifunctional cross-linker Sulfo-SBED at specific locations near the amino terminus of our modified bipartite transit peptide. Once available, we will investigate whether Hsp93, or other members of the putative chaperone complex, are in close physical proximity to the trapped translocation intermediates that they can be cross-linked to them.

86 Characterization of the anti-microtubule drug supersensitive Arabidopsis mutant 28-2b

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Microtubules assemble into highly organized arrays that are essential for nuclear migration, cytokinesis, and cell expansion. In plants, microtubules form a cortical array that lines the periphery of each cell and plays a role in regulating and directing cell wall deposition and cell expansion. Little is known about the mechanisms underlying the organization of this array, or even how it carries out its functions. To learn more, we are taking a molecular genetic approach by characterizing an Arabidopsis mutant (line 28-2b) whose cortical microtubule arrays are hypersensitive to disruption by the anti-microtubule drug oryzalin. On agar growth medium containing 150 nanomolar oryzalin, wild type Arabidopsis roots grow normally, while 28-2b root tips swell and stop growing due to disruption of cell expansion and division. We have mapped the 28-2b mutation to a 45 kb interval on the lower arm of chromosome three, and are working to identify the affected gene. Further characterization of this mutant should provide insights into how cortical microtubule arrays are assembled and affect cell expansion.

87 FZL, an FZO-like protein in plants, links thylakoid morphogenesis to chloroplast division

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FZO is a mitochondrial fusion protein in fungi and metazoa. However, an FZO-like protein in *Arabidopsis*, FZL, is targeted to chloroplasts. *fzl* knockout mutants show a chloroplast division defect with heterogeneity in chloroplast size and thinner thylakoid sacs with disorganized stacking. An *FZL-GFP* gene rescued the *fzl* mutant phenotype. Overexpression of FZL-GFP causes very long thylakoid sacs with considerably less stacking. FZL-GFP is a membrane protein present in both the thylakoids and chloroplast inner membrane. FZL-GFP is mainly localized to punctate or vesicle-like structures associated with chloroplasts and infrequently in the cytosol, but is not associated with mitochondria. The level of FZL-GFP expression correlates with the number of punctate and vesicle-like structures on the surface of the chloroplast, suggesting that these structures are coincident with FZL and that FZL may be involved in their formation. Mutation of a conserved lysine residue in the GTPase domain abolishes the punctate localization pattern and the ability of FZL-GFP to complement *fzl* mutant. We suggest that FZL is an important determinant of the thylakoid morphology and the observed phenotype in *fzl* may reflect abnormal thylakoid and envelope membrane properties in the mutant.

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88 A structure-function study of COP9 Signalosome subunit 7

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CSN7 is a component of the COP9 signalosome (CSN). *csn7* (*fus5*) mutants show a *constitutive photomorphogenic* phenotype. A two-hybrid screen using CSN7 as bait identified a number of CSN7-interacting proteins, including other CSN subunits.The CSN subunits interacted with a central region of CSN7, in the predicted "PCI motif", while the other proteins interacted with either the N or C termini. To further define this "PCI motif", a series of stringent biochemical assays were carried out, including limited proteolysis, analytical gel filtration and circular dichroism spectroscopy. From these studies we identified a stable core domain in CSN7. These studies, together with analysis of transgenic plants that express this domain showed that the CSN7 "core" specifically mediates interactions with other CSN subunits both *in vitro* and *in planta*. One of the CSN7 interactors that does not interact with the "core" is the small subunit of the ribonucleotide reductase, RNR2a, a component of the RNR holoenzyme. This is the key enzyme for reducing NTPs to dNTPs, supplying building blocks for DNA after DNA damage or for cell cycle. This interaction was confirmed both *in vitro* and *in planta*. To further address the biological significance of the CSN7-RNR2 interaction, a *csn7* mutant specifically deficient in its ability to interact with RNR2 was generated.

89 Formation and function of prevacuolar compartments in the *Arabidopsis* embryo

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Seed storage proteins and processing proteases have been found inside prevacuolar compartments in the *Arabidopsis* embryo. Interestingly, other prevacuolar/endosomal compartments lacking storage proteins also coexist in the same embryo cells. In order to understand the origin and functions of these compartments, we have studied high-pressure frozen/freeze-substituted samples of *Arabidopsis* embryos by means of electron tomography and immunolabeling techniques. The storage protein-containing prevacuolar compartments originate in close proximity to the *Trans* Golgi network(TGN), by the fusion of at least two types of Golgi-derived vesicles. As more vesicles fuse, the prevacuolar compartments enlarge (up to ~ 500 nm in diameter) and become multivesicular. These changes in size and morphology appear to be accompanied by an increase in their luminal proteolytic activity. We have observed up to four Golgi stacks arranged with their TGN compartments facing to each other. These clusters of Golgi stacks delimit areas highly enriched in prevacuolar compartments at different stages of maturation. Whereas the participation of these compartments in the secretory pathway is evident as they transport cargo from the Golgi to the protein storage vacuole, their involvement in the endocytic pathway has not been explored. In addition, the function of the other prevacuolar/endosomal compartments lacking storage proteins was not known. We have found that endocytosed plasma membrane proteins localized to the endosomal compartments devoid of storage proteins but not to the prevacuolar compartments that contain storage proteins. We postulate that two populations of prevacuolar/endosomal compartments coexist in *Arabidopsis* embryo cells and that they greatly differ in their involvement in the secretory and endocytic pathways. Supported by an Antorchas Foundation grant to M.S.O.

90 AtTBPs in *Arabidopsis* encode proteins that bind plant telomeric DNA and induce DNA bending in vitro

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Telomeres are the specialized nucleoprotein structures that comprise the natural ends of linear eukaryotic chromosomes and ensure their complete replication and stability. Telomeric DNA-binding proteins (TBPs) are crucial components that regulate the structure and function of eukaryotic telomeres and are evolutionarily conserved. We have identified two homologues of AtTBP1 (for *Arabidopsis thaliana* telomeric DNA binding protein 1), designated as AtTBP2/TRFL9 and AtTRP2/TRFL1, which encode proteins that specifically bind to the telomeric DNA of this plant. These proteins show extensive homology with other known plant TBPs. Northern analysis demonstrated that these *Arabidopsis* TBP genes are ubiquitously expressed, as previously described for AtTBP1. The isolated C-terminal segments of these proteins were capable of sequence-specific binding to duplex telomeric plant DNA in vitro. DNA bending assays using the *Arabidopsis* TBPs revealed that AtTBP1 and AtTBP2 have DNA-bending abilities comparable to that of the human homologue hTRF1, and higher than those of AtTRP1 and AtTRP2. *Arabidopsis* TBPs possessed a highly conserved region C-terminal to the Myb domain called domain E that is absent in hTRFs. Studies of deletion mutants of domain E of AtTBP1 demonstrated that domain E, besides SMTBD, is required for binding plant telomeric DNA and defines a novel class of proteins in *Arabidospsis*. Assuming these proteins are involved in mediating or regulating plant telomere structure and function, the critical question that remains to be answered is whether *Arabidopsis* TBPs bind plant telomeres in vivo. Further studies will be required to determine the actual function and physiological relevance of AtTBPs and AtTRPs in the plant.

91 AtCYCA2;3, a key regulator in the termination of endoreduplication rounds in Arabidopsis

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Plant cells frequently undergo endoreduplication, a process in which chromosomal DNA is successively duplicated in the absence of mitosis. Although the entry into and cessation of endoreduplication are tightly regulated, the regulatory mechanisms of these processes remain unclear. We found that the promoter of an Arabidopsis cyclin A gene, AtCYCA2;3, is active in developing trichomes, which undergo endoreduplication, as well as in proliferating tissues. Loss-of-function mutations of the gene semi-dominantly caused increases in ploidy levels of various organs. Consistent with this, expression of the fusion protein AtCYCA2;3-GFP in the mutant background reduced the ploidy levels in a dose-dependent manner. Moreover, a mutation in the destruction box of AtCYCA2;3 stabilized the fusion protein in the nuclei and enhanced the effect of reductions in ploidy. We propose that the expression level of the AtCYCA2;3 protein acts as a key parameter in the termination of endoreduplication rounds through the signal cascade for DNA replication licensing.

92 Molecular feature of *Arabidopsis* Rhomboid proteins: their subcellular localization, proteolytic activity and substrate specificity

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Intramembrane proteolysis is a fundamental mechanism to regulate various aspects of cellular functions. Rhomboid-1 is an intramembrane serine protease that cleaves *Drosophila* Epidermal Growth Factor Receptor (EGFR) ligands with its transmembrane region to release active growth factors. Rhomboid genes are present in nearly all the sequenced genome of archaea and eukaryotes, including higher plants. Despite the divergence of the sequence, proteolytic activity and substrate specificity are conserved throughout evolution. Here we report the molecular feature of two plant Rhomboid genes, *AtRBD1* and *AtRBD2*, from *Arabidopsis thaliana*. Like *Drosophila* Rhomboid-1, both of them were localized to the Golgi apparatus. AtRBD1 could cleave *Drosophila* ligand Spitz and Keren in mammalian cells and then soluble ligands were released into the medium, while AtRBD2 did not show proteolytic activity. AtRBD1 did not cleave Transforming Growth Factor α, suggesting that substrate specificity is also conserved in plant. This is the first evidence of regulated intramembrane proteolysis (RIP) in plant.

93 ER-to-Golgi transport in higher plant cells: New structures and a new mechanism

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Plant Golgi stacks travel along actin filament bundles in a 'Stop and Go' pattern of movement (Nebenführ *et al. Plant Physiol*., vol.121, 1127-42). This Stop and Go pattern is characterized by alternations between rapid straight displacements (go phase), and random wiggling at set locations (stop phase). Wiggling Golgi stacks are associated transiently with ER export sites marked by the Sec13 protein, a component of the COPII vesicular transport machinery (Yang *et al. Plant Cell*, in press). This suggests that the ER-to-Golgi transport in plant cells takes place during transient interactions between these two compartments.

We have investigated Golgi stacks and the ER in *Arabidopsis thaliana* and alfalfa root meristem and columella cells using electron tomography and immunoelectron microscopy. ER export sites (tER sites) were identified by the presence of budding COPII vesicles that are labeled by antisera against AtSar1p. A fraction of the Golgi stacks were located close to these ER export sites and, most interestingly, the ER export sites appeared to be connected to the *cis*-side of the Golgi stacks through a ribosome-excluding matrix. ER export sites tend to be organized around the *cis*-side of close Golgi stacks. All the COPII vesicles were surrounded by a ribosome-excluding matrix both while they were budding and during their transfer to the Golgi. Significant numbers of COPII vesicles (up to 12) were seen within the Golgi matrix of docked Golgi stacks and these always displayed a small, irregularly shaped *cis*-most cisterna. Golgi stacks not connected to ER export sites were also found. Their matrices contained few COPII vesicles and lacked *cis*-cisternal assembly intermediates. We did not find any evidence for long-range transport of COPII vesicles, intermediate compartments between the ER and Golgi stacks, nor direct connections between ER and Golgi membranes. In root columella cells, the ER is confined to cortical region of the cytoplasm. Some Golgi stacks in the cell cortex were also observed to be coupled to ER export sites via a matrix containing COPII vesicles and displayed assembling *cis*-cisternae; those in the cell center lacked these structures.

Together, these findings demonstrate that COPII vesicle-mediated transport occurs during the "stop" phase of Golgi movement, and that the process of stopping involves the formation of transient links between the ribosome-excluding matrix of forming COPII vesicles and the *cis*-side of the Golgi matrix. COPII vesicles are transferred within this matrix structure. (Supported by NIH grant GM61306 to LAS)

94 Identification of A NAM/CUC2-Like Protein That Interacts with A Receptor-Like Protein Kinase in Arabidopsis

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Calcium (Ca^{2+}), a universal second messenger, regulates diverse cellular process in all eukaryotes. Transient influx of Ca^{2+} constitutes an early event in the signaling cascades that trigger plant defense responses. However, the downstream components of defense-associated Ca^{2+} signaling are largely unknown. By screening of the pathogen-treated *Arabidopsis* cDNA expression library with HRP conjugated calmodulin as a probe, we identified calmodulin binding NAM/CUC2-like protein (CBNAM) which encodes a putative transcription factor with 534 amino acids. By using truncated versions of fusion proteins and a synthetic peptide of CBNAM, we demonstrated that a region 27 amino acids of CBNAM binds to calmodulin in a Ca^{2+}-dependent manner. To determine the specific DNA sequence necessary for CBNAM binding, we employed a polymerase chain reaction mediated random binding site selection method. Based on the analysis, CBNAM binds four classes of sequences (CGTT, GCTT, CCTT, and GGTT). In yeast two-hybrid, CBNAM interact with the putative receptor protein kinase. Interestingly, the kinase assay showed that RLK was able to phosphorylate CBNAM. The RLK gene is strongly induced by a bacterial pathogen and salicylic acid. Our results suggest that both RLK and CaM may regulate a NAM-like protein in plant defense signal pathway.

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95 Functional Analysis of AtORC1a, Arabidopsis thaliana Origin Recognition Complex 1a

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The origin recognition complex (ORC) is required as the initiator for DNA replication in eukaryotes. ORC is composed of six components and essential for DNA replication. ORC1 protein is the largest subunit in ORC and highly conserved essential protein in eukaryotes. In the Arabidopsis thaliana genome, two ORC1 homologues, AtORC1a and AtORC1b, were encoded (Masuda HP, et al., 2004). This was the first finding that there were multiple homologues of ORC1 gene in one genome. However the functions of these genes are not characterized.

In this study, we report the function of AtORC1a in vivo. GFP fused AtORC1a was localized in nuclei. AtORC1a gene complemented the yeast ORC1 gene function in budding yeast cells. These results suggested that AtORC1a has the function as a subunit of initiator for DNA replication. Phenotypic analyses using RIKEN Ds insertion tagged line, such as analysis of cell cycle progression and expression analysis of AtORC1a by real time PCR and promoter GUS fusion are currently in progress. Function of AtORC1 in DNA replication and plant development will be discussed.

96 Endoplasmin is less promiscuous than BiP but displays significant chaperone activity

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The endoplasmic reticulum (ER) contains a number of molecular chaperones that optimise folding and assembly of newly synthesised secretory proteins. These chaperones include BiP (a member of the HSP70 family) and endoplasmin (a member of the HSP90 family). The activity of BiP in the plant ER is fairly well characterised whereas very little is known on endoplasmin function. We have prepared an antiserum against a fusion protein containing the C-terminal 95 amino acids (excluding the KDEL signal) of *Arabidopsis* endoplasmin. The antiserum recognises a polypeptide of 97 kDa in *Arabidopsis* and tobacco extracts. This polypeptide is practically absent in the *shd Arabidopsis* T-DNA mutant, that lacks detectable levels of endoplasmin mRNA, confirming that it represents this chaperone. However, mature roots as well as tunicamycin-treated *shd* seedlings contain endoplasmin protein at a low steady-state level, demonstrating that the *shd* mutant is not a null allele.

Using the antiserum we have established that a much lower amount of newly synthesised polypeptides is associated with endoplasmin than with BiP, both in *Arabidopsis* and tobacco. Following inhibition of glycosylation by tunicamycin treatment, the synthesis of endoplasmin is induced in *Arabidopsis* seedlings but not in tobacco protoplasts. Because this treatment induces the synthesis of BiP in both systems, we conclude that the unfolded protein response acts in part differently on the expression of the two chaperones.

Analysis into whether a greatly reduced presence of endoplasmin in the *shd Arabidopsis* mutant is compensated by BiP showed that no effect was seen in the steady-state level of this chaperone in mature *shd* mutant tissue compared to the wild-type. Furthermore, the synthesis rate of BiP was not affected in *shd* mutant compared to wild-type seedlings but its steady-state level was markedly increased. This indicated a compensatory effect on BiP's turn-over rate in highly active tissue when endoplasmin is not present at its normal level, attributing some important role to the latter. Thus the chaperone activity of endoplasmin was assessed in comparison to BiP. Over-expression of each of these proteins in tobacco protoplasts in absence or presence of tunicamycin showed that endoplasmin displayed a minor but significant *in vivo* chaperone activity on the synthesis rate of a model secretory protein.

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97 Role of complex N-glycan biosynthesis in plant osmotic stress response

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Protein N-glycosylasion is a multistep process involving core-oligosaccharide assembly at the cytosolic face of the ER, core-oligosaccharide transfer to the nascent polypeptide chain and trimming in the ER lumen, followed by final glycan modifications in the Golgi. Genetic analyses indicate that the ER-localized oligosaccharide transferase and glucosidases are essential for osmotic stress tolerance (1) and cellulose biosynthesis, respectively. The role of N-glycan modification in the Golgi, however, has been undeciphered in plants, mainly because appreciable phenotypic alteration has not been detected in mutant plants (*cgl1*) lacking Golgi N-acetylglucosaminyltransferase I that is essential for N-glycan modifications (2).

Using a modified root-growth assay system, we have determined that *CGL1* is required for growth of Arabidopsis roots under high-osmotic-stress conditions. In the presence of hyperosmotic stress, *cgl1* root exhibited greater growth inhibition than wild type roots. The growth inihibition coincides with abberant root tip morphology indistinguishable from osmotic-stress-sensitive *stt3a* mutants, which are defective for a catalytic subunit isoform of oligosaccharyltransferase in the ER (1). Unlike *stt3a* mutations, mutated alleles of *cgl1* did not cause constitutive activation of unfolded protein response (UPR), indicating that the growth arrest of root tips under osmotic stress is regulated by a distinct mechanism independent of UPR signaling in ER. Although *stt3a* and *cgl1* seedlings each grow normally without osmotic stress, *stt3a/cgl1*-double knockout plants exhibited a synergistic phenotype of slow growth and aberrant root morphology even without stress. These results indicate that complex N-glycans are indispensable for optimal plant growth and development. Experiments are in progress to prepare a collection of T-DNA insertion lines that are defective in each N-glycan modification step, and to identify determinants of osmotic stress tolerance in the N-glycan modification pathway using these lines.

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98 A role for Dynamin-Related Protein 1C in root hair and pollen tube growth

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Polarized membrane trafficking is critical to the life of a plant. It is required for both cell division and expansion, including the anisotropic growth of stigmatic papillae, pollen tubes and root hairs. Members of the Arabidopsis dynamin-related protein 1 (DRP1) family have been shown to have a role in polarized cell expansion and cytokinesis. One family member, DRP1C, may have a role in polar membrane trafficking during root hair and pollen tube growth. *drp1C* pollen are shriveled and fail to germinate, demonstrating a requirement for DRP1C in pollen development. A functional DRP1C-GFP fusion protein is localized to the tips of growing root hairs, the tips of expanding pollen tubes, and the cell plate during cell division. Pharmacological studies suggest that DRP1C localization at the tip of expanding root hairs is dependent on the actin cytoskeleton, the presence of PIP(4,5)P2 and an active secretory pathway. The lethality of *drp1C* pollen currently prevents genetic verification of DRP1C's role in pollen tube growth and root hair development; however, based on DRP1C-GFP localization and homology to other DRP1 proteins, we hypothesize that DRP1C plays a critical role in polar cell expansion in root hairs and pollen tubes.

99 In *Arabidopsis thaliana* the High-affinity nitrate transport in the roots is regulated by *NAR2.1* expression

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The genome of *Arabidopsis thaliana* contains two genes *AtNAR2.1* and *AtNAR2.2* that are similar to the *Chlamydomonas NAR2* gene. In *Chlamydomonas* the *NAR2* protein is not involved in transport of nitrogen by itself yet it is required for the high-affinity nitrate uptake. In *Arabidopsis* the mRNA of *AtNAR2.1* is the predominant one with over 99% of the total *NAR2* RNA. The *AtNAR2.1* mRNA was induced 6-fold with nitrate treatment. Similar Nitrate-induction was not observed in the T-DNA mutant plants that had the *AtNAR2.1* promoter region disrupted. As a result of the disruption, the expression levels of *AtNAR2.1* were much lower in the mutant seedlings when compared with the wild type. Under similar conditions the expression of *AtNAR2.2* was very low and constitutive. Also the disruption of *AtNAR2.1* did not result in enhanced expression of *AtNAR2.2* so there was no compensation at all. Moreover there was reduced nitrate-induction of *AtNRT1.1* and *AtNRT2.1* in the *Atnar2.1* mutant seedlings. Under low-nitrate steady state condition there was a 67% reduction in the HATS influx in the mutant seedlings. When the nitrogen deprived mutant plants were induced by nitrate for 6-hours the inducible high-affinity influx (iHATS) influx was reduced by 92%. Under similar conditions there was no effect on the influx via Low-affinity transport system (LATS). All these results clearly indicate that in *Arabidopsis* plants presence of functional *AtNAR2.1* gene is required for the iHATS nitrate influx in the roots.

100 Potential involvement of MATE protein AtNIC4 in hormone transport of *Arabidopsis thaliana*

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The multidrug and toxin extrusion (MATE) family of membrane proteins occur in archeae, bacteria, yeast, animals and plants. The genome of *A. thaliana* encodes at least 56 MATE proteins subdivided into five subfamilies. One of the subfamilies contains the eight *AtNIC* genes (*A. thaliana* novel ion carrier). Only five MATE proteins (ALF5, AtDTX1, TT12, EDS5, FRD3) from *A. thaliana* were initially characterised (Li *et al*., 2003), but their functional roles *in planta* are still not known. All MATE proteins of *A. thaliana* show sequence homology to the functionally characterised transporter NORM of the marine bacterium *Vibrio parahaemolyticus*. NORM seems to function as a secondary carrier catalyzing substrate/cation antiport (Paulsen *et al*., 1996). Here we describe the functional analysis of AtNIC4. Heterologous expression in *Saccharomyces cerevisiae* and *Xenopus laevis* oocytes suggests an involvement of AtNIC4 in transport of monovalent cations. For the functional characterisation of AtNIC4 *in planta* we localised and modulated its expression in *A. thaliana*. Tissue specific northern analysis and promoter-GUS studies show, that AtNIC4 expression is detectable in leaves, flowers and roots. Overexpression of *AtNIC4* in transgenic plants under the control of the CaMV 35S promoter causes accelerated flower stalk development and an almost complete absense of apical dominance. Suppression of *AtNIC4* by RNA interference leads to delayed flower stalk development and flowering. The plants develop larger leaves and the number of stalks and flowers is strongly reduced in comparison to wild type plants. The subcellular localization of AtNIC4-GFP fusion proteins was studied in *Nicotiana tabacum* BY2 cells and *A. thaliana* leaf protoplasts. It was shown that the AtNIC4 protein is localized in peroxisomes. The data collected so far suggest an involvement of the AtNIC4 protein in the distribution of growth- and development-promoting substances. The expression pattern, the localisation in peroxisomes and the dramatic changes in plant architecture, suggest a possible function of AtNIC4 in the distribution of plant hormones like auxin, or hormon specific transport inhibitors like flavonoids. Ongoing studies include the screening for interacting proteins in yeast and the analysis of transgenic plants showing tissue specific expression or inducible overexpression of *AtNIC4*.

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101 TPK/KCO family: K+ channels in Arabidopsis thaliana

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In *Arabidopsis thaliana* six members of structurally divergent potassium channels have been identified by sequence homology to AtTPK1, formerly described as AtKCO1 (Czempinski *et al.* 1997). The five TPK-like proteins present four transmembrane segments and two K^+-selective P-domains as their basic subunit structure. AtKCO3 presents only two transmembrane segments and a single K^+-selective P-domain, like the Kir-family. Among the six channels, five show the presence of Ca^{2+}-binding motifs in the C-termini suggesting that these channels might be involved in calcium-dependent signalling pathways. In their N-termini some TPK channels exhibit a putative 14-3-3 binding site and at least TPK1 shows a highly basic region (KR region) of unknown function close to the 14-3-3 binding site.

Our project aims to analyse the function of the TPK/KCO channels using molecular, biochemical and physiological methods in transgenic plants including detailed expression analysis, subcellular localisation studies and reverse genetic approaches. Studies on AtTPK/KCO-GFP fusion proteins demonstrated that e.g. AtTPK1 is localised in the vacuolar membrane (Czempinski *et al.* 2002) and AtTPK4 in the plasma membrane (Becker *et al.* 2004). Methods like the Split-YFP-system, RET and THS are used to investigate protein interaction within the family and with regulatory proteins, e.g. 14-3-3 proteins. Results from the molecular characterisation of this gene family will be presented.

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102 Cell growth and morphogenesis are affected in the Arabidopsis midget mutant

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To acquire their shape plant cells are using regulatory mechanisms that precisely coordinate processes of cell polarity establishment, directional cell expansion and growth. However, the molecular basis of the cell morphogenesis control in plants remains poorly understood.

We identified a T-DNA tagged midget mutant that displays defective root hair differentiation and less branched trichomes. MAP4-GFP marker line revealed a disturbed microtubule organisation suggesting that the improper cell morphogenesis in the midget mutant may be mediated by impaired cytoskeleton organisation. GL2:GUS reporter expression is not changed in the midget mutant suggesting that MIDGET acts downstream or independently from the TTG-WER-GL3-EGL3 cell specification pathway. To learn more about the mechamisms of cell shape formation we plan to uncover the regulatory pathway that involves the MIDGET gene. As a first step we performed a mutagenesis screen for mutants that restore root hair formation in the midget mutants. From the 35 000 EMS mutagenised midget seeds 58 suppressor lines were isolated. Genetic and molecular analysis of the isolated midget suppressors will help to define regulatory steps in root-hair cell morphogenesis.

103 Subcellular localization and activity regulation of Arabidopsis type-II RACs

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Plant RACs are a subfamily in the Rho superfamily of small GTPases. Subcellular localization and function of Rhos depend on posttranslational lipid modifications and interaction with RhoGDIs. Most Rho proteins undergo prenylation that is required for their association with the membrane and interaction with RhoGDIs. Plant RACs have been divided into two subgroups designated type-I and II. Type-I RACs are prenylated whereas type-II RACs are palmitoylated but not prenylated. Unlike prenylation, no canonical sequence motifs are known for plamitoylation. Furthermore, palmitoylation has been suggested to inhibit the interaction between Rhos and RhoGDIs. Mutational analysis was used examine the association of type-II RACs with the plasma membrane and their interaction with RhoGDIs. Association of type-II RACs with the plasma membrane depends on a conserved bipartite sequence motif comprised of a non-polar helical domain and a proximal polybasic domain. Interaction between RACs and RhoGDIs were examined by yeast two-hybrid and in planta Bimolecular Fluorescence Complementation (BiFC) assays. A Type-I RAC interacted with RhoGDI in both yeast and plants. Interestingly, only the activated GTP-bound form of the RAC interacted with RhoGDI, in contrast to previous reports from yeast and animal systems. Interactions between type-II RACs and RhoGDIs were weak in either yeast two-hybrid or plant BiFC assays. Mutational analysis revealed that differential ability of type-I versus type-II RACs to interact with RhoGDIs results from their divergent C-termini. These results indicate that type-II RACs are not regulated by RhoGDI and acquired unique membrane targeting/association mechanisms.

104 Function and regulation of ARP2/3 complex during Arabidopsis epidermal development

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The specialized shape of cells is the means by which tissues and organs carry out unique functions. It is known that the cytoskeleton and vesicle trafficking play key roles in polarizing cell growth. But a molecular description of these important processes is incomplete. In non-plant cells the Actin-related protein (Arp) 2/3 complex is an essential seven-protein machine that generates branched actin filament networks. The Arabidopsis "distorted group" of trichome morphology mutants offer a powerful experimental entry into actin-dependent morphogenesis. All the "distorted group" mutants fail to properly organize the actin cytoskeleton. Their trichome branches swell in a stage-specific manner and are reduced in length, which is phenocopied by exposing wild-type cells to actin-disrupting drugs. At the whole plant level distorted mutants have a reduced fresh weight and hypocotyl length, defects in cell-cell adhesion in the shoot. Surprisingly, mutations that cause trichome distortion do not noticeably affect tip-growing cells such as pollen tubes and root hairs. "Distorted group" genes encode subunits of two different heteromeric complexes, WASP family verprolin homologous protein (WAVE) and ARP2/3, that directly affect one of many pathways that lead to actin filament nucleation. Mutations in *WURM (ARP2), DISTORTED1 (ARP3), CROOKED (ARPC5), and DISTORTED2 (ARPC2)* have been identified in different labs (Le et al. 2003; Mathur et al. 2003ab; Li et al. 2003; El-Assal et al. 2004; Saedler et al. 2004). Arp2/3 requires activation and is positively regulated by the WAVE complex. We are now testing for the presence of a seven-subunit ARP2/3 complex and are attempting to understand its function in cells. To attain these objectives we have generated a functional epitope-tagged version of an ARP2/3 subunit. We will present our biochemical data of the assembly status and localization of the putative ARP2/3 complex and ideas about how ARP2/3 might function in the plant cell.

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105 Identification of a CaM-Regulated Ca2+-ATPase (ACA11) That Is Located in Vacuole Membrane in Arabidopsis

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As one of active Ca^{2+} transporter, Ca^{2+}-ATPases attribute to the removal of Ca^{2+} from cytosol so that the cytosolic Ca^{2+} concentration can be maintained a low level. Here, we isolated a cDNA from *Arabidopsis*, designated *ACA11* (for autoinhibited Ca^{2+}-ATPase 11) that encodes a Ca^{2+}-ATPase. Only N-terminal deleted ACA11p (de-regulated) was able to complement not only a yeast vacuole Ca^{2+} pump mutant (K473) in high Ca^{2+} media but also a yeast triple mutant (K616) in Ca^{2+} depleted media. The *ACA11* transcript was detected in all tissue examined. The vacuolar membrane localization of ACA11p was determined by the localization of ACA11p tagged with green fluorescent protein in the protoplast and plant root tips by confocal fluorescence microscopy. *ACA11* KO mutant partially suppresses stomatal opening or closing in the light and ABA, respectively. Our results imply that ACA11p belongs to a vacuole localized Ca^{2+} pumps that is regulated by the N-terminal regulatory domain (calmodulin binding domain) and plays in a major role of cytosolic calcium oscillations in *Arabidopsis*.

This work was supported by MOST of Korea to the Environmental Biotechnology Research Center.

106 Generation of marker-free transgenic tobacco plants without the application of selection pressure

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The presence of marker genes in transgenic plants for the production of plant-made pharmaceutical (PMP) or other agricultural application is undesirable. Tobacco (*Nicotiana tobaccum* L.) and other *Nicotiana* species have shown great potential for PMP productions, and development of a marker-free transformation system for tobacco would be very beneficial for this application. We are interested in developing a marker-free tobacco transformation system by not applying selection pressure in the process. In our study, leaf explants were infected with an Agrobacterium carrying either a marker-less binary vector, or a binary vector with a marker gene. The infected explants were incubated on shoot-induction medium without selective compounds for shoot induction. The induced shoots were analyzed by PCR or *gusA* histochemical assay and those that were PCR and *gusA* positive were rooted and transferred to the greenhouse for T1 seed production. The T1 plants were assayed histochemically for *gusA* expression and inheritance, and by Southern hybridization to characterize the integration of transgenes. We have conducted six experiments, each involving three binary vectors and 50-100 leaf explants. T1 plants from 44 primary events have been assayed histochemically so far for *gusA* expression. T1 plants from 31 of the 44 events were found to be *gusA* positive. Of the 31 events, 26 transmitted the *gusA* activity to the T1 generation in a Mendelian 3:1 ratio. The T1 plants from more primary events are being assayed histochemically for *gusA* activity, and Southern analysis will be followed for those *gusA* positive events. The efficiencies of transformation and the percentage of escapes will also be determined.

107 Identification and characterization of nuclear localization and export signals of the rice NAP1 family proteins

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Nucleosome Assembly Protein 1 (NAP1) is a highly conserved histone chaperone which is involved in assembly and remodeling of the chromatin. Our previous studies showed that some tobacco NAP1 proteins were localized in the cytoplasm while the rice Orysa;NAP1;1 was localized in both the cytoplasm and nucleus. Here, we identified a leucine-rich sequence as a bona fide nuclear export signal (NES) and a lysine-rich sequence as nuclear localization signal (NLS), which are located at the N- and C-terminal regions of Orysa;NAP1;1, respectively. Mutation in the NLS resulted in exclusion of the Orysa;NAP1;1 protein from the nucleus whereas mutations in both the NLS and NES resulted in de novo nuclear accumulation of the protein. Orysa;NAP1;2 that lacks a C-terminal NLS was localized in the cytoplasm while mutation in the conserved N-terminal NES did not result in nuclear accumulation of the protein. Moreover, Orysa;NAP1;3 that does not contain any lysine-rich NLS sequences was localized in both the cytoplasm and nucleus. In contrast to the other examined tobacco and rice NAP1 proteins, Orysa;NAP1;3 can be phosphorylated in vitro by casein kinase 2α (CK2α). However, this phosphorylation was unlikely to be responsible for nuclear import of the Orysa;NAP1;3 protein because mutation of the phosphorylation site, which was identified to be located at the very C-terminal region of Orysa;NAP1;3, did not block nuclear accumulation of the protein. Together, our results provide important information about the intracellular localization and signals involved in nucleocytoplasmic shuttling of the rice NAP1 proteins.

108 Division Rules: polar development of microtubule arrays in the arabidopsis epidermis

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The orientation of the division plane is critical for plant form because cells are immobilized by cell walls. The orientation of new cell walls is also central for spacing stomata in the Arabidopsis epidermis. To analyze how microtubule arrays are established in different epidermal cell types we analyzed the establishment of the division plane and cytokinesis in plants harboring alpha-tubulin::GFP construct. We found that division-related microtubule arrays originate and develop with a distinct polarity that differs from the classical centrifugal model.

109 Genetic Approaches to Myosin Gene Function

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Myosin proteins function as molecular motors that drive the ATP-dependent movement of cellular components along actin filaments. Vascular plants encode two different types of myosin, referred to as class VIII and class XI. Class XI myosins show structural similarity to class V myosins from animals and fungi and are thought to be responsible for organelle movement during cytoplasmic streaming in plant cells. The *Arabidopsis* genome encodes 13 class XI myosin genes. Ten of the class XI myosin genes form five pairs of closely related sister sequences. Some of these sequence pairs appear to have arisen in recent genome duplication events, while others do not conform to the pattern of chromosomal duplications. The reasons for the relatively large number of myosin XI isoforms present within a single plant species are not known. We are addressing this question with a combination of genetic, molecular and cell biological approaches.

Promoter-reporter (GUS) constructs revealed specific expression for at least some of the genes. For example, *pXI-J::GUS* was expressed almost exclusively in anthers and pollen, whereas *pMYA1::GUS* and *pMYA2::GUS* had much broader expression patterns. We have isolated homozygous T-DNA insertions lines for most of the 13 genes but could not observe mutant phenotypes for any of the lines. In addition, in all cases tested, intracellular motility of organelles appeared normal. This may suggest functional redundancy of these myosin genes in Arabidopsis. However, RT-PCR analysis has revealed nearly normal mRNA levels for several homozygous insertion lines. In these cases, we have discovered a duplicate (inverted) insertion in the gene which may be responsible for the loss of the inserted sequences from the mRNA.

To overcome some of the problems associated with (recessive) T-DNA insertion mutants we are also generating (dominant) RNA silencing constructs. So far, we have isolated one silenced line for *MYA1*. RT-PCR analysis confirmed the specificity of the silencing effect for only this gene in the family. This plant also displayed no obvious mutant phenotype and had normal organelle movement. We are now in the process of generating double mutants (T-DNA and/or silencing) of closely related gene pairs to test for possible redundancy between these family members.

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110 Identifying target pathways of Sortin 1, a synthetic compound that affects biogenesis and protein targeting to the vacuole

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Chemical genomics is an approach in which diverse chemical libraries are screened for bioactive compounds and the affected pathways are identified using genomic approaches. Using an assay in yeast for the aberrant delivery of a vacuole marker protein, we identified low mass compounds known as Sortins (Sorting Inhibitors) that also result in the secretion of vacuolar proteins in Arabidopsis (Zouhar et al, 2004). Sortins affect vacuole morphology and root growth in Arabidopsis seedlings in a reversible manner. Such reversible phenotypes are a powerful advantage of chemical genomics that would be difficult to achieve using conventional mutational approaches particularly given that in plants vacuole biogenesis is an essential process. Having identified the Sortins as valuable probes of endomembrane pathways, we are now in the process of identifying the pathways and molecular targets of one of the compounds known as Sortin 1. Structure-activity studies indicate that specific substructures of Sortin 1 are responsible for bioactivity. These results are being combined with multiple approaches to identify the target pathways including screens for resistance and hypersensitivity in an EMS-mutagenized Arabidopsis population expressing the tonoplast marker delta-TIP fused to GFP. The marker permits the identification of mutants that are resistant or hypersensitive to Sortin 1 in terms of vacuole morphology as well as root growth. We are also taking advantage of cross-kingdom similarities by screening an indexed deletion yeast collection. These combined strategies will allow us to understand the molecular details of protein sorting to the vacuole and vacuolar biogenesis.

1. Zouhar J, Hicks GR, and Raikhel NV. Proc Natl Acad Sci U.S A. (2004): 101(25): 9497-9501.

111 Control of actin organization by the ITB3 protein in *Arabidopsis*

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We are using *Arabidopsis* trichomes as a model to probe the mechanisms underlying the cytoskeletal control of plant cell shape. We isolated a new class of trichome mutants, the *irregular trichome branch* (*itb*) mutants, that show changes in trichome branch position and branch length. Using indirect immunofluorescence and confocal microscopy, we found that actin organization was profoundly disrupted in developing trichomes of *itb3* mutants. Treatment of wild-type plants with the actin stabilizing agent, Jasplakinolide (Jas), had no effect on developing trichomes, but trichome development on Jas-treated *itb3* plants was blocked prior to trichome branching. This result suggests that the wild type ITB3 product competes with Jasplakinolide, and that contrary to earlier reports, proper actin organization is important for the earliest stages of trichome development. Positional cloning of the *ITB3* locus showed that it encodes a protein of unknown function that is highly conserved in all land plants, but is absent from algae, fungi and animals. Results on the role of ITB3 as a novel actin depolymerizing factor will be presented.

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112 Characterization of the Arabidopsis thaliana PEX11 gene family

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Peroxisomes are dynamic and important organelles within all plant species, playing an integral role in many essential cellular processes such as the glyoxylate cycle, H_2O_2 degradation, and β-oxidation of fatty acids. Although much is known about the biochemical reactions facilitated by peroxisomes, many other aspects of peroxisome function and biogenesis remain largely enigmatic. A key area of peroxisome research is peroxisome biogenesis, which is mediated by the peroxin *(PEX)* genes. However, the mechanism underlying peroxisome proliferation is still largely elusive. To understand how peroxisome proliferation is controlled in plants, the present study focuses on the five-member *PEX11* gene family in Arabidopsis thaliana. Fluorescent microscopy using CFP-fusion proteins within an YFP-PTS1 background plant determined that each PEX11 localized to peroxisomes. Interestingly, members of the *AtPEX11* gene family play discrete roles in peroxisome proliferation. Over-expression of the *AtPEX11* family members resulted in distinct changes in peroxisome morphology. An *AtPEX11a* T-DNA insertion mutant is embryonic lethal, suggesting that this gene is essential and also plays an independent role in peroxisome proliferation. Transcript levels of each of the genes were observed using RT-PCR analysis and promoter-GUS fusions in different tissues and also during different environmental stimuli and stresses. Some of the genes responded to a light treatment and all the *PEX11s* were upregulated during senescence. To determine the effect of reduced *PEX11* transcript levels, plants were transformed with RNAi constructs and the transgenic plants are being characterized. The data from these studies suggest that Arabidopsis *PEX11* genes play non-redundant and perhaps contrasting roles in peroxisome division and proliferation.

113 Cell Wall Integrity is Essential for Normal Cortical Microtubule Organization

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It has long been implied that there is a connection between the cell wall and the microtubule cytoskeleton. For example, disorganization of cortical microtubules with microtubule destabilizing drugs causes isometric cellular swelling. Additionally, in some types of expanding cells, microtubules and newly deposited cellulose microfibrils exhibit similar orientation.

In order to identify components regulating cortical microtubule dynamics and cellulose deposition, we isolated Arabidopsis mutants that are hypersensitive to the microtubule-destabilizing drug oryzalin. We are focusing on two mutants with clearly aberrant cortical MT organization. The first mutation, which causes sensitivity to both oryzalin and isoxaben (a cellulose biosynthesis inhibitor), is in Cellulose Synthase 6 (PRC1). The second mutation, which causes both oryzalin and cold sensitivity, is in the KOR gene encoding a β 1-4 glucanase. The double mutant prc1/kor has a severe growth defect in the root and apparently lacks cortical MTs in week old seedlings. The properties of these mutants suggest the existence of a feedback from the cell wall to the cytoskeleton. We are in the process of measuring MT dynamics in the cesa6/prc1 background.

To probe further the relationship between cellulose synthase and the cytoskeleton in living cells, we generated a CESA6-YFP fusion that complements prc1. CESA6-YFP localizes to the cell cortex in distinct puncta that define linear transverse arrays and move at steady velocities. We are in the process of characterizing this marker by measuring particle dynamics, assaying reactions to various drugs, photobleaching analysis and performing dynamic studies of the relationship between this marker and the cortical MT cytoskeleton.

114 Functional analysis of the plant exocyst complex

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The exocyst (Sec6/8 complex) is eight subunits protein complex conserved among eukaryotes. Characterised in yeast and mammals, it was shown to participate in the spatial regulation of exocytosis. Acting as a tether, the exocyst physically links secretory vesicles with localised plasma membrane subdomains before fusion. Homologues of each exocyst subunit have been identified in the Arabidopsis thaliana genome (Elias et al. 2003). In addition, Segui-Simarro and collaborators (2004) recently described a putative exocyst-like complex involved in vesicles tethering in Arabidopsis.

In this study, we describe Arabidopsis thaliana RNAi lines in which we were able to suppress the expression of Sec3 and Sec5 exocyst subunits. A mutant phenotype was observed in early plant development under stress conditions. Western blots on protein extracts from Sec3 RNAi lines, using polyclonal antibodies raised against Sec3, Sec5 and Sec6, revealed a decrease in the amount of protein of each investigated subunit, suggesting a role for Sec3 in the stability of the complex. Using immunofluorescence, we also observed that Sec3 and Sec6 epitopes co-localise at the growing tip of tobacco pollen tubes. In addition, we used Sec5 antisense oligonucleotides to analyse the exocyst function in tobacco pollen development.

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Elias et al. 2003 Cell Biol Int 27:199

Segui-Simarro et al. 2004 Plant Cell 16: 836

115 The nuclear-encoded ClpR2 and ClpR4 subunits of the plastid Clp protease complex are essential for development of A. thaliana

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Proteolysis in plastids is important for protein processing, protein homeostasis, and regulation of plastid gene expression. Plastids in roots, petals and leaves of *Brassicacea* contain tetradecameric Clp protease core complexes, consisting of five different serine type protease Clp proteins (ClpP1, 3-6), four ClpR proteins lacking the conserved catalytic residues (ClpR1-4) and two proteins of unknown function (ClpS1-2). Using biochemical techniques, we have demonstrated that this Clp complex -isolated from chloroplasts and non-green plastids from roots and petals- migrates as a single 350 kDa complex. In contrast, mitochondria contain a homo-tetradecameric ClpP2 complex (Peltier *et al*. (2004) JBC 279, 4768-4781).

Here we report on the characterization of T-DNA insertion mutants in ClpR2 and ClpR4. A T-DNA insertion in the 5'UTR of ClpR2 leads to delayed shoot development, a yellow/pale green phenotype, reduced Photosystem II efficiency and thylakoid formation, and accumulation of carotenoids and plastoglobules. T-DNA insertion in the second intron of ClpR4 results in (nearly) complete loss of ClpR4 gene expression. The homozygous plants are a seedling-lethal with reduced embryo development. However, they can be partially rescued on media with sucrose when grown under low light fluencies. TEM analysis shows that chloroplast biogenesis is strongly disrupted. Surprisingly, both *clpr2* and *clpr4* mutations have no apparent effect on roots, despite expression of both genes in wild-type plant roots. Both mutants were complemented with their respective genes, confirming the correlation between phenotype and T-DNA disruption of expression for these ClpR non-proteolytic subunits.

We conclude that both ClpR2 and R4 are essential members of the ClpPRS complex and that they are needed for plant development.

116 The plant UBX-domain containing (PUX) protein family regulates the function of Arabidopsis CDC48, a conserved essential AAA-ATPase

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CDC48/p97/VCP is a conserved and essential hexameric AAA-ATPase that functions as a molecular chaperone in numerous diverse cellular activities. CDC48/p97/VCP activity is recruited to specific functions through its interaction with adapter proteins. We have taken a biochemical approach of affinity chromatography and MALDI-TOF mass spectrometry to identify protein regulators of AtCDC48 function during plant cytokinesis. Two previously uncharacterized proteins, PUX1 and PUX2, have been identified. There are 15 members of the PUX (Plant UBX-domain containing) protein family in Arabidopsis. Recent work in non-plant systems has suggested a role for the UBX-domain, an ubiquitin-like protein fold, as a specific interaction domain for CDC48/p97/VCP. Our work in Arabidopsis demonstrates that all tested PUX proteins (1-4 and 11) physically interact with AtCDC48 and therefore support a hypothesis that the PUX protein family is a class of AtCDC48 regulators.

Analysis of PUX1 shows that it is a soluble protein that co-fractionates with non-hexameric AtCDC48 from plant cell extracts and inactivates AtCDC48 *in vitro* through hexamer disassembly. Two null insertion mutant alleles of PUX1 have been identified and show no apparent morphological abnormalities, however, data indicates that these plants have an accelerated growth rate. We propose that PUX1 elicits it negative effect on growth by promoting the inactivation of AtCDC48 function *in vivo*.

Analysis of PUX2 demonstrates that it is a peripheral membrane protein that interacts with AtCDC48 *in vitro* and co-fractionates with membrane-associated but not soluble AtCDC48 *in vivo*. Deletion analysis of PUX2 protein domains reveals that its PUG domain, a protein domain found in protein kinases, N-glycanases and other proteins with nuclear localization, is required for interaction with AtCDC48. Biochemical reconstitution and immunolocalization data suggest that PUX2 facilitates the interaction of SYP31 and AtCDC48, thereby regulating an AtCDC48 membrane-associated function. Analysis of PUX2 loss of function plants will be presented.

117 Interfering with *MOR1* expression causes microtubule disruption and abnormal morphological development

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Microtubule-associated proteins (MAPs) are important modulators of microtubule assembly properties. Mutations in MOR1, the *Arabidopsis thaliana* homologue of the pan-eukaryotic Dis1/TOG family of MAPs, cause temperature-sensitive cortical microtubule disorganisation and associated loss of growth anisotropy [Whittington *et al.* (2001) Nature 411:610]. Analysis of the MOR1 amino acid sequence suggests that the microtubule-binding domains defined in other MAPs are not present in the Dis1/TOG family. However, comparison of MOR1 and other Dis1/TOG family proteins defined conserved domains along the length of MOR1. These conserved regions of MOR1 were tagged with the yellow fluorescent protein (YFP) to functionally dissect the properties of the protein *in vivo*. Whether expressed transiently in leek epidermal cells, or stably in arabidopsis, the YFP-MOR1 fusion proteins remained cytoplasmic and did not bind microtubules. These results indicate that the entire length of MOR1 may be needed for binding to microtubules. Stable integration of YFP-MOR1 fusion constructs in some arabidopsis seedlings triggered the development of *mor1* mutant-like phenotypes such as left-handed twisting of organs, distorted trichomes and isotropic cell expansion. These morphological defects were accompanied by cortical microtubule disruption. Unlike the reversible phenotype of temperature-sensitive *mor1* mutants, the affected transgenic seedlings progressed to gross morphological abnormalities and premature death. This increased phenotypic severity indicates that mutant mor1-1^{L174F} protein retains considerable function at restrictive temperature. These abnormal phenotypes are likely due to co-suppression of the endogenous *MOR1* gene triggered by expression of the *YFP-MOR1* transgenes.

118 Identification and Functional Analysis of Two Small Heat-Shock Proteins from Plant Peroxisomes

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Our knowledge on regulatory proteins from plant peroxisomes including heat-shock proteins (Hsps), protein kinases and phosphatases, proteases and glutathione transferases is rather limited, and it is currently not known how the activity and turnover of matrix enzymes is regulated. Our interest focuses on small Hsps and protein kinases. Small Hsps are a ubiquitous superfamily of Hsps characterized by the relatively small mass of the polypeptide chain and the presence of a conserved C-terminal alpha-cystallin domain. Small Hsps are active in several cell compartments but have not been localized to peroxisomes in any organism. We identified two small Hsps with putative peroxisomal targeting signals type 1 or 2 (PTS1 or PTS2) in the Arabidopsis genome. Subcellular targeting analysis using fusion proteins with yellow and cyan fluorescent protein as well as biochemical subfractionation and proteome studies demonstrated that both sHsps, namely AtHsp15.7 (At5g37670) and AtAcd31.2 (At1g06460) are localized in the plant peroxisomal matrix. AtAcd31.2 is unique in possessing both a functional PTS1 (PKL>) and a functional PTS2 (RLx5HF). Yeast complementation studies indicate that the function of AtHsp15.7 is largely conserved. Expression studies suggest a general and a stress-inducible function of AtAcd31.2 and AtHsp15.7, respectively. Double k.o. mutants deficient in both sHsps are currently being characterized phenotypically under various stress conditions to figure out if and how the peroxisomal sHsps are involved in stress tolerance, protein denaturation and/or membrane protection.

119 The RHL1-RHL2-RHL3 protein complex is required for plant endoreduplication

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Endoreduplication is a common process in eukaryotes that involves the amplification of chromosomal DNA without intervening mitoses. The resulting larger, higher-ploidy nucleus is often associated with an increase in cell size. In a search for mutants deficient in endoreduplication, we found sets of dwarf mutants in *Arabidopsis*: *root hairless 1, 2, 3* (*rhl1, rhl2, rhl3*) and *hypocotyl 6, 7* (*hyp6, hyp7*). Unlike wild-type seedlings, in which some cells can undergo four rounds of endoreduplication and reach 32C, rhl and hyp mutants' cells can reach a ploidy of only 8C. All of these mutants are epistatic to various over-endoreduplicated trichome mutants such as *try, kak* and *rfi*, suggesting that their gene products are essential for the successful progression of endocycles in *Arabidopsis*. By positional cloning, we found that RHL1/HYP7 encodes a plant specific, nuclear protein whereas RHL2 and RHL3/HYP6 encode plant homologs of the archaeal DNA topoisomerase VI (topo VI) subunits A and B, respectively. Further genetic, in vivo and in vitro studies suggested that RHL1 is a novel DNA binding protein and is an essential component of the plant topo VI complex. Topo VI complexes are type II DNA topoisomerases that are capable of DNA decatenation, the ATP-dependent separation of two entwined DNA molecules, and relaxation of supercoiled DNA. Our study suggests that a similar activity of the topo VI complex is required to ensure efficient DNA replication during endoreduplication in *Arabidopsis*. We are currently investigating how topo VI functions during endoreduplication and how this process is controlled.

120 The role of SNAREs and vesicle trafficking in plant cytokinesis and cell-plate formation

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Cell division is a fundamental process for all organisms. It is a required step for almost all aspects of growth and development. Plants perform the final step of division (cytokinesis) in a process mediated by a novel organelle, the cell plate, that is newly synthesized at each division. An apparently identical cytokinetic process is found in all land plants and many types of algae, and in cases where particular proteins are known to be involved, these proteins appear to be conserved in all plants examined thus far. Though morphological analysis indicates that vesicle trafficking and Golgi-derived vesicles are important for cell plate formation, very little mechanistic knowledge of how this process works is known. There is also some evidence of roles for other organelles in cell plate formation, though again, little is known for certain. One aspect of vesicle trafficking known for all eukaryotes is the role of SNARE proteins in the selectivity and fusion of vesicles with target organelles. SNAREs form heterotetrameric complexes that are derived from a v-SNARE on a donor organelle and 3 t-SNAREs on the target, and thus can identify the vesicle trafficking pathway they mediate by their intracellular localization. Because SNAREs represent a reasonably well-understood system, they provide a valuable target for understanding not only cell plate formation, but also in implicating other organelles in this essential process. My lab has identified two new classes of SNARE proteins that localize to the cell plate in dividing cells (the NPSN- and SYP7-gene families, each having 3 members). Aside from the cell plate, in both dividing and non-dividing cells, these SNAREs are also found on organelles such as the plasma membrane, the Golgi and early-endosome-like structures. Through immunoprecipitation, we have shown that the members of these groups represent a novel SNARE complex on the cell plate of dividing cells, as well as on other organelles in interphase cells. This distribution across several organelles aside from the cell plate may offer insight into the roles of these other organelles in cytokinesis. Analysis of mutants in these individual SNAREs indicate substantial redundancy in the NPSN-gene family, but surprising independence of functions in the SYP7-gene family, where individual members appear to have essential roles not only during cell division, but also in the pollen.

In total, these SNAREs provide tools that can be used to characterize the role of endosomes in cell plate formation, as well as to identify specific cargo that may follow the pathway mediated by this new SNARE complex.

121 Genetic Analysis of SECY1 and SECY2 Function

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Bacteria have one form of SecY, which along with SecE and SecG, forms the SecYEG translocon in the bacterial plasma membrane. The SecYEG translocon is responsible for export of proteins out of the bacterial cell. *Arabidopsis thaliana* contains two divergent forms of SecY, which we have designated SECY1 and SECY2. In previous studies, SECY1 was shown to be targeted to the thylakoid membranes in the chloroplast. SECY2 sequence includes a putative transit peptide so presumably this protein is also targeted to plastids, although that has not been experimentally demonstrated. RT-PCR analysis revealed that *SECY1* and *SECY2* have similar expression patterns. Both genes are expressed in dark and light grown shoots and light grown roots and shoots. Although SECY1 and SECY2 proteins appear to be in the same organelles and same organs, genetic analyses indicate that they play different roles in plant development. Mutations in *SECY1* cause a seedling lethal phenotype, while mutations in *SECY2* result in embryo lethality. To investigate whether this is due to expression differences, we performed promoter swap experiments. The *SECY1* promoter driving *SECY1* genomic sequence could rescue *secY1* mutants. Likewise, the *SECY2* promoter driving *SECY2* genomic sequence could rescue *secY2* mutants. The *SECY2* promoter driving *SECY1* sequence could not rescue *secY2* mutants, although the same construct could rescue *secY1* mutants. We conclude that differences in function are more likely to form the basis of the different mutant phenotypes than expression differences. As a first step towards determining function, we are using an epitope-tagging approach to localize SECY2 in cellular membranes. We have shown that SECY2-T7 fusions can rescue *secY2* mutants; therefore we now have the appropriate material to proceed with cell fractionation experiments. Supported by UW-Madison Graduate School.

122 Chemical genomics of the endomembrane system in Arabidopsis

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Characterization of the plant endomembrane system is challenging because mutations in many of the genes result in lethality phenotypes regardless of family size. We used a chemical genomics approach to identify compounds that disturb the endomembrane system by analyzing the gravitropic response in our primary screen (Surpin et al., PNAS, 102: 4902). One of the compounds identified in this screen, chemical ID 5850247, inhibits the gravitropic response and blocks the trafficking of the GFP:-TIP marker to the tonoplast. Thus, GFP is localized to the Endoplasmic Reticulum in GFP:-TIP seedlings that have been grown in the presence of this chemical. We have determined that this compound blocks the auxin response as indicated by a DR5::GUS assay, which explains the strong effect that this chemical has on the gravitropic response. However, chemical 5850247 is unable to block the induction of the cold/ABA-responsive RD29A promoter by cold treatment, indicating that chemical 5850247 is not a ubiquitous inhibitor of gene expression. Several approaches are being followed to understand the activity of this chemical. First, the effect of this compound on targeting marker proteins is being examined and preliminary experiments have shown that the compound may affect the targeting of membrane proteins but not soluble vacuolar proteins. Second, resistant and hypersensitive mutant screens have been initiated in Arabidopsis and yeast with the goal of identifying the signaling pathway that is targeted by this chemical. Characterization of selected mutants that have been identified in these screens will be presented.

123 Characterization of SIAMESE, a putative cell cycle regulator involved in endoreplication

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Recessive mutations in the *SIAMESE (SIM)* gene of *Arabidopsis* results in the production of multicellular trichomes, individual nuclei of which have reduced levels of endoreplication. This phenotype is strikingly different than wild-type trichomes, which are single cells with a nuclear DNA content of approximately 20-32C. These observations suggest that *SIM* is required to suppress mitosis as part of the switch to endoreplication in trichomes. We show that *SIM* encodes a 14kD protein that is a member of a small *Arabidopsis* gene family of four members. Homologs exist in other dicots and in monocots. These proteins share a motif with the KRP cell cycle inhibitor proteins, and have another potential cyclin binding motif as well. An eGFP::SIM fusion is localized to the nucleus. *In situ* hybridization and an enhancer trap reporter demonstrate that SIM is expressed throughout the shoot apical meristem, in leaf primordia, and in the elongation zone of the root. Overexpression of SIM from the 35S promoter results in slow-growing plants with narrow leaves and enlarged epidermal cells that have an increased DNA content. Our current hypothesis is that SIM functions as a CDK inhibitor, repressing the G2-to-M transition when active. Current experiments are aimed at examining SIM's interactions with cyclins and CDKs to elucidate the function of SIM in the cell cycle.

124 Analysis of monoubiquitin-dependent protein degradation in *Arabidopsis*

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In recent years monoubiquitin-dependent protein degradation has emerged as a new pathway to downregulate or process various proteins. This pathway utilises the cell's endomembrane system to sort monoubiquitinated targets into the lumen of the yeast vacuole or the animal lysosome respectively where they are accessible to proteases. An important step is the recognition of the ubiquitinated targets and their sorting into internal vesicles of the multivesicular body (MVB). This process is mediated by the ESCRT I-III complexes in yeast and animals. The key component of ESCRT-I is Vps23/TSG101 whose UEV domain recognises monoubiquitin. Here we show that the ortholog of *Vps23/TSG101*, the Arabidopsis ELCH protein is able to bind to monoubiquitin and is part of a high molecular weight complex. *ELCH* interacts genetically with the bona fide ubiquitin ligase *KAKTUS*. Our genetic and molecular work of the *ELCH* gene suggests that vacuolar protein degradation exists in plants.

Katzmann, 2001; Babst, 2002; Babst, 2002;
Swanson, 1998; Bassham, 2000; Xie, 1998; Bowers, 2004

125 Determining which glutamate receptors mediate depolarization responses to glutamate and glycine in *Arabidopsis* root and hypocotyl cells

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The *AtGLR* genes in Arabidopsis are homologous to glutamate receptor channels that mediate rapid cell-to-cell communication in the central nervous systems of animals. Our studies show that application of glutamate or glycine to wild-type Arabidopsis root and hypocotyl cells induces a large, transient depolarization of the plasma membrane and a coincident increase in cytoplasmic calcium. To learn which if any of the 20 *AtGLR* genes is responsible for this ligand-gated electrophysiological phenomenon, T-DNA insertion mutations in 18 *AtGLR* family members were isolated and their electrical responses to ligand were measured. Most mutants give responses indistinguishable from wild type but two separate alleles of *atglr3.3* display severely reduced depolarizations in response to glycine or glutamate in both the hypocotyl and root. The small depolarization that remains in the *atglr3.3* seedlings is sustained, pH-dependent and not specific to any amino acids tested so far. These characteristics are consistent with the hypothesis that the depolarization in *atglr3.3* is due to amino acid/proton symport. Mutations in the *AtGLR3.4* gene specifically reduce the hypocotyl response to glycine. Together, these results indicate that GLR members have different ligand-binding specificities. Additional evidence of this differential sensitivity was obtained by testing which ligands caused desensitization. Treatment of hypocotyl cells with glutamate greatly diminished the cell's ability to respond to subsequent treatment with either glutamate or glycine. Glycine similarly desensitized the hypocotyl cell to a subsequent glycine treatment. However, glutamate after glycine was fully effective. One interpretation is that all GLR channels can be activated by glutamate (resulting in desensitization) but glycine activates (and therefore desensitizes) only a subset.

126 The Power Of Chemical Genomics To Study the Link Between Endomembrane System Components and the Gravitropic Response: Target Identification Studies With 2-Anilinophenazine

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Chemical genomics is a powerful approach to dissect processes that may be intractable using conventional genetics due to gene lethality or redundancy. Recently, a link has been established between endomembrane trafficking and gravitropism. To understand this link, we screened a library of 10,000 diverse chemicals for compounds that affected the gravitropism of Arabidopsis thaliana seedlings positively or negatively. Four compounds were found to cause aberrant endomembrane morphologies. One of the chemicals (5403629) was structurally similar to the synthetic auxin 2,4-D, whereas the other three chemicals had unique structures. An in vivo functional assay using the reporter B-glucuronidase under the auxin-inducible DR5 promoter confirmed that the unique compounds were not auxins. Interestingly, one of the unique chemicals (5850247) appeared to decrease responsiveness to auxin, whereas another (5271050, or 2-anilinophenazine) was similar to pyocyanin, a bacterial metabolite that has been suggested to target the endomembranes of yeast (Surpin et al., 2005, PNAS 102, 4902-7). We are currently engaged in target identification studies for compound 5271050. Our multi-pronged approach is to screen a yeast deletion library for strains that are hypersensitive to 2-anilinophenazine and then examine orthologous Arabidopsis T-DNA lines for hypersensitivity. We will also initiate genetic screens for EMS mutants that are hypersensitive and resistant to 2-anilinophenazine. Our ultimate goal is to identify the molecular target(s) of 2-anilinophenazine and understand how vesicle trafficking affects gravitropic signal transduction.

127 Autophagic Nutrient Recycling in Arabdiopsis thaliana Directed by the ATG8 and ATG12 Conjugation Pathways

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Autophagy is an important mechanism for non-selective intracellular breakdown whereby cytosol and organelles are encapsulated in vesicles, which are then engulfed and digested by lytic vacuoles/lysosomes. In yeast, this encapsulation employs a set of autophagy (ATG) proteins that direct the conjugation of two ubiquitin-like protein tags ATG8 and ATG12 to phosphatidylethanolamine and the ATG5 protein, respectively. Although important during various stages of growth and development, the molecular mechanisms underpinning autophagy in plants are just beginning to be uncovered. By reverse genetic analyses of the single *Arabidopsis* genes encoding ATG7 - the enzyme responsible for ATG8/12 activation, and ATG5 - the target of ATG12 conjugation, we show here that the ATG8/12 conjugation system is important for survival under nitrogen (N) and carbon (C) limiting growth conditions. The *atg7* and *atg5* mutants are hypersensitive to low N and C starvation, which is accompanied by a more rapid loss of organellar and cytoplasmic proteins. However, multiple isoforms of ATG8 were elevated in the mutants and retained during this period of protein turnover, suggesting that ATG8 is consumed during autophagy. Using a GFP-ATG8a fusion in combination with concanamycin A, an inhibitor of the lytic function in the vacuole, we detected the accumulation of autophagic bodies inside the vacuole. This accumulation was enhanced by starvation but blocked in the *atg7* background. The use of this fusion in conjunction with *atg* mutants may provide an important marker to track autophagic vesicles *in planta* during nutrient remobilization.

128 *PARTING DANCERS*, a gene, requires for the homologous recombination during meiotic prophase I in *Arabidopsis thaliana*

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Homologous chromosome interactions during meiotic prophase I, namely pairing, synapsis and recombination, are important for proper chromosome segregation into four gametes. In yeast, during meiotic recombination the crossovers arise in two pathways, interference sensitive and interference insensitive, and non-crossover events take place independent of the crossover events. Several genes, which are required for formation of crossover pathway intermediates, have been identified. However, only few genes are known for the resolution of double Holliday junctions. In recent years, *Arabidopsis* has proven to be very useful in identifying components of the machinery governing plant meiosis as well as meiotic process in general. Here we describe the isolation of *parting dancers*, a mutant in *Arabidopsis* with clear male meiotic defects leading to reduced male fertility. The male meiocytes showed normal early prophase I stages. However, at diakinesis stage, the mutant formed randomly distributed, reduced number of crossovers which resulted in univalent chromosomes instead of five bivalents. The defects in bivalent formations led to abnormal chromosome distributions in aberrant tetrads during later stages of the meiosis. Transmission electron microscopic analysis revealed that the mutant had normal level of synaptonemal complexes and late recombination nodules at pachytene stage suggesting that the mutant was defective in bivalent formation in a post-synaptic manner. The results, in connection with the current model of homologous recombination in meiosis, suggest that this gene may be important for promoting crossovers in interference sensitive pathway possibly by regulating the formation or resolution of double Holliday junction intermediates.

129 Expression and function analyses of the AtPLDzeta2 gene

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Phospholipase D (PLD) is the enzyme that hydrolyzes phosphatidylcholine to generate phosphatidic acid and choline. The Arabidopsis genome encodes twelve PLDs, which are classified into ten plant-specific PLDs and two eukaryote-general PLDs by their domain structures. The former PLDs, which contain a C2 domain, are known to play a role in signal transduction for responses to environmental stresses. As for the latter PLDs, which contain PX and PH domains, AtPLDzeta1 has been proposed to play a role in root-hair cell development. In this study, we investigated biological function of AtPLDzeta2, the other eukaryote-general PLD of Arabidopsis. We examined the expression pattern of its gene histochemically using a GUS reporter system.

The 1.5-Kbp fragment from the initiation codon of the AtPLDzeta2 gene showed promoter activity mainly in pollens, whereas an upstream fragment of the AtPLDzeta1 gene had strong promoter activity in various tissues but not in pollens. This result suggests that AtPLDzeta2 functions in pollen-tube development as AtPLDzeta1 does in root-hair development. We obtained two independent T-DNA insertion lines of the AtPLDzeta2 gene. Results of mutant analysis will also be reported.

130 Analysis of Exo70 exocyst subunits family function in plants

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Conserved protein complex called the exocyst has been originally discovered in yeast. It is shown to participate in tethering of secretory vesicles to the specific plasma membrane domains. Plant genomes sequence analysis revealed the presence of homologues to all eight exocyst subunits (Elias et al. 2003). In most organisms Exo70 subunit is encoded by only one gene. However in Angiosperms, we have identified whole family of genes/proteins homologous to the Exo70 subunit. The Arabidopsis EXO70 family contains 23 paralogues, in rice at least 30 family members are present. Physcomitrella patens ESTs revealed at least five EXO70 genes, suggesting expansion of the EXO70 family very early in the land plant evolution. Phylogenetic comparative analysis shows, that plant EXO70 genes can be classified into 9 major groups. We analysed Arabidopis T-DNA insertional mutants in several EXO70 genes and this analysis is far from complete. We found surprisingly one homozygous mutant with a phenotype in spite of extensive expression overlap found by the analysis of public expression data based on the Affymetrix DNA chip.

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Elias et al. 2003 Cell Biol Int 27:199

131 Composition and Function of the Arabidopsis WAVE Complex During Epidermal Morphogenesis

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The actin cytoskeleton is an essential regulator of growth and development in eukaryotic cells. In plant cells, actin filaments organize the cytoplasm by positioning organelles and providing tracks for transport. Spatial and temporal control of actin filament nucleation and turnover is likely to control cell expansion, but our understanding of this complicated process is poor. The "distorted group" of trichome morphology mutants provides powerful genetic tools to better understand pathways that control actin filament nucleation and morphogenesis in a variety of cell types. Distorted mutants fail to organize a population of loosely aligned cytoplasmic actin bundles during trichome branch elongation. At the molecular level, trichome distortion is caused by mutations in either WAVE or Arp2/3 complex subunit genes. Arp2/3 is a highly conserved seven-protein complex that generates branched actin filament networks. In animals Arp2/3 is essential. Surprisingly, in Arabidopsis, Arp2/3 does not appear to be essential, and trichomes are the only epidermal cell type that is dramatically affected by Arp2/3 loss of function. Cell-cell adhesion, pavement cell shape, and fresh weight are also altered in a variety of shoot organs. Purified Arp2/3 is inactive and requires the presence of activating proteins for actin filament nucleation activity. The heteromeric WAVE complex is one such activator. WAVE complex was originally isolated from vertebrate cells, and is composed of the subunits NAP1, Sra-1/PIR121, ABI2, HSPC300, and Scar/WAVE. The WAVE complex is thought to regulate the localization and/or stability of the Scar/WAVE subunit. Scar/WAVE is the subunit that directly interacts with and activates Arp2/3. In addition to causing similar trichome distortion when mutated, the Arabidopsis WAVE subunit genes *GRL (NAP1)*, *PIROGI/KLUNKER (SRA-1)*, and *DIS3/SCAR2* encode proteins that employ evolutionarily conserved domains and binary interactions in the pathway to WAVE complex assembly. However, the composition and regulation of the WAVE complex in vivo is not known in plant cell. For example, there are 4 ABI1-like genes and 5 SCAR genes in Arabidopsis and we still need to figure out which of these proteins function in the context of a WAVE complex. We will present results from our recent genetic and biochemical experiments that are aimed at better understanding the composition and regulation of the WAVE complex in vivo.

Reference: Szymanski D.B. (2005) Current Opinion in Plant Biology 8:103-112

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132 The *Arabidopsis ROCK-N-ROLLERS* gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation

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Recent study of meiosis has suggested that the molecular mechanism of the homologous chromosome recombination may be highly conserved among eukaryotic organisms. In budding yeast *Saccharomyces cerevisiae*, the cytological structure where meiotic recombination happen, so called crossover, can be formed by two genetic pathways: one *MSH4*, *MSH5* and *MER3*-dependent, interference-sensitive pathway and one *MUS81*, *MMS4*-dependent, interference-insensitive pathway. The former pathway occupies the majority of the overall crossover formation and makes the crossover distributed evenly along the chromosomes. The later pathway, on the contrary, occupies a minor partition and makes the crossover randomly distributed. In mouse and *Arabidopsis* the homology of *MSH4* have been isolated and it has been proven *MSH4* is indispensable for normal level of occurrence of crossover. However, it is unknown if other crucial factors of cossover formation, such as *MER3*, also are important to meiosis in animal and plant. Here, we report that we have isolated an *Arabidopsis MER3* homolog gene. The high similarity of amino acid sequence and same conserved domain organization of the encoded protein, compared with MER3 of budding yeast, strongly suggest it is the MER3 homolog of *Arabidopsis*. Tissue RT-PCR and RNA in situ experiment show this gene is preferentially expressed in the male and female meiocytes, implying its function in meiosis. We have investigated three allelic T-DNA insertional mutants of this gene and found all of them have the same phenotype in fertility and meiosis, though in different levels. Detailed cytological study has shown that the male meiocytes of these mutants are defective in the formation of homologous bivalent. This gene is named as *ROCK-N-ROLLERS (RCK)* to reflect the mutant phenotype of chromosomes going through the meiotic "dance" as either pairs or individual. Furthermore, the number of crossover formation, or chiasmata, is greatly reduced and the residual chiasmata are distributed randomly, as in an interference-insensitive pattern in the mutant meiocytes. So the gene *RCK* is indispensable for the interference-sensitive crossover formation. What we have found about the function of *RCK* in *Arabidopsis* parallels the function of *MER3* in the budding yeast, and provides further support to the concept that some partitions of the meiotic mechanism are very conserved during evolution.

133 A Novel Genetic Screen to Identify Chloroplast Import Mutants

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To broaden our understanding of protein translocation into the chloroplast, we have developed a novel transgene-based genetic screen in *Arabidopsis thaliana*. Our screen depends on a key enzyme in the shikimate pathway, 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, the physiological target of the herbicide glyphosate ("Roundup"). This pathway occurs in the chloroplast, yielding aromatic amino acids and many secondary metabolites. Because EPSP synthase is synthesized as a precursor (TP-EPSP) in the cytosol, it must be imported into the chloroplast to perform its function. Glyphosate also enters the chloroplast and inhibits the interaction of EPSP synthase with one of its substrates. A mutant form of EPSP synthase (EPSP*) is more resistant to glyphosate. Since EPSP* must enter the chloroplast to confer herbicide tolerance, we predicted that if its precursor, TP-EPSP*, remains in the cytosol because of a mutation in the import pathway, plants would be converted from an herbicide tolerant to a sensitive phenotype. We confirmed this prediction in studies with transgenic lines expressing TP-EPSP* or EPSP*, without a transit peptide, as a control. Thus, the loss of herbicide tolerance has been used in our screen as an initial indicator of a defect in the import pathway, allowing the rapid analysis of mutagenized plants expressing TP-EPSP*. Plants or leaf disks were assayed to assess if TP-EPSP* was imported and conferred sufficient levels of glyphosate tolerance when compared to the control. The fate of GFP fused to a ferredoxin transit peptide (FD5-GFP) was monitored in the same transgenic plants to substantiate that an import mutation had occurred. This double-reporter screen has identified in the M2 generation (30,000 EMS mutagenized plants expressing TP-EPSP* and FD5-GFP) five import mutants where both reporters are mislocalized. The import mutants fall into two classes: either GFP is in the nucleus or the cytosol. Backcrosses with wildtype show that the mutations are recessive and not intragenic. Backcrosses with the parental line are underway to pursue phenotypic analyses of the mutants, and initiate mapping experiments to identify the genes responsible for the import defects. This forward genetic strategy offers a new approach to explore how the cell regulates the selective import of proteins required for chloroplast biogenesis.

134 Disparate Roles for the Regulatory A Subunits in Arabidopsis PP2A

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Protein phosphatase 2A (PP2A) is a key regulator of cellular transactions dependent upon reversible protein phosphorylation. The heterotrimeric PP2A complex comprises a catalytic subunit and regulatory A and B subunits that modulate enzyme activity and mediate interactions with other proteins. The *RCN1* (*ROOTS CURL IN NAPHTHYLPHTHALAMIC ACID*), *PP2AA2* and *PP2AA3* genes constitute the Arabidopsis regulatory A subunit gene family, and our earlier work has shown that the RCN1 protein plays a cardinal role in regulation of phosphatase activity. PP2AA2 and PP2AA3 functions are 'unmasked' when RCN1 is absent. Double mutant phenotypes reveal specific requirements for proper phosphatase regulation during vegetative and reproductive development. Plants lacking functional PP2AA2 and PP2AA3 and heterozygous for an *rcn1* mutation show reduced vigor and fail to segregate homozygous triple mutant progeny, suggesting that at least one functional A subunit gene is essential for viability. *YFP-RCN1* protein fusions expressed under control of the native *RCN1* promoter provide complementing *RCN1* activity in yeast and in planta and exhibit a 'typical' seedling expression pattern with peak expression in primary root tips, lateral and adventitious root primordia and tips, and lower but detectable expression in hypocotyls and shoot meristems. We are using these fusions to analyze the specificity of A subunit function in vivo.

135 Arabidopsis SERK1 receptor recycling mediated by CDC48A

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One of the interacting partners of the Arabidopsis Somatic Embryogenesis Receptor-like Kinase 1-complex found in MALDITOF experiments was the Cell Division Cycle protein AtCDC48A. Co-immunoprecipitations and Yeast Two Hybrid screening confirmed these findings. CDC48 is an AAA ATPase (ATPases Associated with various cellular Activities) involved in membrane fusion events between ER and vesicles, and degradation of ubiquitinated proteins. A novel role for AtCDC48A is the quality control of endoplasmatic reticulum associated degradation (ERAD) of proteins. The active form of the protein is a hexamer. Currently we are investigating the possible role of CDC48A in the recycling and degradation of the SERK1 receptor. Fluorescence lifetime imaging-based Forster Resonance Energy Transfer detection is used to confirm the interaction between SERK1 and CDC48A, which is located at the peripheral membrane, but also in the nucleus and regions in the cytoplasm, probably ER. FRET was detected between the receptor and the membrane-located CDC48 protein only, in specific regions. Our conclusion is that CDC48A is a true interacting partner of the SERK1 receptor. A dominant negative mutant of the CDC48A protein could assist to further define biologically importance of this gene in the degradation pathway of the receptor. The future questions that we would like to address are the relationships between SERK1, CDC48A and other members of the SERK1 protein complex, described earlier in our group, like the Kinase Associated Protein Phosphatase KAPP and 14-3-3lambda. Receptor activation in plant cells and in intact plants by means of activation through e.g phosphorylation would give further information on the role of this receptor complex.

136 Expression of Response Regulators during reproductive Development in Arabidopsis

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Two-component systems provide a basic stimulus-response coupling mechanism to allow organisms to sense and respond to changes in environmental conditions. In its simplest form, the two-component signalling system is composed of two elements: a histidine kinase protein and a response regulator. In the *Arabidopsis* genome 24 putative response regulators (classified as type A or type B ARRs) have been identified based on sequence homology in addition to a number of pseudo-response regulators. We are studying the spatial and temporal expression of 3 of these genes -*ARR22*, *ARR24* and *ARRE*, during the cours e of reproductive development in *Arabidopsis* using semi quantitative RT-PCR and GUS fusion strategies. *ARR22* is a novel type A *Arabidopsis* response regulator whose ectopic expression, driven by the 35SCaMV promoter generates dwarf plants. *ARR22* contains 2 introns, one in the 5'UTR and the second within the ORF and its expression is primarily restricted to the seed-funiculus junction in young and mature siliques. The gene *ARR24* exhibits 70% similarity with *ARR22* and expression is primarily limited to pollen grains within young and mature anthers. Single KO lines for the two response regulators (*ARR22* and *ARR24*) as well as the double KO line for both genes have been generated. Analysis of the single KO lines has failed to identify a mutant phenotype and the double KO line is also indistinguishable from wild type plants suggesting that these genes are unlikely to display overlapping functions. *ARRE* is a gene classified as a hypothetical protein that shows 75% similarity to the first and last elements of the *ARR22* ORF. *ARRE* may possibly act as a pseudo-response regulator based upon its similar temporal and spatial expression pattern and sequence structure to *ARR22*. The possible roles of these three genes in the reproductive development of Arabidopsis is discussed.

137 SPIKE1, a DOCK-family protein, is a Guanine Nucleotide Exchange Factor for Rho Of Plants (ROP) and positively regulates the WAVE-ARP2/3 pathway

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The plant cytoskeleton, consisting of microtubules and actin filaments, has multiple functions during cell and tissue morphogenesis. For example, stable actin filament bundles act as a scaffold for the positioning and trafficking of organelles. The functions of actin filament nucleation and filament turnover during development are not known. We are using the genetics of trichome distortion to understand the mechanisms one important actin filament nucleation pathway. Trichome growth has distinct and highly constrained requirements for intact actin and microtubule cytoskeletons. For example, the distorted group of trichome mutants identified the heteromeric WAVE and Arp2/3 complexes that define one actin-dependent morphogenesis pathway. *spk1* was isolated from a family screen for mutations that cause both trichome distortion and seedling lethality. The reduced trichome branch number, pavement cell and leaf shape defects, as well as the sporophytic sterility of *spk1* plants are not observed in other "distorted group" mutants, suggesting that SPK1 has additional functions. SPK1 belongs to the DOCK family of GEFs. In animals, DOCK proteins activate Rho family small GTPases and elicit changes in the actin cytoskeleton through unknown mechanisms. An evolutionarily conserved DHR2 domain defines the DOCK family, and is sufficient for GEF activity. Arabidospsis encodes 11 ROP small GTPases that control endomembrane and cytoskeleton dynamics in a variety of cell types. However, the signaling pathways and exchange factors that positively regulate ROP are not known. We learned that SPK1 specifically binds to the ROP family of GTPases and that the DHR2 domain is necessary and sufficient for this activity both in vitro and in vivo. SPK1 binds promiscuously to ROPs, but with a high degree of selectivity for inactive or GDP-bound forms. Recombinant, full length SPK1 has potent nucleotide exchange activity. These data strongly suggest that SPK1 positively regulates ROP. The swollen, distorted-like trichome phenotype of *spk1* suggests that one function of SPK1 is to positively regulate the ROP-dependent WAVE-Arp2/3 pathway during trichome morphogenesis. Arp2/3 is a conserved 7-subunit complex that requires an activator such as the heteromeric 5 subunit WAVE complex to nucleate networks of actin filaments. We will present a variety of genetic and biochemical data that are consistent with the hypothesis that a SPK1-WAVE-ARP2/3 pathways controls actin-dependent growth in plants.

138 Functional characterization of OST1 kinase : a key element of ABA signalling pathway in Arabidopsis guard cells

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The *ost1* mutant has been isolated in a genetic screening based on infrared thermography to detect stomatal closure defects in response to drought (1). The *OST1* gene has been cloned and encodes a SnRK2 kinase (2) homologous to AAPK (ABA Activated Protein Kinase) which had been described in *Vicia faba* guard cells (3). OST1 is a central component of ABA signalling pathways in *Arabidopsis* guard cells, as *ost1* mutants are completely unable to close their stomata in response to ABA. Moreover, OST1 is specific for ABA signalling in guard cells. Indeed, other responses to ABA, such as germination inhibition, and responses of guard cells to other factors, such as light and CO_2, are not impaired in *ost1* mutants. The important and specific function of OST1 in ABA-mediated drought resistance led us to investigate the molecular mechanisms regulating OST1 activity.

We produced a recombinant OST1 in *E. coli* and succeeded in purifying the native kinase. The recombinant OST1 has kinase activity and can autophosphorylate. To our knowledge, it is the first time that a SnRK2 kinase can be purified in a native active form when produced in *E. coli*. We focused our studies on intramolecular mechanisms which regulate OST1 activity. On the one hand, we are interested in the importance of autophosphorylation in this regulation. We have developed a mass spectrometry approach in order to identify residues which are targets of OST1 autophosphorylation. On the other hand, we plan to study the structure/function relationships in the C-terminus domain of the kinase which shares no homology with other described proteins. We are generating several truncated versions and point mutated forms of OST1 protein. First, the *in vitro* activity of all the OST1 variants produced in *E. coli* will be tested. In parallel, we will test the ability of the OST1 variants to complement *ost1* mutants guard cells phenotypes. *In planta* studies on these lines will reveal the functional importance of the residues or domains tested. This work will provide a strong basis for future studies on interactions between OST1 and its putative regulators or partners in order to better understand the function of OST1 in ABA signalling.

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139 Accumulation and biological activity of enzymatically and non-enzymatically formed oxylipins

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Plant oxylipins are derived from linolenic acid by enzymatic or non-enzymatic pathways. The best characterised enzymatically formed oxylipins are 12-oxophytodienoic acid (OPDA) and jasmonic acid. Both are molecules with important signalling functions in development and stress responses in plants. Less is known about the non-enzymatically formed oxylipins. We are investigating the accumulation and function of phytoprostanes, which are non-enzymatical oxylipins with structural similarity to OPDA.

Pathogen attack leads to the generation of reactive oxygen species which triggers the formation of phytoprostanes. In order to support this hypothesis, levels of phytoprostanes, non-enzymatically formed hydroxy fatty acids, OPDA and jasmonic acid are analysed after infection with *P. syringae*. The accumulation is investigated in different mutants affected in the production of or sensitivity to reactive oxygen species.

Based on their structural similarity to OPDA, signalling functions for the phytoprostanes have been proposed. Some biological activities of phytoprostanes have been shown. We will present data on the inhibition of root growth, induction of gene expression and camalexin accumulation by phytoprostanes in comparison to OPDA and jasmonic acid.

140 Ethylene Signaling and EIN3 Protein Levels in Arabidopsis

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The hormone ethylene regulates numerous aspects of growth and development in plants. Central to the ethylene signal transduction pathway in Arabidopsis are two transcription factors EIN3 and EIL1, that together activate a number of downstream effector genes. Recent studies have shown that the breakdown of the EIN3 and likely EIL1 proteins is regulated by ethylene. In the absence of ethylene, EIN3 is rapidly degraded by the ubiquitin/26S proteasome pathway using an SCF E3 complex containing the EBF1 and 2 F-Box proteins to direct selective ubiquitination. Ethylene blocks this step allowing EIN3 levels to rise. *ebf1 ebf2* double mutants display a strong growth arrest in addition to containing elevated levels of EIN3. This arrest is partially relieved in the *.ein3-1* mutant, suggesting that EIN3 and likely EIL1 are the sole targets of this SCF E3 complex. Detailed growth kinetics of etiolated *Arabidopsis* seedlings have shown that there are two phases to the growth inhibition by ethylene. The first transient phase is independent of EIN3 and EIL1, whereas the second phase requires both for long term growth inhibition. Using this system, we found that EBF1 and 2 are involved with the onset of and recovery from the second phase of growth inhibition. In particular, the *ebf2-3* mutant seedlings as well as *35S::EIN3* seedlings overexpressing EIN3 exhibit a strong delay in recovery following removal of exogenous ethylene. In adult plants, we find that the phenotypes of the *ebf1-3* and *ebf2-3* mutants are additive with those of *ctr1-1*, suggesting that ethylene signaling affects the EIN3/EIL1 targets and not the activity of the SCF E3s. Our findings support a model where the regulated turnover of EIN3, and possibly EIL1, by SCF^{EBF1} and SCF^{EBF2} are key checkpoints in ethylene signaling.

141 The Protein Kinase Genes AtMAP3Kepsilon1 and AtMAP3Kepsilon2 are Required for Pollen Development in Arabidopsis thaliana

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We have used reverse-genetic analysis to investigate the function of MAP3Kepsilon1(MAP3Ke1) and MAP3Kepsilon2 (MAP3Ke2), a pair of closely-related Arabidopsis thaliana genes that encode protein kinases. We determined that this pair of functionally-redundant genes cannot be transmitted through the male gametes. Microscopic analysis revealed that mature map3ke1;map3ke2 pollen grains are not viable. By contrast, viability of the female gametophyte is not affected by the map3ke1;map3ke2 double-mutant combination. Genetic complementation using a wild-type copy of the MAP3Ke1 locus and tetrad analysis performed using the Arabidopsis quartet mutation demonstrated that the pollen-lethal phenotype is caused by segregation of the map3ke1;map3ke2 double-mutant combination at meiosis. Analysis of pollen ultrastructure by transmission electron microscopy revealed that the first abnormal feature to appear during the development of double-mutant pollen was a breakdown of the plasma membrane. By using a translational fusion between the yellow fluorescent protein (YFP) and MAP3Ke1, we determined that MAP3Ke1 localizes to the plasma membrane and the cytoplasm in root-tip cells of Arabidopsis. These results suggest that MAP3Ke1 and MAP3Ke2 may play a role in maintaining the integrity of the plasma membrane in Arabidopsis.

142 *EER2* encodes a novel factor required for proper modulation of ethylene responses in Arabidopsis leaves and etiolated seedlings

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Significant progress has been made toward elucidation of the ethylene-signaling pathway through isolation of Arabidopsis mutants that are ethylene insensitive or have a constitutive ethylene response. In order to identify novel components of this pathway, we have taken the unique approach of isolating Arabidopsis mutants with enhanced ethylene responsiveness (*eer* mutant class), which is a phenotype that we have found to consist of both an increase in sensitivity and an exaggeration of response to ethylene. Screening for mutants with an *eer* phenotype has resulted in identification of at least three new *eer* mutants including *eer2*, which has between a two- to three-fold increase in ethylene sensitivity and an extreme response to saturating levels of ethylene in etiolated seedlings. Molecular analysis has revealed that the *eer2* mutation negatively impacts the ethylene-dependent induction of several ethylene-regulated genes including *AtEBP*, *basic chitinase*, and *PDF1.2*, indicating that EER2 is required for proper regulation of these and possibly other ethylene-inducible genes, some of which may function as part of a predicted mechanism for resetting the ethylene-signaling pathway following perception. We have recently isolated the *eer2-1* mutation, which represents a single nucleotide change that results in an amino acid substitution, along with several T-DNA insertion alleles at this locus. Currently we are working to determine the biochemical and molecular role of the EER2 factor in modulating ethylene responses. Work includes overexpression analysis, subcellular localization, double mutant analysis, and analysis of possible interactions with known components of the ethylene signaling pathway such as EIN2. We will also present work that is in progress regarding two additional *eer* mutants, *eer3* and *eer4*. This will include our phenotypic characterization and map-based cloning efforts related to each of these additional mutants. By isolating and characterizing such mutants, we hope to further the understanding of how this critically important signaling pathway is regulated in Arabidopsis and plants in general.

143 Withdrawn

144 Photobiological and Electrophysiological Studies of Glutamate-Like Receptors in Etiolated Hypocotyls of *Arabidopsis*

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The *Arabidopsis* glutamate receptor-like (*AtGLR*) genes are similar to those encoding the ligand-gated ion channels that mediate fast responses at glutamatergic synapses in the mammalian brain. The *AtGLR* gene family has 20 members and is divided into three phylogenetic clades. Since their discovery in 1998, the physiological roles and functions of these genes in the plant has been an open question. Some early results were consistent with a role for this gene family in light signaling, a topic our lab studies in the seedling hypocotyl. We have demonstrated, using photoreceptor mutants, pharmacology, and electrophysiology, that blue light acting through phototropin and cryptochrome induces Ca^{2+} and Cl^{-} fluxes across the plasma membranes of etiolated hypocotyls. These ionic events are thought to be components of a process that inhibits hypocotyl growth based on kinetics data from pharmacological treatments and photoreceptor mutant analysis. Glutamate and glycine also cause a membrane depolarization in the etiolated hypocotyl that resembles the blue-light-induced membrane depolarization in both magnitude and time-course. Both the glutamate- and glycine-induced depolarizations are *GLR*-dependent based on the responses of *AtGLR* insertion mutants to these ligands. It has also been found that glycine causes a calcium spike in etiolated hypocotyls and that the glutamate response of the light-grown root may include both calcium and anion fluxes. These data led to the hypothesis some *AtGLR* family members participate in blue-light signal transduction. To test this idea, *AtGLR* mutants are being assayed for their blue-light-induced depolarization response. Blue-light-induced growth responses of *AtGLR* mutants are also being analyzed computationally with a custom image-analysis algorithm (See abstract by Miller et al.) Although this work is in its early stages, some concrete evidence linking the *AtGLR*s to blue-light signaling events has been obtained. Blue light modifies the depolarization induced by glutamate in the etiolated hypocotyl. The depolarization triggered by glutamate is 1.5-fold larger and much longer (sustained instead of transient) when the ligand is applied after a pulse of blue light compared to dark controls. The *GLR* dependence of this enhancement by blue light is being determined using our *AtGLR* insertion mutant library. The physiological significance of this blue-light enhancement of the glutamate response is being explored with high-resolution growth measurements of the developing seedling.

145 Transcriptional and posttranscriptional regulation of RNS1 in response to wounding and ABA stress

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Herbivore feeding produces physical damage to plants. As microbial pathogens may also attack at wound sites, plants have evolved complex systems of defense against herbivores and pathogens. Many of these responses are inducible and are activated specifically as a result of wounding. In *Arabidopsis thaliana*, oligogalacturonides (OGAs) and jasmonic acid (JA) are the main regulators of the signaling pathways that control the local and systemic wound response, respectively. We have previously found that the extracellular ribonuclease RNS1 defines a novel pathway that is independent of JA and OGA. Here we show that abscisic acid (ABA), which has been shown to induce wound-responsive genes in other systems, also induces RNS1. In the absence of ABA signaling, wounding induces only approximately 45% of the levels of RNS1 mRNA that are induced when ABA pathways are operational, although significant levels of RNS1 and other wound-induced nuclease activities still accumulate. These results indicate that two pathways, one of which is ABA dependent, act synergistically to induce the expression of *RNS1*. Preliminary results using transgenic plants expressing reporter genes under the control of the *RNS1* promoter and 5' untranslated region suggest that the ABA-independent pathway mainly controls *RNS1* expression at the transcriptional level. Conversely, the ABA pathway regulates RNS1 accumulation at a posttranscriptional level. The isolation of several ABA-hypersensitive mutants that correspond to RNA-binding proteins prompted the idea that ABA signaling is linked to RNA metabolism. Our results are further support for RNA metabolism as a main component of ABA signaling.

146 Identification of Movement Domains in SHORT-ROOT

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Cell-to-cell communication is necessary for development and differentiation of multicellular organisms. In the Arabidopsis root the formation and differentiation of the ground tissue requires the activity of two related transcriptional regulators, *SHORT-ROOT* (*SHR*) and *SCARECROW* (*SCR*). SHR is an organizing signal regulating the division of a group of stem cells in the root apex through the activation of SCR. Both SHR and SCR are GRAS family proteins that are present in partially overlapping domains within the root. In the areas of the root where the expression of the SHR and SCR proteins does not overlap, these proteins show differences in subcellular localization and the ability to traffic between cells. Comparison of transcription with protein localization shows that SHR protein moves from the stele into the surrounding ground tissue. This is not true for SCR. The SHR protein is present both in the cytoplasm and nuclei of stele cells; whereas SCR is restricted to nuclei. As a way of determining what sequences within SHR and SCR are responsible for these differences, we have performed structure/function analysis of both the SHR and SCR proteins. We have identified a domain within SCR that is required for nuclear localization both in the stele and the ground tissue. Surprisingly this domain is independent of the predicted nuclear localization signal. We have also identified multiple regions within SHR that are required for proper subcellular localization and movement of the protein. Significantly, addition of these domains to SCR are able to affect subcellular localization and in some instances confer movement.

147 Protein-protein interaction involving of ethylene receptor of Arabidopsis

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The plant hormone ethylene is perceived by a five member family of receptors (ETR1, ERS1, ETR2, ERS2, EIN4) in Arabidopsis. Although there is redundancy in the function of ethylene receptors, each receptor may also contribute differently to the ethylene response. To investigate the ethylene receptors at the protein level, a TAP tag was added to the C-terminus of each ethylene receptor.The TAP tag enables the detection of the tagged receptors by Western blot and also allows for purification of receptor protein complexes. Analysis by gel-filtration indicates that each ethylene receptor participates in a protein complex. Ethylene induces a 200-KDa change in the size of the ERS1 protein complex, but has not apparent effect upon the size of the ETR1 protein complex. Deletions of ETR1 indicate that multiple domains of the receptor are involved in protein-protein interactions. To better define components of the receptor complexes, we examined the ability of receptors to interact with each other as well as with the Raf-like kinase CTR1, analyzing their associations in complexes isolated from Arabidopsis. ETR1 was found to preferentially interact with ERS2 and ETR2, compared to ERS1 and EIN4. CTR1 was found to associate with all members of the ethylene receptor family tested. Our study indicates that ethylene receptors perceive ethylene and initiate signaling as components of high-molecular-mass protein complexes. These protein complexes share some common components such as CTR1, but the ethylene-stimulated change in size of the ERS1 protein complex suggests that the composition may also vary in a dynamic fashion.

148 Genetic and Physiological Analysis of the Brassinosteroid Transcriptional Regulator, BZR1

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Maintenance of brassinosteroid (BR) signal transduction and homeostasis is important to plant development and growth. Recently, components of the BR signaling pathway have been discovered and characterized. These components include cell surface receptor kinases (BRI1 and BAK1), an intracellular kinase (BIN2) and phosphatase (BSU1), and a family of nuclear proteins (BZR/BES family). The BZR1 protein binds a conserved sequence in the promoters of known BR-regulated genes and acts to repress gene expression. Additionally, microarray studies suggest that BZR1 controls a subset of the BR-regulated genes that are important for proper BR homeostasis and response. Detailed genetic and physiological studies demonstrate roles for BZR1 and BR in regulation of various aspects of plant development. For example, BZR1 acts to maintain proper axil angles, maintain proper stem elongation, and control flowering timing through both its role in feedback suppression of BR biosynthesis and its role in growth promotion. A group of *bzr1-1D* suppressor mutants has been identified. These mutants suppress different phenotypes of *bzr1-1D*. They are being studied to further understand how BR signaling, and specifically BZR1, controls various developmental processes.

149 The role of light-mediated RNA degradation in the regulation of the Arabidopsis circadian clock

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The best studied of the biological systems for timekeeping are the circadian clocks that control rhythms that have a period of around 24 hours. Recent years have seen a tremendous increase in our understanding of circadian clocks in several model organisms ranging from cyanobacteria to mice. The classic model of a circadian system comprises three components- the input pathways, the oscillator and the output pathways. The core of the circadian system, the oscillator that generates the rhythms, is a feedback loop of positive and negative elements. The input pathways allow the oscillator to be set (entrained) by environmental changes, such diurnal dark/light transitions and changes in temperature. The output pathways control a wide range of processes in organisms across the taxonomic spectrum. Crucial to the correct function of the circadian system is that the oscillator can be entrained accurately by the organism's environment. This is achieved by close control not only of the accumulation of the components (transcription and translation) of the circadian system but also of their degradation. Protein degradation has been shown to be very important for circadian regulation in a number of organisms. The regulation of degradation of oscillator transcripts is potentially another important level of control in the circadian system. However, very little is known about RNA stability in the clock system of any organism. Circadian clock associated 1 (CCA1) is a single myb transcription factor that has been shown to play a central role in the Arabidopsis circadian oscillator. Using transgenic plants that constitutively express CCA1 (CCA1-ox), we can show that the CCA1 transcript is rapidly destabilized by light. We propose that light-regulated degradation of CCA1 may be an important means by which light can entrain the circadian oscillator. We have generated chimeric CCA1 molecules in order to identify the instability determinants in CCA1 transcripts. We are also examining which photoreceptors are important for mediating the light regulation of CCA1 RNA stability. Daily fluctuations in temperature are also known to entrain the circadian oscillator and we are looking at the effects of temperature changes on CCA1 stability. Our goal is to understand the mechanisms for the regulation of CCA1 RNA degradation and its role in entrainment of plant circadian rhythms.

150 Arabidopsis CSN5A and CSN5B subunits are present in distinct COP9 signalosome complexes that play unequal role on plant development

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The COP9 signalosome (CSN) is an evolutionarily conserved multisubunit protein complex involved in variety of signaling and developmental processes through the regulation of protein ubiquitination and degradation. A known biochemical role attributed to CSN is a metalloprotease activity responsible for the de-rubylation of cullins, core components for several types of ubiquitin E3 ligases. The CSN's de-rubylation catalytic center resides in its subunit 5, which in *Arabidopsis* is encoded by two evolutionary conserved genes, *CSN5A* and *CSN5B*. Here we show that CSN5A and CSN5B subunits are assembled into distinct CSN complexes in vivo, which are present in drastically different abundance, with CSN^{CSN5A} appearing to be the dominant one. Transgenic CSN5A and CSN5B proteins carrying a collection of single mutations in or surrounding the metalloprotease catalytic center are properly assembled into CSN complexes, but only mutations in CSN5A result in a pleiotropic dominant negative phenotype. The extent of phenotypic effects caused by mutations in CSN5A is reflected at molecular level by impairment in CUL1 de-rubylation. These results reveal that three key metal-binding residues as well as two other amino acids outside the catalytic center play important roles in CSN de-rubylation activity. The characterization of *csn5a* and *csn5b* homozygous insertion mutants confirmed that CSN^{CSN5A} is predominantly responsible for the de-rubylation of CUL1 throughout development, even though the observation that the pleiotropic phenotype of the *csn5a csn5b* double mutant is more severe then the one of the *csn5a* mutant alone unreveals a minor function for CSN^{CSN5B} in cullins de-rubylation. Taken together, our data provides physiological evidence on a positive role of CSN in the regulation of *Arabidopsis* SCF E3 ligases through RUB de-conjugation, and highlights the unequal role that CSN^{CSN5A} and CSN^{CSN5B} play in controlling the cellular de-rubylation of cullins.

151 Combining genomics and reverse genetics to identify and characterize components in sugar-response pathways

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Sugar signaling represents a relatively unknown area within plant response networks. To find more genetic components of sugar-response pathways, Affymetrix GeneChip experiments were performed. The results from these experiments were used to identify genes that are regulated by glucose and/or sucrose, but not by equi-molar concentrations of sorbitol. Glucose and sucrose regulated genes were then screened for those predicted to encode proteins with activities commonly associated with response pathways, such as transcription factors, protein kinases and protein phosphatases. Based on these criteria, 189 "target genes" have been identified. To determine which of these target genes act in sugar response, plant lines carrying T-DNA insertions in most of the target genes have been obtained. These mutants are now being screened for defects in sugar response. One assay being used is to plate mutant seeds on minimal media supplemented with sucrose, glucose, or sorbitol to determine which of the mutant lines are insensitive or hypersensitive to the inhibitory effects of high concentrations of exogenous sugars on early seedling development. Quantitative RT-PCR is also being used to screen the mutants for defects in sugar-regulated gene expression. As several sugar-response mutants isolated previously through different mutant screens have displayed defects in phytohormone response or metabolism, we are also characterizing the response of seeds of these new mutant lines to the inhibitory effects of abscisic acid and paclobutrazol on seed germination. Using these approaches, we have identified at least two new, previously unreported mutants in sugar response. The first mutant is insensitive to high concentrations of exogenous sugar during early seedling development, whereas the second mutant is hypersensitive to exogenous sugar during the same developmental period.

152 Sugars and Phytohormones Regulate Seed Germination and Early Seedling Development via a Complex Signaling Network

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Metabolism and development are tightly linked in plants' life cycles. Sugars, in addition to playing a primary role in plant metabolism, also affect an array of plant developmental processes. For example, plants undergo profound metabolic and developmental transitions during seed germination and early seedling development. Regulation of these transitions and other processes occurs via a complex signaling network that receives input from several chemical signals. These chemical signals have long been known to include abscisic acid, which inhibits seed germination, as well as ethylene and gibberellin, which promote seed germination. Recent work by our lab and others has shown that soluble sugars also affect seed germination. Even low to moderate concentrations of exogenous sugars (e.g. 30 mM glucose) cause a significant delay in the rate of germination of wild-type Arabidopsis. This effect is not due to alterations in the osmotic potential of the media, as even substantially higher concentrations of sorbitol do not exert similar effects. Several lines of evidence also suggest that sugars and phytohormones interact in the regulation of seed germination. For example, several sugar-insensitive (*sis*) mutants of Arabidopsis also exhibit alterations in phytohormone response and/or metabolism. The *sis1* mutant is allelic to the ethylene constitutive response mutant, *ctr1*. The *sis4* mutant is allelic to the abscisic acid deficient mutant, *aba2*, and the *sis5* mutant is allelic to the abscisic acid insensitive mutant, *abi4*. Finally, the *sis2* mutant displays resistance to the gibberellin biosynthesis inhibitor paclobutrazol, suggesting that *sis2* may be defective in gibberellin response or metabolism. Findings that exogenous glucose greatly exacerbates the negative effects of paclobutrazol on seed germination also suggest interactions between sugar and phytohormone response pathways. Possible models for these interactions will be discussed. However, sugars may also regulate seed germination and early seedling development via mechanisms that are relatively independently of phytohormones. For instance, two other *sis* mutants, *sis3* and *sis7* both exhibit wild-type or near wild-type responses in all phytohormone assays conducted to date. Currently, the signaling pathways through which *sis3* and *sis7* exert their functions are being studied.

153 Multiple Tyrosine Residues Are Critical In Gibberellin-Sensitivity Of DELLA Protein, RGL2

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DELLA proteins are gibberellin (GA)-responsive negative regulators of growth and development in plants. Out of five DELLA proteins in Arabidopsis, RGA (GFP-fused) undergoes degradation in response to GA via 26S-proteasome, whilst GAI and RGL1 (both GFP-fused) were reported relatively insensitive to GA. This study shows that DELLA protein RGL2, expressed in BY2 cells, also undergoes GA-induced degradation via proteasome pathway. In addition, GAI, RGL1 and RGL3, when expressed as myc-tagged proteins, also undergo GA-induced degradation. Thus all five Arabidopsis DELLA proteins (GAI, RGA, RGL1, RGL2 and RGL2) respond to GA-induced degradation. Previous studies suggested that phosphorylation of tyrosine might play a critical role in GA-induced degradation of barley DELLA protein SLN1. Treatment of BY2 cells, expressing GAI, RGA, RGL1 and RGL2 proteins, with inhibitors of tyrosine kinases resulted in strong resistance to GA-induced degradation. Treatment with alkaline phosphatase resulted in greater mobility of RGL2 protein in SDS-PAGE essay demonstrating that RGL2 exists in a phosphorylated form. Alignment of 10 known DELLA proteins from different plants resulted in 8 tyrosine (Tyr) residues, which were completely conserved and were considered potential target residues for phosphorylation. Mutation of these residues into alanine in RGL2 demonstrated that Tyr52, Tyr89, Tyr223 and Tyr435 were critical for GA-sensitivity. Mutation of these critical tyrosine residues into glutamate also resulted in loss of GA-responsiveness, whilst mutation into conservative phenylalanine residue resulted in retention of the GA-sensitivity. Since phenylalanine cannot be phosphorylated, we suggest that not RGL2 but possibly other proteins in GA-signaling pathway may be targeted for tyrosine phosphorylation to influence its GA sensitivity.

154 Isolation and characterization of a bZIP transcription factor from Arabidopsis regulating the endoplasmic reticulum stress response

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Proteins synthesized in the endoplasmic reticulum (ER) are correctly folded before translocation. If protein folding is incorrect, genes for ER-resident chaperones such as BiP and calnexin are induced. This phenomenon is known as the ER stress response or unfolded protein response. We have been studying molecular mechanism of the ER stress response in Arabidopsis. Since basic leucine zipper (bZIP) transcription factors are involved in the ER stress response of yeast and animals, we assumed that it might be also the case in plants. Then, we analyzed transcripts of 75 genes encoding putative bZIP in the Arabidopsis genome and identified a bZIP gene AtbZIP60 induced by tunicamycin that is potent inhibitor of N-linked glycosylation and causes the ER stress response. Other reagents that induce the ER stress response also induced AtbZIP60 transcripts. AtbZIP60 encodes a predicted protein of 295 amino acids with a putative transmembrane domain near its C-terminus after a bZIP domain. A truncated form without C-terminal region fused with GFP localized to the nucleus. Expression of truncated AtbZIP60 clearly activated any of three BiP and two calnexin promoters but did not an HSP70 promoter in a transient assay in protoplasts. The induction was considered to be through cis-elements, P-UPRE and ERSE, that are often found in promoters of ER chaperone genes. Interestingly, truncated AtbZIP60 also appeared to induce the expression of AtbZIP60 through an ERSE-like sequence in the promoter of AtbZIP60. However overexpression of full length AtbZIP60 did not function, suggesting digestion of AtbZIP60 protein triggers activation of the transcription activity. With these results we propose the following model. AtbZIP60 is synthesized at a low level as a precursor protein that may be anchored in the ER membrane under unstressed conditions. Sensing ER stress by an unknown mechanism, the N-terminal domain of AtbZIP60 is cleaved and translocated to the nucleus. Soluble AtbZIP60 activates transcription of target genes through either P-UPRE or ERSE. Transcription of AtbZIP60 is also activated through an ERSE-like sequence to amplify the signal. In a T-DNA tagged mutant of AtbZIP60, induction of some genes induced in the ER stress response in wild type is repressed. However, several genes are still induced in the mutant even though truncated AtbZIP60 activated their promoters suggesting redundant pathway of the ER stress response in Arabidopsis.

155 Degradation of the Auxin Response Factor, ARF1

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The 23 Arabidopsis auxin response factors (ARFs) mediate auxin-regulated gene expression through binding auxin response elements (AuxREs) in promoter regions of auxin-regulated genes (1,2). Their roles are modulated by Aux/IAAs, of which there are 29 in Arabidopsis (2). The Aux/IAA-ARF interaction requires Aux/IAA domains III and IV and similar C-terminal domains found in most ARFs (1,3). A model for auxin-regulated gene expression suggests that ARFs permanently occupy the AuxREs and when auxin levels are low, Aux/IAA proteins generally repress auxin response by forming heterodimers with ARFs, blocking their function (4). When auxin levels are high Aux/IAAs are degraded, through auxin-induced interaction with the SCF^{TIR1} complex (5). Degradation of Aux/IAAs allows the formation of ARF homodimers and in the cases of Q-rich ARFs, transcription of the auxin responsive genes. It is also possible that Aux/IAA dimerisation prevents Aux/IAAs from forming Aux/IAA-ARF dimers and therefore regulates transcription. To further analyse the integration of control mechanisms affecting auxin-regulated gene expression we have begun to study ARF degradation.

ARF1 was discovered through its ability to bind an artificial palindromic repeat of the conserved TGTCTC AuxRE found in the promoter of the soybean *GH3* gene (1,6). We have found that ARF1 is capable of conveying a half-life of approximately 3 hours on the otherwise long-lived firefly luciferase (LUC) protein in transgenic 6-8-day-old Arabidopsis seedlings. As previously observed for Aux/IAA degradation (5,7), ARF1 degradation is inhibited *in vivo* in the presence of proteasome inhibitors. Further investigation is underway to determine whether degradation of ARF1 is dependent on the class of E3 ligases called SCFs. Our results indicate that ARF degradation, while slower than that observed for Aux/IAA proteins is significant and provides initial evidence for further control mechanisms associated with the highly regulated responses to the plant hormone auxin.

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156 MALDI-TOF/MS based identification of proteins present in the Arabidopsis Somatic Embryogenesis Receptor-like kinase 1 complex

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One of the plant receptor like kinases (RLK) is the Arabidopsis thaliana Somatic embryogenesis Receptor Kinase 1 (AtSERK1). AtSERK1 overexpression results in an increased embryogenic competence of explants. We used a proteomics approach to determine the composition of the AtSERK1 signalling complex in vivo. The receptor was fused to cyan fluorescent protein and expressed in wild type plants under its native promoter. Using Blue Native Gel Electrophoresis we showed that AtSERK1 is involved in a protein complex of 300 to 500 kDa. The receptor complex was immunoprecipitated from seedlings, co-immunoprecipitating proteins were analysed by mass spectrometry and identified peptides were matched with Arabidopsis protein database to determine the correlating parent proteins. This proteomics approach confirmed the presence of kinase associated protein phosphatase-KAPP and AtCDC48A previously identified by yeast two-hybrid screen.

Several additional proteins were found such as another member of the 14-3-3 family, 14-3-3nu, two other RLK the AtSERK3 (BAK1) and BRI1, the MADS-box transcription factor AGL15 and an uncharacterised zinc-finger protein. Several of the proteins were subsequently confirmed to be in a complex with AtSERK1 by alternative methods such as co-immunoprecipitation using specific antibodies raised against some of the interacting partners and by FRET. These studies show that the method used here could be applicable for other plant membrane protein for which a GFP-tagged version is available.

157 GCR1, GPA1 and prephenate dehydratase are required for blue light-induced production of phenylalanine in etiolated Arabidopsis

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The long term goal of our laboratory is to understand the signal transduction mechanism(s) by which the Blue Low Fluence (BLF) system regulates the initial stages of leaf development. The aromatic amino acids, phenylalanine and tyrosine, and the intermediate metabolites that derive from them can account for up to 30-40% of the mass of an individual plant. We have previously established that GCR1 and GPA1 have roles in the BLF system. As part of a larger study we identified several proteins that can interact with GPA1 including prephenate dehydratase (PD1). This enzyme catalyzes the conversion of prephenate to phenylpyruvate, which in turn is converted to phenylanine. This specific pathway, while common in bacteria and fungi has not been explored in higher plants. Potential roles for PD1 in blue light regulation of phenyalanine synthesis and/or early seedling development were addressed by use of insertion mutants, yeast two hybrid and co-precipitation studies, photobiology, and biochemistry. PD1 is located in the cytoplasm of etiolated arabidopsis. Our data indicate that irradiation of etiolated seedlings with blue light increases phenylalanine synthesis by five-fold. Insertion mutants in GCR1, GPA1 and PD1 indicate that all are essential for this increase. We have used HPLC analysis to confirm that PD1 converts prephenate to phenylpyruvate and that the rate of PD1 activity is enhanced through its physical interaction with pre-activated GPA1. Spectral analysis and optical sectioning of insertion mutants for GCR1, GPA1 and PD1 indicate the loss of several specific, localized, UV-absorbing compounds likely to derive from phenylalanine.

158 The GCR1, GPA1, Pirin1, NF-Y-A5/B9 signal chain mediates both blue light and ABA responses in Arabidopsis

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Different classes of plant hormones and different wavelengths of light act through specific signal transduction mechanisms to coordinate higher plant development. While a great deal of progress has been made, full signal transduction chains have not yet been described for most blue light, or abscisic acid-mediated events. Based on data derived from T-DNA insertion mutants, yeast two-hybrid and co-precipitation assays, we report a potential signal transduction chain leading to blue-light-mediated Lhcb expression in etiolated Arabidopsis: GCR1 (the sole Arabidopsis protein coding for a potential G-protein coupled receptor), GPA1 (the sole Arabidopsis G? subunit), Pirin1 (one of four members of an iron-containing subgroup of the cupin super family), NF-Y-A5 (an NF-Y-A CCAAT-box binding protein subunit) and NF-Y-B9 (LEC1; an NF-Y-B CCAAT-box binding protein subunit). We also report the unexpected finding that the same signaling mechanism is responsible for the hypersensitive response of seed germination to the plant hormone abscisic acid.

159 Cloning and characterization of FB1-resistant (*fbr*) mutants from activation tagging populations

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Programmed cell death (PCD) is important for many key processes occurring during the life cycle of plants, including development and plant defense responses. However much remains to be learned about the molecular mechanisms regulating PCD. We used forward genetics to identify genes that participate in plant PCD. The fungal toxin fumonisin B1 (FB1) elicits PCD in *A. thaliana*, and was used as a tool to identify FB1-resistant (*fbr*) mutants by virtue of their ability to develop in the presence of the toxin. Two *fbr* mutants, 91A and 93E2, were identified from activation tagging T-DNA mutant populations.

Plant DNA flanking the 93E2 mutant T-DNA insertion was rescued by inverse PCR. A T-DNA insertion was found on chromosome 2 near the 5'UTR region of a JAR1-like gene (At2g47750), whose family members adenylate plant hormones including jasmonic acid and auxin. Experiments aimed at determining the possible role of this gene in causing the *fbr* phenotype were initiated before subsequent genetic analyses revealed that the 93E2 *fbr* phenotype was not a result of this T-DNA insertion. To assess the function of this uncharacterized gene, RNAi lines were generated and root length assays were performed in the presence or absence of indole acetic acid (IAA). Several RNAi lines were more sensitive to 5μM IAA, showed greater primary root length in the absence of IAA, and exhibited early flowering compared to the wild-type Col-0. These phenotypic differences correlate with At2g47750 expression levels in the RNAi lines.

Plant DNA flanking the T-DNA insertion in 91A was rescued by PCR walking. An insertion was found in the intergenic region between CLE19 (At3g24225) and putative pectate lyase (At3g24230) genes. A Salk T-DNA insertion line (094120) with an insertion near the one found in 91A also displays the *fbr* phenotype. RT-PCR revealed no differences in the expression of the CLE19 gene (At3g24225) between wild type and 91A plants, and no transcript was observed for the pectate lyase–like gene, consistent with a lack of ESTs. However, several transcripts corresponding to the intergenic region were identified in the MPSS database, and RNA secondary structure predictions revealed significant hairpin stem-loop structures should form from RNA derived from the intergenic region. Long stem–loop precursor molecules are a characteristic of microRNAs (miRNA), which regulate gene expression at the level of both transcription and translation. Experiments aimed at determining whether the T-DNA insertions disrupt miRNA precursors to cause the *fbr* phenotype and their potential targets will be presented.

160 Loss of function mutation of COBRA, which is a determinant of oriented cell expansion, invokes cellular defense mechanism in Arabidopsis thaliana

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We have identified an Arabidopsis T-DNA insertion mutant that results in complete loss-of-function of the COBRA gene, which encodes a putative glycosylphosph atidylinositol (GPI)-anchored protein that modulates cellulose deposition and oriented cell expansion in roots (Schindelman et al., 2001). Unlike the previously described point-mutational alleles of COBRA (cob-1, cob-2 and cob-3) that had defective cell elongation only in the root tissue, the new loss-of-function mutant allele (named "cob-4") exhibits abnormal cell growth throughout the entire plant body and accumulates massive amounts of stress response chemicals such as anthocyanins and callose. To gain further insight into the mechanism by which COBRA affects cell growth and physiology, we compared the whole-genome gene expression profile of cob-4 plants to that of wild-type plants. Consistent with the mutant phenotype, many genes involved in anthocyanin biosynthesis were up-regulated in the cob-4 plants, whereas genes involved in cell elongation were down-regulated. The most striking feature of the gene expression profile of cob-4 was the massive and coordinate induction of defense- and stress-related genes, many of which are regulated by the plant stress signal jasmonic acid (JA). The expression of genes involved in salicylic acid (SA)-dependent defense pathway also was increased significantly in the cob-4 plants, whereas genes involved in ethylene (ET) signaling were not. Indeed, the cob-4 plants over accumulated JA by nearly 8-fold compared to wild-type plants. However, a double mutant with aos (defective in JA biosynthesis) exhibited a similar phenotype to the single cob-4 mutant, suggesting that the activation of the JA pathway is not required for the phenotype of cob-4 mutant. This mutant provides evidence that the plant respond to sensory inputs from the cell expansion/growth.

161 CTR1, a MAPKKK that is a negative regulator of the ethylene-signaling pathway, is a target of phosphatidic acid

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Phosphatidic acid (PA) has only recently been identified as an important signaling molecule in eukaryotes. In plants, its formation is triggered following various biotic and abiotic stresses including wounding, pathogen attack, drought, salinity, chilling and freezing. In the past few years, several protein targets of PA have been identified including the mammalian MAPKKK, Raf-1. CTR1 is a plant MAPKKK that is homologous to Raf-1 and functions as a negative regulator of ethylene signaling. Ethylene is a key hormone in promoting processes such as ripening, senescence, and response to pathogens. Here we present evidence that CTR1 is a PA target and that CTR1 activity may be regulated *in vivo* by binding to PA. The CTR1 kinase domain was found to bind to PA directly and specifically. PA binding blocks an intramolecular interaction between CTR1's kinase and N-terminal domains and addition of as little as 1 nmole PA severely inhibits CTR1 kinase activity *in vitro*, both in terms of substrate phosphorylation and autophosphorylation. Although the Raf-1 PA-binding motif is conserved in CTR1, mutations that eliminate this motif in CTR1 did not reduce PA binding nor did they affect the capability of PA to inhibit CTR1 activity *in vitro*. Our deletion studies reveal that the PA-binding site in CTR1 actually resides in the last 170 aa of the protein. Based on our analysis, this region has higher affinity for PA than PA-binding domains found in other proteins such as Raf-1 and TAPAS. Although we have not found that ethylene induces PA accumulation in either cells or seedlings, KO mutants of the PA-producing enzyme phospholipase D (PLDα1) exhibit altered morphology following ethylene treatment, including reduced apical hook formation. This is consistent with PA inhibiting CTR1 kinase activity *in vitro* and suggests a positive effect of PA on ethylene responses. Based on the biochemical and physiological evidence obtained, a model will be presented for the role of PA in ethylene signaling.

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162 WRKY target genes of WRKY53 transcription factor during leaf senescence in Arabidopsis thaliana

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Senescence is not a chaotic breakdown but highly regulated and leads to death of whole organs or eventually the entire plant. Only few data is available concerning regulatory factors controlling leaf senescence. The Arabidopsis WRKY transcription factor family is involved in the regulation of gene expression during pathogen defense, wounding, trichome development, and senescence. It was already shown that WRKY53 plays a key role in early senescence (Miao *et al.*, 2004). By using recombinant WRKY53 protein and genomic DNA we isolated several candidate target genes including other transcription factors, also members of the WRKY family like WRKY06, WRKY13, WRKY15, WRKY18, WRKY22, WRKY29, WRKY42, and WRKY62. The binding of different WRKY promoters by recombinant WRKY53 protein was confirmed in vitro. Furthermore it was shown that WRKY53 could act either as transcriptional activator or transcriptional repressor and that WRKY53 negatively controls its own expression. Altering expression of WRKY53 in overexpressing and knockout lines resulted in altered expression of the WRKY target genes. Promoter-reportergene-lines and results of northern blot analyses revealed similar expression patterns between *WRKY53* and its target genes. To examine whether there is a regulatory network, we analyzed WRKY expression in some overexpressing WRKY transgenic plants and several knock out lines by RT-PCR or northern blot analysis.

References

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163 Non-cell-autonomous Signaling and Developmental Control Mediated by AtPAPK1

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One mode of intercellular communication in plants involves trafficking of macromolecules between cells through specialized intercellular channels, termed plasmodesmata. This exchange of proteins and RNA is thought to be important in orchestrating physiological and developmental programming in a non-cell autonomous manner. Although little information is available at the molecular level, protein phosphorylation has been implicated as one potential mechanism regulating this fundamental process. As a direct test for this hypothesis, we have isolated a putative plasmodesmal-associated protein kinase (PAPK) from tobacco cells. Importantly, PAPK phosphorylated, in vitro, a subset of viral and endogenous non-cell-autonomous proteins including Tobacco mosaic virus movement protein (TMV MP) and Arabidopsis transcription factor, LEAFY. Subcellular localization studies identified possible Arabidopsis PAPK homologs including PAPK1. Targeting of AtPAPK1 to plasmodesmata was suggested by co-localization with TMV MP of which plasmodesmal association is established (Lee, J.-Y., Taoka, K., Yoo, B.-C., Ben-Nissan, G., Kim, D.-J., and Lucas, W.J., 2005, manuscript submitted). AtPAPK1 appeared to associate with vesicle-like structure which moves along the cytoskeleton. These results suggest that targeting of AtPAPK1 to plasmodesmata may involve vesicles and cytoskeletal components. Furthermore, ectopic expression of AtPAPK1 in Arabidopsis severely altered the growth and developmental patterns of the transgenic plants suggesting important role of PAPK1 in non-cell autonomous signaling. Thus, AtPAPK1 represents a novel plant protein kinase, targeted to plasmodesmata, that likely participates in regulating macromolecular trafficking between plant cells. The use of AtPAPK1 as a valuable molecular tool for dissecting the plasmodesmal targeting mechanism and its role in non-cell-autonomous signaling will be discussed.

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164 PINOID acts as a positive regulator for auxin efflux

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Auxin is the unique phytohormone that moves organ-to-organ with high polarity. This polar movement is achieved by asymmetric localization of auxin-transporting (efflux or influx) proteins in the plasma membrane. PINs represent the auxin efflux carriers, and their molecular actions are likely to be linked with PINOID (PID), a serine/threonine protein kinase. We have studied to identify the detailed relationship between PID and PINs in auxin physiology. Because root hair development is sensitively affected by auxin, the Arabidopsis root hair single cell system was adopted for our experimental purpose. Overexpression of PID (PIDox), specifically in the hair cell using the AtEXP7 promoter, suppressed root hair development. This led us to hypothesize that PIDox depleted the intracellular auxin level by facilitating the activity of auxin efflux carriers, which subsequently resulted in the suppression of root hair development. Pharmacological studies with PIDox transformants consistently showed that the PID effect on root hairs is closely associated with auxin efflux activity. Root hair-specific overexpression of a PIN gene (PINox) also strongly suppressed root hair development, further supporting the results acquired with PIDox transformants. In addition, subcellular localization study of PID and PIN using GFP fusion technique and pharmacological methods indicates that the two proteins would interact in proximity. Our results demonstrate the auxin efflux activity in the in vivo single cell level and suggest that PID acts as a positive regulator of auxin efflux carriers.

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165 Effects of Increased ER-localized Ca2+on Recovery From Osmotic Stress

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Developing drought tolerant crop plants is an important agricultural goal because of the increasing global demand for water and declining aquifer reserves. Several calcium regulated protein kinases have been identified that affect drought tolerance. These kinases are regulated by transient calcium oscillations in the cytosol of plant cells that occur in response to a wide range of environmental stimuli. Cytosolic calcium concentration is maintained at a low level by Ca2+ transport channels and calcium buffering proteins localized in the intracellular compartments. We used a calcium binding peptide (CBP) derived from calreticulin to increase calcium levels in the endoplasmic reticulum (ER) (1,2). The CBP was fused to the green fluorescent protein (GFP) and expressed in Arabidopsis. Compared to GFP control and wild type plants, the CBP plants showed increased root growth and better survival under drought conditions in sorbitol containing medium and in soil. Analysis of gene expression demonstrated that CBP expression in the ER increased transcription of some drought-inducible genes under normal growth conditions. These results identify a new link between intracellular calcium levels, root growth, and drought tolerance.

166 Modulation of Brassinosteroid Signaling by an Atypical Basic Helix-Loop-Helix Protein

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Brassinosteroids (BR) are a unique class of plant polyhydroxysteroids that regulate many aspects of plant growth and development. Genetic and molecular approaches have led to discoveries of several key components of the BR signal transduction pathway, including the transmembrane BR receptor BRI1. To identify additional regulators of plant steroid signaling, we carried out an activation-tagging-based genetic screen and identified several Arabidopsis genes whose overexpression suppress the weak *bri1-301* mutation. One of them, *activation-tagged bri1 suppressor 1* (*ABS1*), encodes an atypical basic helix-loop-helix nuclear protein. Genetic study indicated that ABS1 requires BRs and a partial-functional BRI1 to promote plant growth. Interestingly, overexpression of a truncated ABS1 that lacks the basic motif was still able to suppress the *bri1-301* mutation, suggesting that ABS1 modulates BR signaling through sequestering a yet-to-be identified negative regulator. Further investigation of ABS1 and identification of ABS1-interacting proteins will increase our understanding of the nuclear activities of the BR signaling pathway.

167 Hormonal cross-talk and signalling to the Arabidopsis microtubule cytoskeleton requires the POLARIS peptide

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The rate and plane of cell division and anisotropic cell growth are critical for correct development in plants, and are regulated by diverse input mechanisms, involving cross-talk between several hormone signalling pathways. This is clearly evident in for example the *Arabidopsis* root, where cell patterning, expansion and differentiation are axially organized and influenced by auxins, cytokinins, ethylene and gibberellins. The microtubule cytoskeleton is a key mediator of axial cell growth, and is itself a target for hormonal signals. We show that the *POLARIS* (*PLS*) gene of *Arabidopsis*, which encodes a 36 amino acids polypeptide, is required to restrict ethylene signalling to the microtubule cytoskeleton, to allow corrrect cell division and expansion, auxin and cytokinin signalling and root growth. *pls* mutant seedlings exhibit a short root, which can be rescued by the genetic and pharmacological inhibition of ethylene signalling. Free IAA levels and polar auxin transport are reduced in the mutant, which are also rescued in *pls etr1-1* double mutants, showing a role for aberrant ethylene signalling in auxin transport and accumulation. Significantly, *pls* shows reduced responses to the microtubule inhibitors amiprophos methyl and oryzalin, but *pls etr1* double mutants show the response restored to wild-type, and *PLS* overexpressers show enhanced responses. This represents the first evidence of a role for small polypeptides in regulating cross-talk between hormones and the cytoskeleton to regulate growth in plants.

168 Plant Responses to Stresses: Transcript Profiling of Stress/Defense-Related Genes of Arabidopsis thaliana

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Plants, in their natural environment are continuously challenged by pathogens, pests and several other biotic and abiotic stresses. Plant hormones salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) have been implicated in regulating defense mechanisms in plants. These defense mechanisms may be deployed independently or in networking via cross-talk with each other to build up an effective resistance depending on the nature of the pathogen. The identification and characterization of molecular components and genes that underlie plant defense/stress mechanisms is of great significance. In order to characterize the stress/defense response responses, we prepared cDNA microarrays containing about 1600 PCR amplified cDNAs, mainly derived from cDNA libraries enriched for genes differentially expressed in response to variety of biotic and abiotic stresses. These cDNA microarrays were used to identify the stress/defense transcriptome of Arabidopsis grown under various biotic and abiotic stresses to examine the differences and cross-talk between their signaling cascades. Our results demonstrate existence of a substantial network of regulatory interaction and coordination occurring among different plant defense pathways. Comparison of these expression profiles allowed us to identify both shared and stimulus-specific responses. The data from these experiments will be presented.

169 Auxin and TAC1 regulate telomerase through a novel ubiquitin ligase

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Telomeres are specialized nucleoprotein complexes at the ends of eukaryotic chromosomes that are essential for proper chromosome behavior and stability of the genome. Telomerase, a complex ribonucleoprotein with reverse transcriptase activity, synthesizes and maintains telomeric DNA in most eukaryotes. Mutations that affect expression or activity of telomerase inexorably lead to catastrophic destabilization of the genome. Our goal is to identify and characterize genes that maintain genome stability by regulating telomerase activity. We previously identified TELOMERASE ACTIVATOR1 (TAC1) as a transcription factor whose overexpression induces telomerase activity in mature leaves of Arabidopsis, tomato, and rice, without activating the cell cycle. Induction of telomerase by TAC1 can be prevented by reducing concentrations of auxin in Arabidopsis through transgenic expression of iaaL, a bacterial protein that conjugates free IAA to lysine. Other results, including resistance of primary roots to exogenous auxin in *TAC1*-null mutants and auxin-independent growth of callus overexpressing TAC1, indicate that TAC1 not only is part of the previously described link between auxin and telomerase expression, but also potentiates other auxin responses. We recently identified *AtBT2*, a gene encoding a BTB-domain protein, as a direct target of TAC1. Overexpression of AtBT2 alone is sufficient to induce telomerase in vegetative organs. AtBT2, like other BTB-domain proteins, appears to be part of a cullin3-based ubiquitin ligase complex, distinct from the cullin1-based ubiquitin ligase known to mediate many other aspects of auxin signaling. We hypothesize that AtBT2 directs the modification of a telomerase regulator. AtBT2 contains a calmodulin-binding domain at its C-terminus, which provides a plausible connection to auxin in the TAC1/AtBT2 pathway. Because exogenous auxin can increase concentrations of cytosolic calcium, one explanation for the requirement of both TAC1 and auxin in activating telomerase is that TAC1 is necessary to induce transcription of *AtBT2*, and auxin is necessary to maintain a sufficient calcium concentration for AtBT2 function. Treatment with the calcium ionophore A23187 bypassed the requirement for auxin in the TAC1/AtBT2 signaling pathway, presumably by increasing the cytosolic calcium concentration. Together, these results suggest that telomerase activity can be induced in an auxin-dependent manner through a novel ubiquitin ligase complex. Furthermore, auxin appears to affect calcium signaling in this pathway.

170 Identification of genes required for Age-Related Resistance response using a Microarray/Reverse Genetics approach

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Plants respond to pathogen attack with a number of different responses, including Age-Related resistance (ARR). Many plant species, including *Arabidopsis*, become resistant to normally virulent pathogens as they mature. In *Arabidopsis*, growth of *Pseudomonas syringae* pv *tomato* (*Pst*)can be suppressed from 10 to 100-fold in plants expressing ARR. ARR in *Arabidopsis* is a developmentally-regulated pathogen-induced and environmentally-sensitive response. ARR has been correlated with the transition to flowering in some cases or with senescence in others and also with the accumulation of secondary metabolites or defense proteins. ARR in most pathogen-plant interactions has been studied in a descriptive or preliminary fashion except for studies done in *Arabidopsis* in our lab and in tobacco and pepper. SA accumulation is required for ARR, as demonstrated by the ARR-defective phenotypes of the SA-deficient lines, *sid1, sid2*, & NahG. However, SA probably doesn't play its usual signaling role to activate downstream defense gene expression via NPR1, as ARR occurs in the *npr1-1* mutant. Also intercellular washing fluids from plants expressing ARR exhibit anti-bacterial activity to *Pst*, suggesting that SA may accumulate in intercellular spaces and act as an anti-microbial agent during ARR. Both SID1 and SID2 are required for ARR, however we predict that other genes are also necessary for ARR, including a developmentally regulated receptor that becomes activated when *Arabidopsis* matures, allowing the plant to respond to normally virulent *Pst* in a highly resistant manner. A microarray experiment identified a number of genes up-or down-regulated during ARR. We combined the ability to identify many ARR-associated genes (microarray) with reverse genetics (start with gene sequence, then find mutants) by searching the TAIR T-DNA insertion lines. T-DNA insertion lines were identified for some of the ARR up-regulated genes and some of these lines displayed ARR-defective phenotypes, suggesting that these genes play a role in the ARR response. Additionally, classical mutant screening has identified an ARR-defective neutron mutant (*P17*) that can be partially rescued by SA application, and responds normally to *Pst* infection. The semi-dominant nature of *P17* suggests that wild type plants may encode an early positive regulator in the ARR pathway. Functional analysis of the ARR T-DNA insertion mutants is currently underway, and progress towards mapping the *P17* gene will be discussed.

171 Functional differentiation of Aux/IAA proteins, IAA7 and IAA19, involved in auxin responses in Arabidopsis

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Aux/IAA proteins repress activities of auxin response factors (ARF) by forming Aux/IAA-ARF heterodimers. Gain-of-function mutants in seven *Aux/IAA* genes show some overlapping phenotypes, but they are clearly distinct from each other, indicating functional differentiation in *Aux/IAA* genes. Because yeast two-hybrid assay shows promiscuous Aux/IAA-ARF interactions, it may be the specific patterns of gene expression that determine distinct phenotype of each mutant. In an attempt to check this assumption, we compared phenotypes of transgenic Arabidopsis plants which express the mutant cDNA derived from *msg2-1*, *slr-1*, and *axr2-1* (*mIAA19*, *mIAA14*, and *mIAA7*, respectively), under the control of the 2.0 kb promoter of *IAA19* or *IAA7* (*pIAA19* and *pIAA7*, respectively). Under the control of *pIAA19*, *mIAA19* reproduced *msg2-1* phenotypes including defects in tropic responses and low frequency of lateral roots, but it did not cause low fecundity unlike *msg2-1*. The plants which expressed *mIAA7* under the control of *pIAA7* reproduced the dwarfism and agravitropism like *axr2-1* in aerial parts, but it displayed no aberrant phenotypes in roots although *axr2-1* showed root agravitropism, more abundant lateral roots, and smaller number of root hairs. Thus, we confirmed that both *mIAA19* and *mIAA7* transgenes partially reproduced their mutant phenotypes, when they expressed under the control of their own promoters. However, *mIAA19* expression under the control of *pIAA7* had no effects on morphology, although it caused defects in tropic responses. On the other hand, expression of *mIAA14* under the control of *pIAA7* reproduced *axr2-1* phenotypes weakly. Thus, functions of *mIAA19* differed from those of *mIAA7* and *mIAA14*. This result is consistent with a phylogenetic evidence that *IAA7* and *IAA14* are most closely related in *Aux/IAA* genes. Transgenic plants which expressed *mIAA14* under the control of *pIAA19* showed aberrant development of cotyledon; seedlings of the transgenic plants with the most severe phenotype completely lacked cotyledons, and had the fused first leaf. They closely resembled *pin-formed1 pinoid* double mutants (Furutani et al., 2004). The aberrant development of cotyledon has never been observed in either *msg2*, *slr*, or *pIAA19::mIAA19* plants. These suggest that effects of ectopic expression of *mIAA14* by *pIAA19* during embryonic development are clearly different from that of *mIAA19*. These results show functional differentiation between products of *IAA19* and a pair of sister genes, *IAA7* and *IAA14*.

172 Defining a Role for Members of the Arabidopsis Aux/IAA Gene Family

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In the *Arabidopsis* genome, there are 28 *Aux/IAA* genes that encode structurally related proteins. Plants that lack the expression of various members of the *Aux/IAA* gene family have been identified. Interestingly, only subtle, tissue- specific changes in phenotypes have been reported for some of these loss-of-function plants. We have focused on three genes (*IAA5, IAA6, IAA19/MSG2*) whose protein products are more closely related to one another than they are to other members of the Aux/IAA protein family. Plants lacking the expression of each of these genes look like wild type when grown under greenhouse conditions. Given that multiple genes encode similar proteins, one explanation for the wild type growth patterns is that other members of the Aux/IAA protein family compensate for the loss of these three genes. Interestingly, in plate-based assays the triple loss-of-function seedlings generate more adventitious roots, which is consistent with the model that the Aux/IAA proteins are negative regulators of auxin-mediated events. Given this subtle phenotype, differences in the spatial, temporal, or hormonal responses of these three genes during plant growth and development could support the notion of overlapping function. In order to better define and compare the expression patterns of *IAA5, IAA6, and IAA19/MSG2*, we have established a quantitative real-time PCR assay to monitor the kinetics, specificity, and dose-dependent changes in the steady state mRNA levels of these genes. In addition, we are constructing transgenic lines harboring promoter-reporter gene (nuclear localized GFP-GUS) constructs. Using these two systems we are determining the effect that various hormonal and environmental stimuli have on the expression patterns of these genes.

173 brassinosteroids signaling through the multi-heterodimerization between two different families of leucine rich-repeats receptor-like kinases

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Since the identification of BRI1, 25 leucine rich-repeats containing receptor-like serine/threonine kinase (LRR-RLK), as an essential component mediating BR signaling, several additional critical proteins have been reported in recent years including BAK1, a receptor pair with BRI1 in plasma membrane, BIN2, a cytoplasmic negative regulator of BR trasnsduction, nuclear proteins such as BES1/BZR2, BZR1 and their homologs and interactors with transcriptional activation properties, which is making fine web of BR signaling. Moreover, restricted expression pattern in vascular cells of BRI1 homologs, *BRL1* and *BRL3*, indicates the possibility that each plant cell or tissue might have specific subsets of proteins responsible for BR signaling from perception in plasma membrane. To teat this possibility, we investigated that whether the BAK1 homologs, another members of LRR-RLKa! subfamily, can form heterodimers with BRI1 and mediate BR signaling in a similar way as BAK1 did. Full-length BAK2 and BAK5 expressed in yeast showed specific interaction with BRI1 in co-immunoprecipitation experiments and the phosphorylated forms of receptor pairs were impaired when BRI1 was co-immunoprecipitated with the catalytic domain-defective BAK2. Not only the transgenic *bak1* plants containing *BAK2*, *BAK3* or *BAK4* under the *BAK1* promoter rescued characteristic bak1 phenotype with round rosette leaves and short petioles, but also overexpression of BAKs in wild type or weak *bri1-301* plants induced the constitutive BR-responsive phenotype. Given the results from biochemical and transgenic approaches, it is thought that BRI1 can form heterodimer complexes with many BAKs as well as with BAK1, and these specific interactions would lead to the subsets of BR responses in specific plant tissues, even though the different binding affinity of each BAK to BRI1 should be further studied.

174 Activation-tagged suppressors of *phyB-4* identify points of cross-talk between hormones and photomorphogenesis

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Activation tagging, a gene-overexpression mutagenesis tool, has been used to identify extragenic suppressors of the long-hypocotyl phenotype conferred by the photoreceptor mutant *phyB-4*. Seven of these *sob-D* mutants (suppressor of phyB- dominant) have been identified and cloned in the Neff lab to date, some of which implicate cross talk between various hormone signaling pathways and photomorphogenic development. The *sob7-D* mutant phenotype is caused by the over-expression of a brassinosteroid-inactivating cytochrome P450, CYP72C1. Brassinosteroid hormones have also been implicated in photomorphogenesis based on the characterization of a previously identified phyB activation-tagged suppressor, *bas1-D*. We generated single and double null-mutants of *BAS1* and *SOB7* to test the hypothesis that these two genes modulate photomorphogenesis. *BAS1* and *SOB7* act redundantly or synergistically with respect to light promotion of cotyledon expansion, repression of hypocotyl elongation and flowering time in addition to other phenotypes not regulated by light. We also provide biochemical evidence to suggest that *BAS1* and *SOB7* act redundantly to reduce the level of active brassinosteroids such as brassinolide and castasterone, but have unique mechanisms. Overexpression of *SOB7* results in a dramatic reduction in endogenous castasterone levels, and although single null-mutants of *BAS1* and *SOB7* have the same level of castasterone as the wild type, the double null-mutant has twice the amount. Application of brassinolide to overexpression lines of *BAS1* or *SOB7* results in enhanced metabolism of brassinolide, though only *BAS1* overexpression lines confer enhanced conversion to 26-hydroxybrassinolide, suggesting that SOB7 and BAS1 convert brassinolide and castasterone into unique products. Computer modeling of interactions between these two P450s and brassinolide supports our hypothesis that BAS1 acts as a carbon-26 hydroxylase whereas SOB7 is likely to hydroxylate one or more other carbons of this steroid. Yeast and Arabidopsis brassinolide-feeding experiments will be used to further test these models.

175 ginC, a new locus regulating glucose responses

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Studies carried out in recent years have revealed that plants use diverse signaling pathways to sense the environmental conditions and to generate adaptative responses. These signals do not act independently, but in an integrated way with other pathways regulating the metabolism and the development of the plant. A clear example of this integration is represented by the glucose signaling pathway of glucose, which not only generates metabolic responses according to the sugar concentration in the plant but also regulates diverse processes related to the development and the adaptation to abiotic stress situations. We are studying the signaling mechanism of glucose in plants with the aim of understand how different regulatory signals interact in the plant, and developing biological tools that may contribute to improve crop production. For this purpose, we have carried out different screenings to identify gin mutations amongst a collection of Arabidopsis T-DNA insertions. These analyses have identified a new mutation that we have called ginC. The phenotypic characterization of ginC shows alterations in the mutant plant responses to osmotic and saline stress conditions. The seeds ginC are able to germinate in a high salt medium; nevertheless the adult plants display a wilty phenotype, showing a high sensitivity to osmotic stress. Molecular analyses show that ginC mutation affects a dual-phosphatase that we have denominated Ptp2, (although the mechanism of action of this phosphatase remains unknown). Our data indicate that over-expression of the cloned gene ptp2 can complement the ginC mutation, and even give plants to phenotypes opposite to those of the ginC mutant. Plants that over-express ptp2 are more sensitive to the presence of glucose and salt in the medium, and at the same time more resistant to drought that the wild-type plants. Thus, the results obtained in Arabidopsis suggest an important function of the Ptp2 protein in the tolerance response to stress.

176 Structure-Function Studies of *Arabidopsis thaliana* NPH3

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Genetic studies have identified a number of proteins functioning in the signal-response system(s) mediating phototropism in *Arabidopsis thaliana*. One such protein is NPH3, which encodes a novel protein that interacts with the dominant photoreceptor mediating phototropism, phot1. We have taken a multi-faceted approach to identify the biochemical function(s) of NPH3. First, we have generated a number of mutations (both missense point mutations and small peptide insertions) throughout that NPH3 protein and have found that several appear to represent temperature-sensitive (ts) partial loss-of-function alleles; thus revealing domains whose proper conformations are presumably critical for function. Second, we have used two of these ts alleles to identify second-site suppressor mutations that "cure" the initial loss-of-function phenotypes. The ts-nature of the initial mutations suggests that these alleles may affect protein-protein interactions, and thus second-site suppressor are likely to encode proteins that normally interact with NPH3. Third, we are attempting to produce sufficient quantities of highly purified NPH3 for crystallization and subsequent 3-D structure studies. We are currently utilizing *Pichia pastoris* to express His-tagged NPH3 and subsequent chromatography and electrophoresis methods to make highly enriched samples. By obtaining a crystal structure of NPH3 we will be in an ideal position make sense of our genetic studies and to predict further regions of the protein to target for mutagenesis and functional studies. In the end, the structure-function studies described here are aimed at the identification additional members of the phot1-NPH3 signaling complex that will provide important insights into the biochemical function(s) of NPH3.

177 Tissue specific combinatorial control of the ABA response in seeds revealed by expression analysis of the endosperm during germination

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We have shown that the Arabidopsis endosperm plays an active and vital role during seed germination and seedling establishment. Furthermore, we have also demonstrated that lipid breakdown in germinating seeds is inhibited in the embryo by ABA, but not the endosperm, while gibberellins are required in both tissues. Here we report the transcript profiling of endosperm and embryo in germinating Arabidopsis seeds, and the transcriptome response to germination inhibition by ABA and paclobutrazol in both tissues. Transcript profiling of endosperm tissue identifies known and novel endosperm specific transcripts, many of which are also expressed during seed development. We show that while key enzymes in lipid mobilisation are regulated at the level of transcription this is not reflected in the total transcript abundance, and present evidence that this represents selective translation of post-germinatively synthesised transcripts. We also show that at the transcriptional level that both the GA and ABA biosynthetic pathways are present in endosperm tissues suggesting that the endosperm could contribute significantly to overall seed phytohormones. Global transcript analysis supports the view that the ABA response in the endosperm differs markedly from that in the embryo, and reveals the absence of the key ABA signal transduction component ABI4 in the endosperm. The expression patterns of ABI3, ABI4 and ABI5 suggest a model for the differential ABA control of gene expression in the seed.

178 WVD2, a novel microtubule binding protein affecting plant anisotropic cell expansion, morphology and helical handedness

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Arabidopsis *WVD2* was identified by a forward genetic screen as a gene that causes plant and organ stockiness and inversion of helical handedness upon over-expression. Plants with high constitutive expression of *WVD2* are short and thick, show reduced anisotropic cell elongation, dampened root waving on hard-agar surfaces, and have inverted cell file rotation in hypocotyls and roots compared to wild-type. Over-expression of *WVD2* also results in increased thigmomorphogenesis, a developmental reduction in size in response to regular mechanical stimulation. WVD2 is a small (23 kD calculated mol wt), highly charged protein and contains a domain found in TPX2, an unconventional microtubule-binding protein first characterized in vertebrates. Recombinant GST-tagged WVD2 bundles and co-sediments with microtubules in vitro. Localization of WVD2 using 35S::WVD2-GFP transgenic plants and immuno-confocal analysis shows signal typical of cortical microtubules in epidermal cells of leaves, hypocotyls and roots and guard cells of leaves. We will report data on the regulation of WVD2 and WVD2-like (WDL) proteins in response to hormone signaling. WVD2 and other WDL family members comprise a group of novel microtubule-associated proteins that regulate the dimensions and handedness of cells, organs, and ultimately the plant body.

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179 Regulation of light-harvesting complex II phosphorylation via reactive oxygen species in chloroplasts

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The photosynthetic light reactions generate reducing equivalents and reactive oxygen species (ROS) that possess counteracting reducing and oxidizing effects on the reduction state of chloroplasts. To prevent oxidative damage, plants have evolved a complex network of antioxidant systems as well as processes for thermal dissipation of excess light energy. It has also become evident that chloroplast-derived ROS have a crucial function in the cross signaling of various stresses in plant cells. The chloroplast redox state is delicately reflected in the phosphorylation of the Photosystem II light harvesting antenna (LHCII) proteins. The LHCII kinase is activated in light by reduction of cytochrome *b6/f* complex in thylakoid membrane. However, gradual down-regulation of LHCII protein phosphorylation occurs with increasing light intensities, and even short-term oscillations in the chloroplast redox conditions are reflected in the level of LHCII protein phosphorylation. Such dynamic modulations are mediated via thiol-redox regulation involving a thioredoxin-like inhibitory compound and hydrogen peroxide (H_2O_2). Our present study focuses on the role of ROS and their scavenging enzymes in the complex regulation of chloroplast metabolism, and particularly LHCII protein phosphorylation in vivo. In chloroplasts, H_2O_2 is actively formed by photoreduction of oxygen via Photosystem I and superoxide dismutase activities. Chloroplast ascorbate peroxidases (APXs) reduce H_2O_2 to water using ascorbate as an electron donor, and thus play an important role in the complex detoxification systems for H_2O_2. We analyzed Arabidopsis thaliana *npq4-1* mutants deficient in the dissipation of thermal energy, and *t-apx* and *s-apx* mutants lacking the thylakoid-bound and stromal forms of APX, respectively. No significant differences in growth rate, leaf thickness, chlorophyll content or the level of LHCII protein phosphorylation were observed between the mutant and wild type lines after long-term growth under different light intensities. However, upon a shift of plants to different light regimes, the *npq4-1* and *s-apx* plants were able to maintain LHCII protein phosphorylation at light intensities that induced inhibition of phosphorylation in wild type leaves. We propose that accumulation of H_2O_2 in chloroplasts is involved in the maintenance of LHCII protein phosphorylation under elevated light intensities in vivo. The complex redox control of the LHCII protein phosphorylation in relation to H_2O_2 accumulation and high light acclimation will be discussed.

180 The temperature-sensitive CUL1 allele, *axr6-3* identifies previously unknown SCF-regulated signaling pathways

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Selective protein degradation by the ubiquitin-proteasome pathway has emerged as a key regulatory mechanism in a wide variety of cellular processes. The selective components of this pathway are the E3 ubiquitin-ligases which act downstream of the ubiquitin-activating and -conjugating enzymes to identify specific substrates for ubiquitinylation. SCF-type ubiquitin-ligases are the most abundant class of E3 enzymes in Arabidopsis. In a genetic screen for enhancers of the *tir1-1* auxin response defect, we identified *eta1/axr6-3*, a temperature-sensitive mutation in the CUL1 core component of the SCF^{TIR1} complex. The *axr6-3* mutation prevents CUL1 from incorporating into the SCF complex and therefore disrupts SCF complex assembly. In contrast to the previously described *axr6-1* and *axr6-2* alleles, which are semi-dominant and exhibit a seedling-lethal homozygous phenotype, *axr6-3* is a recessive, temperature-sensitive mutation. *axr6-3* displays a pleiotropic phenotype with defects in numerous SCF-regulated pathways including auxin signaling, JA signaling, flower development, and photomorphogenesis. Essentially all of the physiological and molecular phenotypes of *axr6-3* are substantially enhanced by growth at elevated temperatures. We used *axr6-3* as a tool to identify pathways previously unknown to be regulated by SCF-mediated proteolysis and propose new roles for SCF regulation of the far-red light/phyA and sugar signaling pathways. The recessive inheritance and the temperature-sensitive nature of the pleiotropically acting *axr6-3* mutation opens promising possibilities for the identification and investigation of SCF-regulated pathways in Arabidopsis.

181 Characterization of an ectopic cell separation mutant *tfa1-2 'things fall apart'*

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The plant cell wall is dynamic, responding to developmental and environmental cues, and essential for plant structure and basic development. Processes such as cell to cell adhesion and cell separation are examples of this dynamic nature of the plant cell wall. We have identified several ectopic cell separation mutants that we have designated *tfa "things fall apart."* These mutants are all characterized by unregulated cell separation in epidermal, cortical and vascular tissues. This process is most severe in young seedlings affecting the hypocotyl, cotyledon, and first true leaves. Crosses among the mutants indicate that two of these genes are allelic and recessive mutations, and we have designated them *tfa1-1* and *tfa1-2*. *tfa 1-2* was identified from an EMS screen of Arabidopsis seedlings and PCR-based mapping indicates that it is positioned on the right arm of Chromosome I. Expression of several cell wall associated genes has been analyzed by RT-PCR and several show changes in expression in mutant background. Microarray analysis of *tfa1-2* is being done. We will present phenotypic, physiological and molecular characterization of tfa1. We propose that there are several regulatory pathways affecting cell separation programs in the plant. TFA1 may represent a gene regulating a repressor system.

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182 AtPLC5 encodes a plasma membrane associated phosphatidylinositol-specific phospholipase C, that requires for ABA sensory in Arabidopsis

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Phosphoinositide-specific phospholipase Cs (PI-PLCs) are important enzymes in eukaryotes which catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into the two second messengers inositol 1,4,5-trisphosphate and diacylglycerol. A cDNA of the *AtPLC5* protein is similar to those of plant PI-PLCs and animal PI-PLCζ(zeta)(Saunders et al. 2002). The 6 x His-tagged AtPLC5 was expressed in*E.coli* cells, and purified. The recombinant AtPLC5 protein can hydrolyze the substrate PIP2. Hydrolysis PIP2 was Ca^{2+}-dependent. The optimum Ca^{2+} concentration for the enzyme activity was found to be 10μmol/L. The N-terminal region of AtPLC5 was expressed as a recombinant protein and used in generating polyclonal antibody that specifically recognized AtPLC5. Western blot analysis of the cell fractions prepared from*Arabidopsis*plants indicated that the AtPLC5 protein is associated with plasma membrane. Northern blot and promoter-GUS activity analysis shown that, AtPLC5 was expressed mainly in pollen and vasculature tissues of leaves under normal growth condition, and strongly induced in root tips by exogenous ABA. Transgenic lines expressing*AtPLC5* antisense show no growth inhibition by ABA and lines over-expressing AtPLC5 sense show more sensitive to ABA. Results suggest that AtPLC5 encodes a functional membrane associated protein that involves in ABA sensory of *Arabidopsis* plants.

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183 RTE, a novel regulator of the ethylene receptor ETR1 in Arabidopsis

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Ethylene is a gaseous plant hormone that has profound effects on plant growth and development. Genetic analysis has been key to elucidating the ethylene signaling pathway. The isolation of ethylene response mutants in *Arabidopsis* has relied upon genetic screens for insensitive or constitutive response in dark-grown seedlings.

In order to identify new components of the ethylene signaling pathway, a screen was carried out for suppressors of the dominant ethylene insensitive receptor mutant *etr1-2*. *etr1-2* confers weak ethylene insensitivity, and unlike other insensitive receptor alleles, the mutant protein ETR1-2 can still bind ethylene. This screen led to the identification and cloning of the *REVERSION-TO-ETHYLENE –SENSITIVITY-1 (RTE1)* locus. Loss of *rte1* function results in suppression of the *etr1-2* insensitive phenotype, but is unable to suppress the insensitivity caused by stronger dominant mutant receptors that do not bind ethylene. In addition, *rte1* single mutant analysis revealed a phenotype reminiscent of the *etr1* null mutant. Further genetic analysis suggests *RTE1* has a role in the regulation of the ETR1 receptor.

RTE1 is predicted to encode a novel integral membrane protein with homologues in other plants and animals, including one copy in humans. Sequence analysis reveals two highly conserved regions containing invariant cysteine and histidine residues, which are common in metal binding proteins. *Arabidopsis* has a second copy of *RTE*, which we have named *RTE2* (identities (50%), positives (68%)).

In order to further examine *RTE* function, two approaches have been initiated, one is to characterize the *rte2* single mutant and the double *rte1 rte2* null mutant, and the other is to examine ETR1 protein stability and localization in an *rte1* and/or *rte2* background. To date there is no detectable phenotype for the *rte2* null mutant, nevertheless there is a striking phenotype in the dark and light for *rte1 rte2* double null seedlings. Moreover ETR1 abundance does not appear to change in *rte1* plants suggesting other possible mechanisms of regulation.

184 The SUMOylation pathway is required for plant growth

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The covalent attachment of small polypeptides to other proteins has been receiving increasing attention as an important method for post-translational control. Following the discovery of the first member of this group - ubiquitin over 20 years ago, a number of structurally related tags have been identified with diverse roles in various cellular processes. One of these is the SMall Ubiquitin-like Modifier (SUMO). Sumoylation can affect protein localization, protein-protein interactions, and/or protect proteins from ubiquitination and degradation by the 26S proteasome. We previously identified a number of expressed SUMO isoforms in Arabidopsis and detected genes encoding components of the SUMO conjugation pathway that activate and ligate SUMO to proteins. In *S. cerevisiae*, the SUMO pathway is essential. Similarly, analysis of T-DNA lines has revealed that sumoylation is required for the viability of Arabidopsis. Loss of either of the single copy genes encoding C-terminal portion of the SUMO-activating enzyme (SAE2) or the SUMO-conjugating enzyme (SCE1) leads to embryo lethality. SUMO1 and 2 are highly homologous isoforms (92% identical) that become rapidly conjugated to other proteins following various stresses, suggesting they play a role in the stress response. Like *sae2* and *sce1* mutants, double *sum1 sum2* mutant plants are also inviable. Previous reports have shown that sumoylation also regulates flowering time and ABA signaling. Given the number of SUMO functions, it is expected that numerous regulators of plant growth and development are targets. We are working to characterize the targets of the SUMO pathway to further define its role in plant biology.

185 Phosphorylation of NtMAP65-1a by NRK1 MAPK controls microtubule organization

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The NACK-PQR MAP kinase pathway, which is composed by NACK1 kinesin-like protein, NPK1 MAPKKK, NQK1 MAPKK and NRK1 MAPK, is activated during the cytokinseis in cell division cycle of tobacco and arabidopsis. These all components are localized on the equator of phragmoplast that is a plant specific apparatus organized by microtubules during the cytokinesis. Previous results from our laboratory are suggested that the NACK-PQR pathway controls the phragmoplast expansion followed by cell plate formation (1-6). In the present paper, we searched the target proteins that might be phosphorylated by NRK1 MAPK in vitro. We have identified three microtubule associated proteins (MAPs) including NtMAP65-1a that belongs to the Ase1/PRC1/MAP65 family. Some proteins of this family are enriched at the spindle midzone in animal and at the phragmoplast in plants. We defined the phosphorylation sites by NRK1 in NtMAP65-1a, and examined effects of the non-phosphorylated or phosphorylated proteins on microtubule bundling in vitro. Arabidopsis have nine genes belonging to the Ase1/PRC1/MAP65 family, and we also examine whether some of these members can be phosphorylated by NRK1 arabidopsis homolog. We discuss the biological function of NtMAP65-1a phosphorylated by NRK1, and roles of the MAP65 family proteins during cytokinesis.

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186 26S Proteasome-Dependent Cytokinin Signaling

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The Ubiquitin/26S Proteasome Pathway (UPP) controls most of targeted proteolysis, including the conditional removal of key activator and repressor proteins in hormone signal transduction cascades. Regulatory proteins that are degraded by the UPP in response to changes in hormone concentrations have been identified in the abscisic acid, auxin, brassinosteroid, ethylene and gibberellic acid response pathways.

The 26S proteasome mutants *rpn10-1* and *rpn12a-1* have decreased cytokinin sensitivity and offer indirect evidence for proteasome-dependent control of cytokinin signaling. Since both mutations are recessive, it was concluded that the 26S proteasome mediates the degradation of a repressor of cytokinin action [1, 2].

To identify this putative repressor and the factors that control its degradation rate, we have isolated mutations that modify the cytokinin insensitivity of *rpn12a-1*. The *suppressor of rpn12a (sor)* mutations include: (1) intragenic suppressors that have wild type development and cytokinin responses indicating that RPN12a function is restored, (2) extragenic suppressors with a wild type cytokinin response, and (3) extragenic suppressors with a constitutive cytokinin response. Analysis of *sor3*, an extragenic suppressor with a constitutive cytokinin response phenotype, revealed a reciprocal genetic interaction with *rpn12a-1*. Plants carrying the *sor3* mutation resemble wild type seedlings treated with high concentrations of the cytokinin BA. The *sor3* phenotype is more severe in an *RPN12a* compared to an *rpn12a* background, suggesting that the effects of loss of SOR3 function and loss of RPN12a function partially complement each other. This genetic interaction suggests a functional relation between SOR3 and RPN12a. Based on the recessive nature of this mutation, we propose that *SOR3* encodes a repressor of cytokinin growth responses and that its activity is controlled by the UPP.

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187 Reverse-genetic analysis of the Arabidopsis MAP Kinase Kinase Kinase gene AtMEKK1

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MAPK cascades are believed to be involved in the signal transduction pathways that plants use to respond to biotic and abiotic stress. Molecular and biochemical studies have suggested that the Arabidopsis MAP Kinase Kinase Kinase gene MEKK1 is involved in salt and cold stresses amongst others (Mizoguchi et al, 1996; Kazuya-Ichimura et al, 2002). We have used a reverse-genetic approach to investigate the function of the MAP3K gene MEKK1. Arabidopsis plants carrying T-DNA insertions within this gene display a unique phenotype in that optimal growth of the plants occurs under conditions of elevated salinity.

188 Plant Cytokinesis Controlled by A MAP Kinase Cascade and Kinesin-like Protein NACK1

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Tobacco protein kinase NPK1 is the MAPKKK that is required for the formation of cell plates (septa) during plant cytokinesis (1). We have identified tobacco NQK1 as a MAPKK downstream of the NPK1 MAPKKK and tobacco NRK1 as a MAPK downstream of the NQK1 MAPKK (4). We also have identified an activator of NPK1 MAPKKK, which is named NACK1, a M-phase-specific kinesin-like protein (2). Biochemical and genetic analyses have shown that NACK1 activates NPK1 through molecular interaction, the active NPK1 in turn activates NQK1 by phosphorylation, and the active NQK1 activates NRK1 by phosphorylation (1-4). Activation of these three protein kinases occurs only at late M phase of tobacco cell cycle (1-4). Expression of dominant negative forms of these components in tobacco cultured cells and introduction of mutations in Arabidopsis homologs cause the generation of enlarged cells with immature cell plates and multi nuclei (1-4). These results suggest that the NACK1-activated MAP kinase cascade (consisting of NPK1, NQK1 and NRK1) positively controls a step of the cell plate formation during plant cytokinesis. Studies of subcellular localization of NACK1, NPK1 MAPKKK and NQK1 MAPKK have shown that these proteins are localized to the equator of phragmoplast (1-3), a plant-specific cytokinetic apparatus, which consists of microtubules and microfilaments and supports the cell plate formation through the centrifugal expansion of the phragmoplast during cytokinesis. When depolymerization of phragmoplast microtubules is induced, activities of three kinases in the MAPK cascade rapidly decrease (4). These results suggest a role of the NACK1-activated MAP kinase cascade in a microtubule turnover in phragmoplast during plant cytokinesis. We will show regulatory networks that control cytokinesis in Arabidopsis.

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189 Screening for direct target genes of ARR1

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Cytokinins regulate the plant development through cell proliferation and differentiation. It is known that the His-Asp phosphorelay mediates the signal from cytokinin-receptor His kinases and that type-B response regulators including ARR1 directly activate the transcription of cytokinin primary responsive genes. However, the overall feature of signal transduction to cell proliferation and differentiation is still unclear mainly because pathways downstream from the phosphorelay have not been revealed. To obtain information about the downstream pathways, we identified direct target genes. First, we examined known cytokinin responsive genes, and found that ARR1 can directly transactivate all the examined cytokinin primarily responsive genes, including those for type-A response regulators, while ARR1 contributes the responses at different extents depending on genes. Next, using HiCEP analysis, we identified additional ARR1 target genes, all of which were also found to be primary responsive to cytokinins. These results indicate that ARR1 mediates a wide variety of cytokinin responses in the early process of their intracellular signal transduction pathways and suggest the possibility that ARR1 and paralogous type-B response regulators mediate all primary transcriptional responses to cytokinins.

190 Regulation of cellulose synthesis by phosphorylation of the catalytic subunit

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Cellulose is central to plant development and is an economically important target. Despite this it is still unclear how cellulose synthesis is regulated. Significant advances have recently been made in the cloning of genes involved in cellulose synthesis. Three different cellulose synthase catalytic subunits (CesAs) are essential for cellulose synthesis in the secondary cell wall (Taylor *et al* 1999; 2000; 2003). The three secondary cell wall specific CesAs (IRX1, IRX3 and IRX5) are expressed in identical patterns, as shown by promoter reporter gene constructs and by immunodetection using specific antibodies. The three proteins all interact within the same protein complex. The presence of all three catalytic subunits, but not their catalytic activity, is required for the correct interactions between these subunits and for the subsequent targeting of the cellulose synthase complex to the plasma membrane (Taylor *et al* 2003; Gardiner *et al* 2003).

The complexity and size of the cellulose synthase complex is likely to mean that cellulose synthesis is regulated post-translationally. I have recently obtained evidence that IRX3 is phosphorylated and have identified two sites that are phosphorylated *in vivo*. I have mutated these phosphorylation sites in IRX3 to mimic the 'always phosphorylated' and 'never phosphorylated' states and re-introduced these mutated genes into a null *irx3* background. The results of these experiments show that phosphorylation is indeed a mechanism by which cellulose synthesis is regulated. Current work is ongoing to determine the details of the effects of phosphorylation in cellulose synthesis. This latest work will be presented, along with new data regarding the signalling mechanisms responsible for these phosphorylation events.

These results offer the first glimpse into the regulation of cellulose synthesis. This is the first proof that cellulose synthesis is regulated by phosphorylation of the catalytic subunits and is an important advance in our knowledge of the synthesis of this important polymer. The identification of regulatory mechanisms controlling cellulose synthesis will allow a unique opportunity to manipulate the content and composition of cell walls, a developmentally and economically important target for biotechnology.

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191 Analysis of Arabidopsis Type-A Response Regulators in Cytokinin Signaling

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Plant growth and development is regulated by a network of hormones and their downstream signaling pathways. In the current model of cytokinin signaling, histidine kinase receptors transmit the cytokinin signal via histidine phosphotransfer proteins to response regulators in a multi-step phosphorelay that is similar to bacterial two-component signaling systems. The Arabidopsis genome encodes two main classes of response regulators, namely the type-A and B *ARRs*. Type-B *ARRs* have been shown to be transcriptional activators of a subset of cytokinin induced genes. The type-A *ARRs* are a family of ten genes rapidly induced by cytokinin. They encode proteins containing a receiver domain conserved among response regulators, but lacking a recognizable output domain. We have isolated loss-of-function mutations in all type-A ARRs and constructed multiple mutant combinations including septuple mutants. Analysis of cytokinin responses in *arr* mutants indicate functional overlap among type-A ARRs and that these genes act as negative regulators of cytokinin signaling. Further phenotypic analysis of higher order arr mutants suggest that these genes may play a role in shoot patterning and pollen development. Biochemical studies suggest that phosphocompetition is not the only function of these two-component genes. Phenotypic characterization of higher order *arr* mutants, patterns of *ARR* gene expression and biochemical studies will be discussed.

192 Elucidating Functions of Plant Receptor-Like Kinases

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The *Arabidopsis* genome contains a family of receptor-like kinases (RLKs) with over 600 members, yet only a handful have known function. RLKs have been implicated in growth and development, disease resistance, and stress response. Using a high-throughput reverse genetics approach, we have isolated gene knockout lines for 120 RLKs. These mutant lines have been screened for phenotypic alterations in growth, development and responses to stress.

Currently, three different phenotypes have been observed in one RLK subfamily. Two of these phenotypes are from single gene knockout lines and the third is from a double homozygous line. Of particular interest is a mutant we call "lacy", which displays asymmetric leaf morphology, small size, loss of apical dominance, and abnormal trichome development. The second phenotype has been found in two different mutant lines and is characterized by shorter roots and hypocotyls than that of the wildtype. These two lines have insertions in genes that are very closely related. The double homozygous mutant appears to have delayed flowering, evidenced by much larger rosettes and a thicker main stem than in wildtype. Further analysis of the double mutant is currently underway. All four mutant lines have disruptions in receptor-like cytoplasmic kinases (RLCKs).

Phenotypic analysis includes observing growth and development under normal greenhouse conditions as well as subjecting the mutant lines to various hormone treatments including auxin, cytokinin, gibberellin, and ethylene. So far, only the two lines mentioned above with short roots and hypocotyls display differential growth under conditions of exogenous application of auxin and cytokinin. The phenotypes of the two lines under these conditions are the same as under no hormone application, suggesting that these lines may be insensitive to exogenous auxin and cytokinin.

Ultimately, seed from the isolated homozygous lines will be sent to the ABRC for distribution to the scientific community.

193 Unique and Overlapping Expression Patterns Among the Arabidopsis 1-Amino-Cyclopropane-1-Carboxylate Synthase Gene Family members

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The pyridoxal phosphate-dependent enzyme, 1-aminocyclopropane-1-carboxylate synthase (ACS; EC 4.4.1.14), catalyzes the rate-limiting step in the ethylene biosynthetic pathway in plants. The Arabidopsis genome encodes nine ACS polypeptides that form eight functional (ACS2, ACS4-9, ACS11) and one nonfunctional (ACS1) homodimers. Transgenic *Arabidopsis* lines were constructed expressing the β-glucuronidase (*GUS*) reporter genes from each promoter of the *ACS* gene family members in order to determine their pattern of expression during plant growth and development. All genes, except *ACS9*, are expressed in five-day old etiolated or light grown seedlings yielding distinct patterns of GUS staining. *ACS9* expression is detected however in the true leaves of ten day-old seedlings. Overlapping and unique expession patterns were detected for all the gene family members in various organs of one month old plants. Overlapping expression is observed in hypocotyl, roots, various parts of flower (sepals, pedicle, style, etc.) and in the stigmatic and abcission zones of the silique. Exogenous IAA enhances the constitutive expression of *ACS2*, *4*, *5*, *6*, *8* and *11* in the root tip zone. Wounding of light grown hypocotyls tissue inhibits the constitutive expression of *ACS1* and *ACS5* and induces the expression of *ACS2*, *4*, *6*, *7*, *8* and *11*. Examination of GUS expression in transverse sections of cotyledons reveals that all *ACS* genes, except *ACS9* are expessed in the epidermis cell layer, guards cells and vascular tissue. Similar analysis with root tip tissue treated with IAA reveals unique and overlapping expression patterns in the various cell files of lateral cap, cell division and cell expansion zones. For example *ACS8* is uniquely expressed in the lateral root cap cells, whereas *ACS2* in the protoxylem cell files of the cell division zone. Overlapping expession is observed among *ACS2*, *4*, *5*, *6*, *7*, *8* and *11* in the protoxylem and protophloem cell files of the cell expansion zone. IAA inducibility is gene specific and cell file dependent across the root tip zone of the primary root. This limited comperative exploration of *ACS* gene expression reveals constitutive spatial and temporal expression patterns of all gene family members through out the growth period examined. The common and yet distinct gene activity pattern detected reveals a combinatorial code of spatio-temporal co-expression among the various *ACS* gene family members during plant development. This raises the prospect that functional heterodimers are formed in *planta*.

194 Revealing the novel regulation of the COP9 Signalosome (CSN)

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The COP9 Signalosome (CSN) is an eight-subunit nuclear protein complex, which shares homology with the 'lid' of the 26S proteasome. CSN was initially identified in plants and was subsequently shown to be essential for the development and survival of all higher eukaryotes. CSN is known to interact with cullin containing E3 ubiquitin ligases (E3s), and eventually modulate signal transduction pathways through regulation of protein degradation.

In search for novel regulation of CSN, we dissected the function of human CSN, by focusing on subunit 1 (CSN1). The CSN1 C-terminal half, encompassing the PCI domain, was responsible for interaction with CSN2, CSN3, and CSN4 subunits and therefore was required as a scaffold for CSN assembly as a complex. The CSN1 N-terminal half (CSN1N), on the other hand, possessed an intrinsic function of repression on gene expression. CSN1N inhibited the accumulation of *c-fos* and suppressed signal activation at specific responsive elements on the *c-fos* promoter. Moreover, CSN1 repressed JNK1 gene expression leading to suppression of c-Jun phosphorylation in the SAPK/JNK pathway. This regulation of repression was found to be independent from the known protein-degradation mediated regulation. To reveal the underlying mechanisms, we purified interacting proteins directly binding to CSN1N (NBPs) and identified SAP130, DdX15, and CFIm68, which are components of transcription, splicing, and poly (A) formation machinery, respectively. Together this suggests that CSN1 as part of CSN has a previously unidentified role in mRNA processing.

We are currently using *Arabidopsis* to understand the biological roles of this novel regulation in signals transduction on morphogenesis. We have identified the plant homologues of the human NBPs and are creating a series of knockout plants defective in each of the NBP gene. Detailed biochemical analyses on the CSN-NBP interactions in combination with *in planta* research will reveal the novel mechanism of CSN on signal transduction.

195 Ubiquitin C-Terminal Hydrolases 1 and 2 Affect Shoot Architecture in Arabidopsis

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Deubiquitinating enzymes are essential for the ubiquitin/26S proteasome pathway to control the steady-state levels of Ub and Ub-protein conjugates. The ubiquitin C-terminal hydrolase (UCH) family is defined by the presence of a signature active site bearing a Cys/His/Asp catalytic triad. DNA database searches revealed that Arabidopsis contains three UCH genes, designated UCH1 to 3. UCH1 and 2 encode ~330-amino acid proteins that share 74% similarity. Their closest orthologs are members of the UCH37 family, a group defined by an additional conserved C-terminal domain that potentially associates with the 26S proteasome and helps process poly-ubiquitin chains before target degradation by the proteasome. The Arabidopsis UCH2 protein has hydrolase activity *in vivo* and *in vitro* and is capable of cleaving both α- and ε-peptide bonds at the C-terminus of ubiquitin. Mutants with T-DNA insertions in either *UCH1* or *2* are phenotypically normal, suggesting that UCH1 and 2 have redundant functions. However, the double mutant *uch1-1/2-1* displays hyponastic leaves, decreased chlorophyll content and increased shoot apical dominance. In contrast, plants overexpressing UCH1 develop epinastic leaves, increased chlorophyll content, and show decreased shoot apical dominance caused by an increase in axillary growth and reduced internode elongation. These phenotypes suggest that UCH1/2 may help control cell division and expansion by modulating key regulators via their ubiquitination status and subsequent degradation by the proteasome. The auxin signaling pathway has previously been shown to play key roles in cell division/expansion and shoot apical dominance. To test whether the auxin pathway is affected in these mutants, *35S::UCH1/axr1* and *35S::UCH1/axr2* lines were created. These double mutants demonstrated dramatic pleiotropic defects in plant growth, suggesting that UCH1 acts synergistically with AXR1 and 2. In addition, *35S::UCH1* has increased levels of *DR5::GUS*, a reporter of early auxin response genes, while *uch1-1/2-1* has decreased *DR5::GUS* levels. One of the substrates of the UCHs might be the short-lived AUX/IAA proteins, which are known to be degraded by the proteasome. In support of this, the degradation rate of one AUX/IAA reporter protein, *HS::AXR3NT-GUS*, is altered in the UCH mutants. The combined data indicate that UCHs regulate the stability of AUX/IAA proteins, thus influencing auxin signaling and plant development.

196 Exploring the role of Arabidopsis JA-amido synthetase in wound signaling and ozone stress responses

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Jasmonic acid (JA) is a crucial fatty acid-derived plant signal that is involved in both development and in defense responses against biotic and abiotic stress. The importance of JA is well documented, but the molecular mechanism of JA action is still not well understood. The finding that Arabidopsis JASMONIC ACID RESISTANT1 (JAR1) is a JA-amido synthetase that activates JA by conjugating it to isoleucine (Ile) is a novel discovery with important implications in the field of plant hormone biology. The phenotype of *jar1* mutants indicates that the gene is important for defense against pathogens and protection against ozone stress. We have found that mechanical wounding induces *JAR1* mRNA accumulation, and we are determining whether this correlates with the formation of JA-Ile. The timing of conjugate accumulation will be compared to the induction of wound response genes that are dependent on jasmonate signaling. JA also modulates ozone-induced hypersensitive cell death in a *JAR1*-dependent manner. Consequently, *jar1* plants are more susceptible to ozone damage than wild type. It remains to be established whether the conversion of JA to JA-Ile is necessary for ozone tolerance. Investigation of *JAR1* gene expression in response to ozone exposure and complementation of ozone susceptibility of *jar1* with JA-Ile are discussed in this study.

197 Novel transcription factors and E3 ubiquitin ligases involved in brassinosteroid signal transduction in *Arabidopsis*

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Plant steroid hormone brassinosteroids (BRs) play important roles throughout growth and development. Unlike animal steroid hormones that bind nuclear receptors to directly activate target gene expression, BRs are perceived by membrane-localized receptor kinase BRI1 (Kinoshita et al. 2005 Nature 433: 167). Multidisciplinary approaches have been used to study how the BR signal is transduced from the cell surface receptor to nuclear target genes. By screening for *bri1* mutant suppressors, we identified a nuclear protein BES1 that functions downstream of BRI1 and found that BES1's protein level is regulated by BR signaling (Yin et al. 2002 Cell 109: 181). More recently, we established that BES1 is a transcriptional activator that binds to and activates BR-target gene promoters (Yin et al. 2005 Cell 120: 249). BES1 amino-terminal domain is involved in DNA binding and can potentially form a novel basic-helix-loop-helix (bHLH) motif. In addition, BES1 interacts with a typical bHLH protein, BIM1, to synergistically bind to E-box (CANNTG) sequences present in many BR-induced promoters. Mutants of *BIM1* and its close family members display BR-response phenotypes. Thus, BES1 defines a new class of plant-specific transcription factors that cooperate with transcription factors such as BIM1to regulate BR-induced genes. While BES1 protein accumulates in the nucleus in the presence of BRs, BIN2 kinase, a negative regulator in the BR pathway, appears to phosphorylate and destabilize BES1 in the absence of BRs (Yin et al. 2002 Cell 109: 181). To understand how BES1 protein levels are controlled, we performed a yeast two-hybrid screen and identified two BES1-interacting E3 ubiquitin ligases, an F-box protein BAF1 and a RING-finger protein BAR1. Suppression of *BAR1* expression by RNAi leads to weak BR-response phenotypes including increased BES1 protein level, excessive cell elongation and partial resistance to a BR biosynthesis inhibitor. Based on these results, we propose that BAR1 and BAF1 function together to target BIN2-phosphorylated BES1 to the protein degradation pathway. Taking together, our studies establish that BRs signal to regulate the protein levels of a novel family of transcription factors to regulate plant growth and development.

198 Interactions between sugar and phytohormone signaling pathways during seed germination and seedling development

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Sugars, such as glucose, have been shown to affect many aspects of plant growth and development acting not only as nutrients but also as signaling molecules. Exogenous glucose delays seed germination in Arabidopsis thaliana not only in wild type (WT) but also in all the mutants in hormone signaling pathways that have been previously examined. Based on these data it was concluded that plant hormone signaling pathways, Abscisic Acid (ABA), Gibberellin (GA) and ethylene, are not involved in the glucose-induced delay of seed germination (Price et al., 2003; Dekkers et al., 2004). Our study demonstrates that ABA Insensitive 3 (ABI3) gene in ABA signaling pathway and RGA-like 2 (RGL2) and SPINDLY (SPY) genes in GA signaling pathways play important roles in glucose-induced delay of germination. Our results indicate that transcription of ABI3 and RGL2 genes, both acting to inhibit germination, is upregulated by glucose. In addition, we show that ABI3 may have a role in activating RGL2 gene expression and that RGL2 may have a similar function in the regulation of ABI3 transcription. Furthermore, our data support previous findings that different sugars such as the two hexose stereoisomers, glucose and mannose, delay or inhibit seed germination via different branches of hormone signaling pathways. The analyses of post-germination seedling development of wild type plants indicate that glucose may have a stimulatory effect on root and shoot growth. This effect is concentration dependent. Comparison of WT and spy seedling growth on different glucose concentrations suggests that the stimulatory effect of glucose may be partially exerted via GA signaling pathway. The effects of glucose on plant growth and development may be opposite and stage dependent. The inhibitory effect on seed germination seems to be accomplished via activation of ABA signaling pathway, through ABI3, and inactivation of GA signaling pathway through RGL2 and SPY. On the other hand, the stimulatory effect of glucose on seedling growth may involve activation of GA signaling pathway.

199 PDK1 regulation of PINOID family of AGC kinases activity in Arabidopsis

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PINOID (PID) kinase is believed to be a key regulator of auxin-mediated plant development. We are characterizing the role of auto-and transphosphorylation on PID activity. We used recombinant PID protein to demonstrate that intramolecular PID autophosphorylation is required for transphosphorylation by PID of exogenous substrates. Basal levels of PID autophosphorylation are increased after pre-incubation of recombinant PID with total protein extracts from flowers and seedling shoots. PID is a member of the AGC serine-threonine kinase family that is phylogenetically conserved. PID-like mammalian AGC kinases act in a phosphorylation cascade initiated by the phospholipid associated kinase PDK1, which binds its substrates through interaction with the conserved PIF domain. Because PID and other closely related AGC kinases in *Arabidopsis* also contain the PIF domain, we asked if they were substrates for PDK1 phosphorylation. We found that PID family of AGC kinases interact with PDK1 and a subset are phosphorylated. Family members lacking the PIF domain bind to PDK1 but are not substrates, suggesting that the PIF domain may have functions other than substrate docking. PIF domain containing proteins that were phosphorylated by PDK1 showed a dramatic increase in in vitro transphosphorylation assays. We show that this activation requires the phosphorylation of the PINOID activation loop serine S290. These results suggest that phospholipid signaling may mediate auxin-regulated signaling processes. We have analyzed the expression patterns of 16 of the *PID*-like Arabidopsis AGC family and show that these genes have spatially distinct but over-lapping expression patterns. Thus, functional gene redundancy may explain the lack of loss-of-function phenotypes associated with these mutants.

200 Investigating the role of ETA2/CAND1 in regulating SCF complex activity

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Auxin regulates various fundamental processes of plant growth and development. In response to auxin, the SCF^{TIR1} ubiquitin ligase targets members of the AUX/IAA family of transcriptional regulators for ubiquitin-mediated proteolysis. Degradation of the AUX/IAA proteins leads to diverse downstream auxin responses. In an effort to identify new components of the SCF^{TIR1}-mediated auxin response pathway, a screen was conducted for enhancers of the *tir1-1* auxin (*eta*) response defect. *eta2-1* is one of the novel mutants identified in this screen. *eta2-1* plants have several phenotypes related to impaired auxin response . Molecular studies found that these phenotypes are the result of reduced SCF^{TIR1} activity in *eta2-1* mutants (1). Isolation of the *ETA2* gene revealed that it encodes an Arabidopsis ortholog of human CAND1 (Cullin-Associated and Neddylation-Dissociated). Biochemical studies with mammalian cell lines suggest that CAND1 acts as a negative regulator of SCF function by sequestering unmodified CUL1 away from SKP1 and the F-box protein, thus preventing assembly of the SCF complex. In contrast, we find that the *eta2-1* mutation diminishes the ability of CAND1 to interact with CUL1, demonstrating that the interaction between these two proteins is required for SCF activity and that CAND1 positively regulates SCF function. These paradoxical findings have been explained by a model invoking CAND1 in regulating a dynamic cycle of assembly and disassembly of the SCF complex in vivo, through disassociation and association with CUL1. Double mutant analysis with the *axr6-2* and *eta1/axr6-3* alleles of CUL1 reveals additional insight into the interactions between CAND1 and CUL1. Whereas *eta2-1* and *axr6-3* interact synergistically, *eta2-1* suppresses the seedling lethality of *axr6-2*. Biochemical studies suggest that disassociation of CUL1 from CAND1 is disrupted by the *axr6-2* mutation. Molecular studies on examining the effects of these mutations on SCF homeostasis are underway.

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201 The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation

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The phytohormone ABA regulates many complex aspects of plant development including seed maturation, dormancy, germination, and root growth. The B3 domain transcriptional factor ABI3 is a central regulator in ABA signaling, but little is known how this factor itself is regulated. Here, we show that an ABI3-interacting protein (AIP2), has E3 ligase activity and can ubiquitinate itself as well as ABI3 in vitro. AIP2 has a stronger binding affinity for ABI3 B2+ B3 domain than its A1+B1 domain, but only ubiquitinates the latter. The AIP2 E3 activity is abolished by mutations in the RING motif. In double transgenic plants, induced AIP2 overexpression leads to a decrease in ABI3 protein levels. On the contrary, ABI3 levels are significantly elevated upon induced expression of a dominant negative AIP2 RING mutant which interferes with the endogenous AIP2 E3 activity. Moreover, an aip2 null mutant shows higher ABI3 protein levels compared to WT after seed stratification, and is hypersensitive to ABA, mimicking the ABI3-overexpression phenotype. By contrast, AIP2 overexpression plants are more resistant to ABA, mimicking the abi3 mutant phenotype. The aip2 phenotype can be complemented either by expressing the native AIP2 gene or overexpressing 35S-AIP2 gene, but not by a RING mutant AIP2 gene. Our results indicate that AIP2 is a negative regulator in ABA signaling by targeting ABI3 for post-translational destruction.

Key words: Abscisic acid-insensitive 3 (ABI3), ABI3-interaction protein2 (AIP2), ABA signaling, ubiquitination, proteosomal degradation.

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202 Transcriptome analysis of ROP10 small GTPase-mediated abscisic acid signaling in Arabidopsis

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The phytohormone abscisic acid (ABA) modulates a variety of growth and developmental processes and abiotic stress responses, but its signaling that probably involves a complex network is not well defined. Previous results have demonstrated that ROP10, a member of ROP small GTPases, is a plasma membrane-associated negative regulator specifically involved in ABA responses (1). We are currently investigating the signaling mechanism by which ROP10 effects gene expression in the nucleus. Our preliminary results suggest that ROP10 suppresses the ABA-induced expression of MYB2 and MYC2 and several related MYB/MYC (bHLH) transcription factors. However, mRNA expression of *RD29A/B*, *RAB18*, *PP2C* and *ABF2* was not affected in the *rop10* knockout mutant, indicating that ROP10 might specifically switch off the recently established MYB/MYC pathway in ABA signaling (Abe et al., 2003). To test this hypothesis, we are performing a DNA microarray analysis and the results will be presented.

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203 Arabidopsis BRS1 is a secreted and active Serine Carboxypeptidase

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The Arabidopsis BRS1 gene encodes a serine carboxypeptidase II-like protein. Its biological role in brassinosteroid (BR) signal transduction was first established by its capability to specifically suppress a weak brassinosteroid insensitive 1 (bri1) allele, bri1-5, when overexpressed. In order to expand our knowledge of the molecular mechanisms of BRS1 function, the subcellular localization and the biochemical characteristics of BRS1 were determined by using transgenic plants harboring a 35S-BRS1-GFP construct and fusion proteins purified from 35S-BRS1-FLAG plants. BRS1-GFP fusion protein was mainly secreted and accumulated in the extracellular space. Immunological data suggest that BRS1 is proteolytically processed by an unknown endoproteinase in planta. Affinity-purified BRS1-FLAG from transgenic plants show strong hydrolytic activity with a broad P1 substrate preference including basic and hydrophobic groups on either side of the scissile bond. The hydrolytic activity of BRS1 can be strongly inhibited by a serine proteinase inhibitor, PMSF. The pH and temperature optima for the hydrolytic activity of BRS1 are pH5.5 and 50oC, respectively. These data demonstrate that BRS1 is a secreted and active serine carboxypeptidase, consistent with the hypothesis suggested by our previous genetic evidence that BRS1 may process a protein involved in an early event in the BRI1 signaling pathway. To discover in vivo substrate(s) of BRS1, we performed an activation-tagging genetic screen in bri1-5 brs1-1D background. One wild-type like suppressor has been identified. Currently we are characterizing this new suppressor. Possible molecular mechanisms of BRS1 will be discussed.

204 Industry Collaboration and the Novelty and Persistence of Arabidopsis Research

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As intellectual property protection has expanded and the distinction between basic life science research and applied biotechnology erodes, academic *Arabidopsis* labs are increasingly collaborating with firms on research. I argue that these collaborations exert a measurable influence on the ethos of academic science. Specifically, I argue that academic science has a different risk preference than industrial science, influencing its researchers to attempt more novel approaches and maintain greater substantive persistence. As a result, industry ties may entrain the activity of their academic partners.

Using the complete archive of *Arabidopsis* articles from 1974-2002, I develop indices of combinatorial novelty and research persistence. I then infer collaboration with industry through co-authorship of academic articles, co-invention of patents, mentions of industry collaborators or funders in article acknowledgements, and mentions of partnerships between life science companies and the universities housing *Arabidopsis* researchers in the business press. Next, I use panel models to evaluate the impact of industry collaboration in prior years on the novelty and persistence of research in the current one. My findings suggest that industry partnerships make science less novel and influence scientists to be less persistent in their inquiry. These partnerships exert their effects unequally, however, by improving the academic quality of research performed by central, high status scientists and universities while degrading those qualities for peripheral ones. By engaging central scientists on the basis of their academic quality and peripheral scientists on the basis of their time and effort, industry creates a striking, new division of scientific labor.

My presentation will describe further results of this research, including the effect of industry collaboration on the application-orientation of *Arabidopsis* projects, the sharing of *Arabidopsis* resources, the dissemination of *Arabidopsis* findings and the cumulative frontier of *Arabidopsis* knowledge.

205 AraCyc and MetaCyc: databases to survey the metabolic network in plants and microorganisms

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AraCyc (http://www.arabidopsis.org/tools/aracyc) is a single-species metabolic pathway database that visualizes biochemical pathways in *Arabidopsis thaliana*, and displays genes and enzymes on the context of the metabolic network of this model plant. The initial database was computationally predicted by mapping the functional annotations of the Arabidopsis genome onto the pathways in the reference database MetaCyc (http://metacyc.org). Since its creation the database has been extensively updated. Pathways predicted from the Pathway Tools component PathoLogic (http://bioinformatics.ai.sri.com/ptools) were manually validated from the literature, absent pathways were added and unsupported pathways eliminated. Genes and their mappings to pathways are continuously updated with the latest functional annotation of the Arabidopsis genome. The most recent version of AraCyc (release 2.1) comprises 1615 unique genes mapped onto 221 pathways. More than 70 pathways have been manually curated with the ongoing effort to completely and acurately curate all the predicted and validated pathways. Pathways and enzymes are furnished with evidence and citation to assure high data quality. Manually curated pathways contain extensive curator commentary. Structural and functional data extracted from the literature are associated to pathways, compounds, enzymes and genes. Cross reference links are provided from data objects to other databases. Data can be searched and downloaded from the website. A feature of the Pathway Tools software, the Omics Viewer permits overlaying large-scale functional genome data such as gene expression, proteomic, and metabolite profiling data onto the metabolic map to illustrate the results in a broad metabolic context. MetaCyc (http://metacyc.org) is a multi-species database containing non-redundant metabolic data from more than 340 organisms, primarily microorganisms and plants. The latest edition (release 9.0) features 547 pathways, more than 5000 enzymatic reactions and over 2000 enzymes and genes. The pathways are experimentally elucidated and supported by 5500 citation. As part of the ongoing endeavour to increase the breadth of pathway coverage, plant secondary metabolism was added to accommodate the goal of MetaCyc to catalog the universe of metabolism. MetaCyc has also been used as a reference database to predict more than 150 species-specific pathway databases from newly annotated genomes (http://biocyc.org).

206 Latest Developments on the Microarray Data Available at TAIR

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Since 2003, TAIR (http://arabidopsis.org) has served as a public repository of Arabidopsis microarray expression data, accepting data submissions and providing free distribution and shared access to full datasets of both single channel and multiple channel experiments. Major datasets available at TAIR include the public data generated by the Arabidopsis Functional Genomics Consortium (AFGC) and AtGenExpress, a multinational project coordinated by the Arabidopsis Functional Genomics Network (AFGN). In addition, many contributions from individual labs are available. As of April 2004, TAIR contains raw and normalized data for 167 experiments comprised of 1673 arrays and over 36 million data points. Experiments and gene expression profiles can be searched independently via a Web-based query, and the data can be downloaded for local use. Other tools available at TAIR allow mapping of array elements to genes and vice versa, visualization of in-house cluster data, functional categorization of groups of genes, graphical visualization of microarray data on a biochemical pathway diagram, and browsing of experiments based on their annotation. A tutorial with step-by-step instructions for using the different microarray tools available at TAIR is provided at http://arabidopsis.org/help/tutorials/micro_intro.jsp. Also, each tool is hyperlinked to a 'Help' page where detailed descriptions for its use are specified. TAIR accepts submission of array designs and experiments via simple, pre-formatted Excel spreadsheets. Researchers are encouraged to submit their data to TAIR. For information on how to submit microarray data see: http://arabidopsis.org/info/microarray.submission.jsp).

207 Controlled Vocabularies For Describing And Comparing Phenotypes And Gene Expression In Angiosperms

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The Plant Ontology Consortium (POC) (www.plantontology.org) is a collaborative effort of several plant databases and experts in plant systematics, botany and genomics. A primary goal of the POC is to develop simple, yet robust and extensible controlled vocabularies that accurately reflect the biology of plant structures and developmental stages. These provide a semantic framework for meaningful cross-species queries across distinct databases. The current ontology release integrates the diverse vocabularies in use to describe Arabidopsis, maize and rice anatomy, morphology and growth stages. This integration spans two major taxonomic divisions: monocots and dicots. This year, POC will integrate other species groups: legumes, Solanaceae and other plant families. We will present the organizing principles and rules followed in developing the Plant Ontologies. Standards and methods for using the Plant Ontologies to annotate gene expression patterns, microarray experiments and mutant phenotypes will be addressed too. Using our ontology browser, the Plant Ontology AmiGO, over 3500 gene annotations from three species-specific databases, TAIR, Gramene and MaizeGDB, can now be queried and retrieved. We will provide examples from member databases, that demonstrate how the PO supports gene discovery, phenotype prediction, and gene product functions, while also accessing mutant stocks and germplasm. The project is supported by National Science Foundation grant No. 0321666.

208 Report on resource project in RIKEN BRC

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RIKEN BRC

RIKEN BioResource Center was established in 2001 and carried out a resource project on both mammal and plant. Experimental Plant Division collects, preserves and distributes plant resources such as Arabidopsis seeds, plant DNA materials and plant cultured cells. The number of resources preserved is 264,651, and we have already distributed 13,973 materials to the world community. Our major resources are as follows: Arabidopsis full-length (RAFL) cDNA clones, Arabidopsis transposon-tagged lines, Arabidopsis activation-tagged lines, and wild-type Arabidopsis from Sendai Arabidopsis Seed Stock Center. We are going to collect and provide not only Arabidopsis resources but also full-length cDNA clones of other model plants and crops that will be generated in near future.

209 Introducing IGB for Arabidopsis: a resource for Arabidopsis genome data visualization

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The Integrated Genome Browser (IGB, pronounced 'Ig-Bee') is an experimental, Java-based, desktop software program that allows rapid, interactive visualization and exploration of genes, sequence, microarray and other types of data from multiple genomes. Until now, IGB has only been able to display data from animal genomes, simply because Arabidopsis and other plant data have not been available in compatible formats. To remedy this, we created a prototype Arabidopsis genome 'Quickload' server, which is an on-line data repository containing annotations and sequence data that users can load and view in IGB. Currently, the server delivers foundation gene annotations from TAIR, probe-to-genome alignments from Affymetrix' ATH1 GeneChip platform, and reference sequence data from version 5.0 of the Arabidopsis genome.

IGB is designed to allow and encourage loading annotations for an entire genome at once. To make this possible, IGB uses optimized data models that reduce memory requirements, binary file formats that speed up loading time, and a local data caching system. IGB also implements techniques for exploratory data analysis, such as the ability to display and manipulate numerical data alongside gene structures. This feature is especially useful for viewing probe readings from DNA expression microarrays but has other applications, as well. In addition, IGB can load data from user-generated files so long as they conform to one of several compatible formats. We are working to develop tools and documentation that will make the process of file format conversion easier and more transparent.

Information about IGB, including links to download pages and instructions on how to use the program, is available at www.transvar.org/at_annots. To access Arabidopsis data after launching IGB, select the 'Arabidopsis' option under the 'Quickload' tab. To load individual data sets, click the checkboxes to the right. The 'TAIR' option loads gene models, and the 'ATH1' option loads probe sets from the Affymetrix ATH1 chip. To load sequence for an entire chromosome, click the 'Load Entire Sequence' button. To view Web pages describing individual gene models, right-click (Windows, Unix) or control-click (Mac) the gene and select the 'Get Info' option.

IGB is part of the Genoviz open source software development project [sourceforge.net/projects/genoviz], a project dedicated to creating and distributing visualization software for biology and bioinformatics.

210 NASC Germplasm Resources and Ontologies

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The Nottingham Arabidopsis Stock Centre (NASC) along with ABRC (Arabidopsis Biological Resource Center) in the US stores, maintains and distributes over 400,000 lines. Our stocks include a large proportion of insertion mutation lines approaching saturation of the transcriptome, ecotypes, mapping lines, activation tagged inserts, and promoter enhancer traps.

New lines include CATMA lines, RNAi silencing and several large donations from the JIC. A number of smaller donations have also been received from a number of groups as a result of the stock centres' recent donations request.

All of these seeds can be ordered through our online catalogue at http://arabidopsis.info.

NASC are also keen to promote the use of ontologies and standards to enhance the annotation of the data stored in the germplasm catalogue. One of the first steps has been to use the current Plant Ontology (PO) with the Phenotype and Trait Ontology (PATO) to curate germplasm phenotype data using Entity Attribute Value (EAV) descriptions.

Ontologies allow us to standardise the way we describe phenotypes observed in mutant plants. The EAV annotations allow users to search for specific phenotypes and will also retrieve information about mutants that share a similar phenotype. This was almost impossible before ontologies were implemented as the phenotypes are stored in a free text format making them difficult to search and compare. We are now encouraging all donors to use the ontologies to describe any observed phenotypes, special form have been designed and can be downloaded to aid this.

211 Oligogo, A Web Based Primer Design Site for Arabidopsis Gene Families

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The complete annotation of the *Arabidopsis* genome reveals that most genes are members of gene families, some with upwards of a thousand members. In this context, the concept of one gene, one function is being replaced by a more family-centric view where a set of genes may be responsible for any particular function or trait. This underlying family structure of the genome is a central theme in the design of **Oligogo** (http://oligogo.botany.wisc.edu//), a web-based primer design application built to group genes into families to allow researcher to easily organize PCR-based projects in these natural sets.

Oligogo offers a simple web-interface whereby a AGI id (i.e. At1g01010) is used to identify and display all of a gene's family members on a single web page. Genes in these family sets can be displayed with exons and introns shown to scale as they appear in genomic DNA, or as cDNA, with introns collapsed to small space-holder boxes color coded to represent the intron's phase. Presenting genes in family sets provides important visual clues concerning exon, intron, and intron phase conservation between family members which is difficult to see when a gene is viewed in a chromosomal context, or as one gene per page. Further site development will include superimposition of a protein domains on the cDNA cartoon, to help identify relationships between the exon/intron and domain structures.

Once a gene's family has been identified, **Oligogo** allows for facile primer design, display, and output as an Excel style table for ordering. Currently, **Oligogo** provides primers for genotyping T-DNA insertion lines, and primers for real time RT-PCR for mRNA quantification. Primers are dynamically identified from a database which contains primer scores for every position in the genome of sufficient complexity. To ensure a high degree of gene specificity, primer scores within the coding regions of a gene are based on sequence similarity between family memebers as deduced from a multiple sequence alignment of a gene and its six closest relatives. This primer program was originally built to design genotyping primers for T-DNA insertion lines for an NSF funded project to isolate homozygote mutants for 2000 genes, and has been used to design over 1500 genotyping primers with a 99% success rate. This database approach to primer selection ensures speed, and ease of development for future primer sets which will include primers for genomic clone and promoter construction, degenerate primers, RNAi, and microRNA.

212 CAPSGEN and CAPSTRACKER: Programs for the Generation, Storage and Validation of in silico generated Molecular Markers

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We have developed a program for computationally generating CAPS and other markers from EST or genome sequence information from two or more varieties of a given species. We have created a web-accessible database of these in silico generated markers, called CAPSTRACKER, which may be queried based on chromosomal location, marker type, restriction enzyme (in the case of CAPS markers), etc. This database will facilitate map-based cloning in many different ecotypes of Arabidopsis or any other organism for which sequence information from two or more varieties is available. Biologist-friendly features are incorporated into this interface, including virtual gel images of the markers. In addition, we also envision CAPSTRACKER as a community-based resource that biologists can update with information as to whether a given marker works for an ecotype not yet in the CAPSTRACKER database. We also present a program, called CAPSID, that identifies the most parsimonious number of markers to use to genotype a collection of ecotypes. See http://bbc.botany.utoronto.ca.

213 Genomics-Related Stocks Distributed by ABRC

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The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration functional genomics. Several flank-tagged insertion collections are distributed by ABRC. The 152,000 SALK lines from J. Ecker contain insertions in 21,000+ genes. Syngenta Biotechnology, Inc. has donated its "SAIL" collection, from which 38,000 lines are being distributed. This material contributes insertions in 2,500 additional genes plus many second alleles. The Wisconsin Ds-Lox population consists of 10,000 lines which constitute ~1,000 additional genes or second alleles to the unique collection. Full length open reading frame (ORF) cDNA clones are still being received. The total number of full length clones is almost 15,000 including donations from SSP, Salk (J. Ecker), TIGR (C. Town), Peking/Yale and J. Callis. ABRC distributes cDNA clones in the versatile Gateway™ system. Many novel clones arising from 2010 Project research utilizing this system have been received. We are also distributing a number of full length cDNA clones in the pENTR™ vector. We expect to obtain clones of the SSP and Salk collections in a Gateway™ entry vector this year.

We have formatted some of the new cDNA clone resources into plates. For example, 11,000 SSP clones are presently available in this format. We plan to distribute other similar large collections in this way. Additional genome-related stocks received or to be received include: a) 1,500 full length cDNA clones of hypothetical genes; b) expression constructs that have combination translational fusion-promoter reporter function; c) transposon insertion lines, d) transgenic lines, e) RNAi lines and f) BACs from species related to A. thaliana. ABRC distributes large numbers of T-DNA lines that are organized as pools for forward genetic screening, the BAC genomic clones utilized for the AGI sequencing project and EST collections representing ca. 10,000 Arabidopsis genes. The T-DNAs employed to generate our lines include enhancer trap, activation tagging and over-expression constructs, as well as simple insertions. ABRC is supported by the National Science Foundation.

214 The Arabidopsis Biological Resource Center – 2004-2005 Resource Acquisitions and Distribution

Randy Scholl, Emma Knee, Deborah Crist, Luz Rivero, Natalie Case, James Mann, Julie Miller, Garret Posey, Pamela Vivian, Zhen Zhang, Ling Zhou

ABRC, Dept. of PCMB and Plant Biotechnology; The Ohio State University

The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis. ABRC stock information is in TAIR and stocks can be ordered through the database Web site maintained by the Carnegie Institution of Washington (http://arabidopsis.org), with informatics support from the National Center for Genomic Resources (NCGR).

Seed stocks have been added to our collections in the past year, including: A) 10,000 Wisconsin Ds-Lox sequence indexed T-DNA lines; B) 300+ mutant lines; C) stocks from the Chromatin Functional Genomics Consortium and the FTFLP project, D) representatives of A. thellungiella; E) transgenic lines and natural accessions; F) recombinant inbred lines; G) additional TILLING lines; and H) miscellaneous transgenic lines. The T-DNA lines of the SALK, SAIL and Wisconsin collections provide insertions in ~25,000 different Arabidopsis genes, with 15,000+ genes being represented by at least two independent insertions.

New DNA stocks added to the collection include: 1) sequence-validated open Reading Frame (ORF) clones from the J. Ecker, 2) 480 ORF clones from C. Town; and 3) a number of multifunctional vectors that utilize the Gateway™ system. The present ORF collection represents 13,000+ genes. The SSP and Salk ORF collections have been formatted to plates and revalidated by end sequencing. A number of other clones, with diverse functions have been received.

During the past year, ABRC distributed 66,000 seed and 23,000 DNA stocks to over 2,400 researchers in 1,600 laboratories worldwide. Distributions of T-DNA lines contribute to the very high numbers of seed stocks being sent, and the ORF clones represent the most popular DNA stocks.

ABRC is supported by the National Science Foundation.

215 The Botany Array Resource: Using Public Microarray Data to perform e-Northerns, Expression Angling, and Promoter Analyses

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In order to facilitate access to and sharing of gene expression data generated by our Affymetrix facility, we have implemented a web-enabled database based on MIAME and MAGE-ML standards. The database currently contains data for approximately 22,000 genes across ~150 samples. In addition, we have loaded data from NASCArrays and from the AtGenExpress Consortium. Three web-based tools providing several novel features have been developed for querying the the data: an Expression Browser, an Expression Angler, and Promomer for promoter analyses. The tools and the database together comprise the Botany Array Resource – the BAR. The Expression Browser allows users to perform socalled "electronic Northerns", i.e. to input a list of genes, whose expression levels will be selected across all or user-specified experiments in the database. The Expression Angler is a tool for "fishing" for genes with similar expression profiles, across all samples in the database, from NASCArrays, from the AtGenExpress Consortium, or in a user-provided set. The results from these two tools can be displayed in an extensively annotated HTML format, or plain text. The HTML format contains compact tabular representations of tissue type analyzed, plant age, type of experiment, plus expression levels, and automatic hierarchical clustering results. For each gene entered functional categories, annotation, and gene aliases, plus links to TAIR are also provided. Promomer allows users to identify over-represented "words" in a promoter or in the promoters of co-regulated genes. These easy-to-use tools can help guide biological experiments aimed at understanding gene function, functional redundancy, and regulatory networks, and we validate these aspects using recent results from the literature. The manuscript describing the BAR has been accepted for publication in the Plant Journal. See http://bbc.botany.utoronto.ca.

216 High throughput production of full-length ORF clones from low-expressing Arabidopsis genes

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The Institute for Genomic Research

Full-length (FL) cDNAs are essential for functional genomics studies and for refining genome annotation in Arabidopsis. While FL-cDNAs are available for over half of Arabidopsis genes, there are many that have yet to be obtained since no cognate clones have been sequenced from existing cDNA libraries, presumably due to their localized or low expression patterns. At The Institute for Genomic Research, we are targeting these difficult genes for FL-cDNA cloning in order to define the gene structure and produce Gateway® compatible ORF clones for the scientific community. The target list includes 1) genes that are predicted by computer algorithms but exhibit neither experimental evidence of expression in Arabidopsis nor database match to proteins of known function (hypothetical genes), 2) conserved intergenic regions identified by comparative genome analyses (new genes yet to be annotated), and 3) annotated genes without Arabidopsis cDNA support, with little to no expression on ATH1 arrays, and having only protein matches in other plant species (named genes). To validate expression and gene structure of the hypothetical genes and intergenic targets, we have developed a high throughput 5' and 3' RACE cloning pipeline and also explored the effectiveness of sequencing from subtracted and normalized cDNA libraries. ORFs of validated genes are amplified and cloned into the Gateway pDONR221 (Invitrogen) entry vector. Sequence-verified entry clones from this project are being deposited at ABRC. Research supported by the NSF 2010 Project.

217 Functional analysis of flowering-time gene *FD*

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In Arabidopsis, *FLOWERING LOCUS T* (*FT*) gene integrates signals from several distinct pathways that regulate floral transition. We have been interested in factors acting downstream of floral pathway integrator *FT*. *FD* encodes a bZIP transcription factor and preferentially expressed in the shoot apex. FT interacts with FD in vitro and in yeast cells. Several evidences suggest that the activity of FT seems to require protein/protein interaction with FD. Expression of *FD* from leaf-vascular-specific promoter (*pSultr2;1-FD*) could not rescue late-flowering phenotype, although *pFD-FD* transgene fully complemented the *fd* mutation. FD is likely to play a key role in regulation of FT function in the shoot apex via protein-protein interction.

FD contains a potential phosphorylation site by calcium-dependent protein kinase (CDPK) in the C-terminal region. Mutant forms of FD which lack a C-terminal region could not interact with FT in yeast cells and failed to complement *fd* late-flowering phenotype even by over-expression. Role of the C-terminal region in intercellular localization of FD protein were investigated using EYFP:FD fusion proteins. Mutation of C-terminal region caused a speckled nuclear distribution of EYFP in leaf epidermis of Nicotiana benthamiana and in the shoot apex of Arabidopsis. This altered nuclear distribution pattern inside the nucleus was observed in FT-independent manner.

FD is involved in transcriptional activation of the floral meristem identity gene *AP1* redundantly with LFY. In *fd; lfy* double mutant, severe reduction of *AP1* mRNA levels and strong defects in floral specification are observed. Furthermore, in young *35S:FD* seedlings, *AP1* gene was ectopically induced. Up-regulation of *AP1* in *35S::FD* plants require FT function. It is likely that *FD* mediates the action of *FT* in activation of *AP1* expression redundantly with LFY.

These results suggest FT and FD act inter-dependently in the floral transition and activation of *AP1* expression.

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218 The MADS domain factors AGL15 and AGL18 act redundantly to repress flowering in short days

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The transition from vegetative to reproductive development in Arabidopsis can be influenced by environmental factors such as daylength and exposure to cold, or endogenous factors within the plant. Several MADS-domain transcription factors are known to be critical for Arabidopsis to respond appropriately to these environmental and endogenous factors. We can now add the MADS domain factors AGL15 and AGL18 to this group. We showed previously that constitutive expression of the MADS box gene AGL15 causes a variety of phenotypic changes, including a delay in flowering. Constitutive expression of the related gene AGL18 produces similar changes, as well as delayed flowering. To analyze loss-of-function effects, we isolated and characterized *agl15* and *agl18* single mutants and *agl15 agl18* double mutants. Down-regulation of *AGL15* or *AGL18* transcript levels in the single mutants had no effect on flowering time in either long days or short days. However, simultaneous down-regulation of both transcripts, in the double mutants, resulted in early flowering in short day conditions (approximately 40 leaves versus 60 leaves in wild type), but not in long day conditions. Thus, AGL15 and AGL18 appear to act redundantly to repress the transition to flowering, and are important regulators under short day conditions. No other phenotypic changes are apparent in either the single or double mutants. We are currently combining *agl15* and *agl18* mutations with other mutations affecting flowering time to determine which pathway(s) AGL15 and AGL18 are involved in. Supported by USDA NRICGP (2001-35304-10887).

219 Identification of small RNAs in fruit crops

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Small RNA molecules have been shown to be key regulators of gene expression in eukaryotic systems. HortResearch is undertaking research to determine how small RNA-mediated gene regulation in fruit crops and in particular the non-coding RNAs termed microRNAs (miRNAs). Guided by sequence complementarity, the miRNAs interact with target mRNA transcripts to negatively regulate their expression.

Based on the apparent conservation of miRNAs between different plant genera, we have taken the approach of using Arabidopsis miRNA sequences to search the HortResearch Fruit EST databases and identify potential targets. Using various bioinformatics tools a significant number of ESTs/genes whose transcripts are the potential targets for miRNA-mediated regulation have been identified. These putative targets were predicted based on their degree of sequence complementarity to the Arabidopsis miRNA and their putative function.

To complement the bioinformatics approach, and thereby verify the potential miRNA targets, and to discover new targets we have also adopted a direct cloning approach. This has involved the isolation of small RNA molecules from specific fruit tissues. Small RNA molecules have been isolated and sequenced from critical stages of fruit development as determined by the microarray analysis.

220 *Arabis alpina* as a model species to study perennialism

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Most perennials flower more than once and their growth habit is described as polycarpic. This behaviour is in contrast to Arabidopsis, which shows monocarpic development. With the long-term aim of understanding how the regulation of perennialism is achieved, we are studying the flowering behaviour and growth patterns in *Arabis alpina*, a perennial relative of *Arabidopsis thaliana*. The growth pattern in this species shows several characters associated with the perennial phenotype such as, juvenility, extensive axillary branching, conservation of meristems and restricted senescence of reproductive organs. In terms of flowering behaviour, *A. alpina* accessions vary in their response to chilling temperatures. Some accessions show an obligate requirement for vernalisation whereas others do not always require chilling to flower. The role of vernalisation in the control of flowering time and polycarpic growth habit of *A. alpina* has been investigated. We isolated the homologue of *FLOWERING LOCUS C* (*AaFLC*) and we are following its expression as a molecular marker for vernalisation response.

To study further the components involved in the perennial growth habit we have generated a mutagenised population of *A.alpina*

221 A Tapetal Specific NAC transcription factor involved in pollen development

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The *NAC* gene superfamily is a novel class of transcription factors present only in plants. They share a conserved domain defined as the NAC protein domain by Aida et al (1997). The defining genes are *NAM, ATAF1* and *CUC2*. This gene family has been shown to be involved in plant development, defense and abiotic stress responses. We have been studying the expression pattern of *NAC* genes using Affymetrix chips and real time PCR on different *Arabidopsis* plant tissues and have found that the majority of them display complex patterns of expression. Few *NAC* genes are specifically expressed in a single tissue or cell type. However *At1g61110* is noteworthy since it is only expressed in flowers. Other *NAC* genes can play roles in floral development as in the case of *NAP (NAC LIKE ACTIVATED BY PI/AP3)*, which is a direct target of *APETALA3*, a B class floral homeotic gene. Therefore we wanted to study the importance of *At1g61110* in floral development.

Determining the localized expression of *At1g61110* within the flower was our first objective. Promoter GUS fusion analyses were conducted, and the expression was localized to the anther tapetum of *Arabidopsis* plants. T-DNA insertion lines for the tapetal *NAC* gene were obtained from SALK institute but no defects were found in anther development. However, there is evidence that *NAC* genes can act redundantly as in the case of the *CUP SHAPED COTYLEDON* phenotype, therefore the use of a chimeric dominant repressor could be more informative than T-DNA knock down.

We have developed plants bearing a chimeric repressor for our tapetal *NAC* gene and found that fertility in these plants is compromised. Furthermore, over expression lines seem to show defects in flower development. We are in early phase of these studies, but preliminary evidence suggests that mutations in the tapetal specific *NAC* gene (*At1g61110*) can cause defects in tapetum and pollen development that lead to male sterility.

222 Site of action of a floral pathway integrator, FLOWERING LOCUS T

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Flowering in a long-day plant Arabidopsis is regulated by several pathways which converge on transcriptional regulation of the floral pathway integrators including FLOWERING LOCUS T (FT) (Kobayashi et al., 1999). FT is a direct target of CONSTANS, a key transcriptional regulator of the photoperiod pathway, and encodes a protein with similarity to mammalian Raf kinase inhibitor protein (also known as phosphatidylethanolamine binding protein). In rice (a short-day plant), an FT ortholog, Hading date 3a (Hd3a) plays a role in photoperiodic regulation of flowering, suggesting that FT is a conserved regulator of flowering (Kojima et al., 2002).

FT transcription is immediately induced in cotyledon and leaf vascular tissues upon transfer from short-day to inductive long-day photoperiods. Promotion of flowering by FT requires a bZIP transcription factor FD which is preferentially expressed at the shoot apex and activates transcription of APETALA1 (AP1) redundantly with LEAFY (LFY). Several lines of evidences suggest that FT and FD are inter-dependent in promotion of floral transition and activation of AP1 expression.

Since the activity of FD, which is preferentially expressed at the shoot apex, seems to require protein/protein interaction with FT, shoot apex is likely the site of action of FT protein. Consistent with this, restoration of FT function in the whole region or L1 of the shoot apex by FD or PDF1 promoter rescued late-flowering phenotype of ft and severe reduction of AP1 expression and floral defects of ft; lfy. Functional FT::GFP fusion protein expressed in shoot apex by FD promoter was distributed both in nucleus and cytoplasm. Nuclear distribution of FT::GFP fusion protein was also observed in leaf epiderimis of Nicotiana benthamiana after Agroinfiltration. Furthermore, flowering of 35S::FT:GR plants were accelerated upon dexamethasone treatment. These are consistent with the notion that FT protein acts in the nucleus to modulate FD activity. These and other approaches to elucidate cellular and subcellular sites of action of FT will be presented.

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223 TFL1 control of plant architecture: a *cen*tral role?

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The architecture of plants is dependent upon two groups of largely undifferentiated cells, the shoot apical meristem (SAM) and the root apical meristem. The SAM is responsible for all aerial organs formed during the life-span of a plant, maintaining a group of undifferentiated cells at its centre, and giving rise to organ primordia and secondary meristems at its flanks. In *Arabidopsis*, the SAM goes through 3 distinct phases: it makes leaves in the vegetative phase (V), secondary shoots subtended by a cauline leaf in the I1 phase, and flowers when it enters the reproductive inflorescence phase (I2). The identity of the SAM and the length of each phase determine plant architecture.

The *TFL1* gene controls plant architecture by delaying the progression of the *Arabidopsis* SAM through each of its phases. Mutations in *TFL1* result in shortening of all phases and, ultimately, the conversion of the SAM into a terminal flower, a phase normally absent in the wild type. Conversely, plants carrying a *35S::TFL1* transgene have all phases extended, and exhibit a novel phase (I1*) where secondary inflorescences form in the absence of a subtending cauline leaf.

TFL1 is normally expressed only in the shoot, in the centre of the SAM and in the vasculature. The lengthening of all phases observed in *35S::TFL1* plants suggests 2 important models: TFL1 can either act in primordia and secondary meristems to directly affect their fate, or TFL1 can only influence fate by its interactions in the centre of the SAM. In this second model, the effect of *35S::TFL1* is simply due to increased expression in its normal domain. To test which model is correct, we generated plants expressing the gene in floral meristems. Preliminary results show that the activity of the gene may be specific to the centre of the shoot meristem, suggesting it modulates cell fate before they form primordia. Inducible expression of *TFL1* will also clarify when the gene is able to affect phase change, and allow us to test if expression in any one phase only affects that phase.

To extend our knowledge on the control of plant architecture, we are characterising a novel mutant from *Antirrhinum*, which shows common features to *tfl1* and *cen* (the *Antirrhinum* homologue of *tfl1*) such as early flowering and the production of terminal flowers, as well as additional effects, such as conversion of sepals into petals. Isolation of this gene should give further insights into the regulation of meristem identity across distant species.

224 Efforts at Generating Apomixis Using Reverse-Genetics

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Apomixis, or asexual reproduction through seed, has long been sought as a panacea to many current agricultural problems. The facile generation of maternally clonal plants would allow for the fixation of hybrid genotypes, the genetic isolation of transgenic varieties, and the rapid production of new true-breeding lines. However, current efforts to transfer apomixis into agriculturally important species or generate it de novo have failed to yield any viable results. This is mainly due to an incomplete understanding of the mechanism and genetic regulation of apomictic reproduction. We hope to elucidate some of these mechanisms by using a reverse genetic approach to generate apomictic mutants of Arabidopsis. Instead of trying the re-create this system as a whole, our efforts have focused more on replicating individual "steps" or aspects of apomixis: maternal genome duplication, isolation and removal of the parental genome, parthenogenetic development, etc. All of these can be found to varying degrees in other species, e.g. yeast, C. elegans, Drosophila, mouse, etc. In some cases, the genes or mutations responsible for these desirable phenotypes have been cloned and characterized. We then searched the available databases of known and putative Arabidopsis genes for homologous sequences. The Salk Institute Genomic Analysis Laboratory (SIGnAL) T-DNA lines provide knock-out mutations in almost every putative gene of interest we identified. Such in silico experiments have already yielded vast numbers of putative genes involved in reproductive control. It is our belief that the ongoing genetic, physiological, and cytological characterizations of these mutants will allow us to clarify and perhaps duplicate the various mechanisms of apomictic reproduction.

225 Beyond boundaries: LOLLO is required for boundary formation and embryo development

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The LBD gene family has been identified as a whole of 42 Arabidopsis gene (Shuai et al., 2002; Iwakawa et al., 2002), sharing the so called "LOB domain". This domain comprises a cysteine-rich N-terminal end, a central GAS motif and a C-terminal part predicted to form a leucine zipper. One of the members of this family, ASYMMETRIC LEAVES 2, via its interaction with ASYMMETRIC LEAVES 1, has been identified as a strong repressor of the KNOX genes KNAT1, KNAT2 and KNAT6 (Ori et al., 2000; Semiarti et al., 2001; Lin et al., 2003). AS2 is also known to promote the LATERAL ORGAN BOUNDARY gene (LOB), the founder member of the LBD gene family. The LOB gene seems to play a role in the boundary establishment and/or the communication between meristem and initiating lateral organs (Shuai et al., 2002).

Through an activation-tagging mutagenesis, we isolated a new gain-of-function mutant of Arabidopsis thaliana named LOLLO-D (LOL-D), carrying a transposon insertion near the LOL gene that belongs to the LBD gene family. RNA in situ hybridizations show that the LOL gene is expressed in the boundaries between meristem and organ primordia. Overexpression of the LOL gene causes plant dwarfism, the formation of lobed leaves and meristem arrest. Floral organs are also affected: sepals and stamens are underdeveloped and pistils are often curled. Misexpression of a dominant negative version of the LOL gene causes cotyledon fusion, indicating that LOL is required for the formation of boundaries between developing organs. We will present our results on LOL requirement during embryo and later plant development.

226 Sphingosine Metabolites Play A Role in Plant Growth and Development

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A clearer understanding of sphingolipid function in plants is beginning to emerge. While research in a number of labs points to a role for these metabolites and their related enzymatic conjugates in responses like modulation of guard cell aperture, and enhancement of disease resistance, our research suggests these metabolites are important throughout the life cycle of the plant playing a crucial role in development. Our approach to analyzing these metabolites has focused on 3 major enzymes involved in the metabolism of sphingosine-1-phosphate, a key structural and signaling molecule present on the plasma membrane based on research from animal systems. The sphingosine kinase (SGK1), lyase (SGL) and phosphatase (SGP) each represent a potential point of regulation for sphingosine metabolites based on research in animal systems. Based on sequence homology with animal systems, we have identified putative genes for each of these enzymes in *Arabidopsis thaliana*. We have obtained homozygous insertional lines for several of these enzymes and are now characterizing the expression and function of each of these genes through RT-PCR, transgenics and consideration of developmental and growth phenotypes. This research represents a unique effort to study these important metabolites and their regulation throughout the plant life cycle.

227 Two Receptor-Like-Kinases are essential for the control of tapetum development

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Leucine Rich Repeat Kinases (LRR-K) belong to the 650 member Receptor Like Kinase (RLK) family. They are thought to function in the perception of the cell environment and the initiation of intracellular signaling pathways. The functions of most of these genes remain unknown. We report the characterization of two redundant LRR-Kinases. Whole plant analyses showed stronger expression in flowers and fruits compared to somatic tissues. To elucidate their functions, we conducted a reverse genetic approach. Whereas single mutants did not show a clear phenotype, double mutants of the close homologues exhibited obvious fruit growth defects, resulting in the disruption of seed formation. WT pollen reverted this sterility showing a probable stamen development defect or non functional pollen production. In young buds, mutant anthers developed normally but stopped at an early stage close to meiosis. Investigations using confocal microscopy of anthers showed that meiosis occurs but that because of the lack of the tapetal cell layer, microspores abort. These results show that these LRR-Kinases function at an important control point for sporophytic development controlling male gametophyte development. Further analyses of the function of these genes, including an unusual membrane targeting, will be presented.

228 Functional Characterization of FW2.2

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fw2.2 is one of the few QTLs that have been isolated thus far in plant. It is also the first gene known to control fruit size in a crop plant. *fw2.2* encodes a protein with no sequence homology with any protein of known function. Yet it is known to be a regulator (either directly or indirectly) of cell division (Frary et al. 2000, Cong et al.2002, Liu et al. 2003). Computational modeling had earlier suggested that the FW2.2 protein might have a similar structure to RAS proteins which are found in mammalian cell cycle control and possess the GTP-binding ability (Frary et al. 2000). To shed light on the possible function of FW2.2, we have conducted a series of experiments aimed at elucidating cellular location and function of the FW2.2 protein. To examine whether FW2.2 has the GTP-binding activity, nucleotide exchange assays were performed (Leonard et al. 1994). The binding [35S]GTPγS ability of the recombinant proteins GST-FW2.2, GST-2TK (as a negative control) and GST-CDC42 (as a positive control) was measured after 10min incubation. The results demonstrated that the amount of CDC 42 protein–bound radioactivity rapidly reached extremely high level (1,350,295 cpm), while that of the FW2.2 protein and the negative control protein GST-2TK binding activity was at very low levels (1,499 and 1,681 cpm, respectively). Thus, FW2.2 is not a GTP-binding protein. Additional analysis of the protein annotation predicted that FW2.2 is a novel protein containing two transmembrance domains and may be coupled with cell membrane. To this prediction, GFP/FW2.2 fusion protein was bombarded into young tomato leaves. The transient expression of GFP/FW2.2 was observed in the plasma membrane under a confocal fluorescence microscope, indicating that FW2.2 is localized at plasma membrane. Further, the yeast two-hybrid screening has revealed that there are about 90 putative positive candidate proteins which might interact with the FW2.2 protein. Among which many proteins have been found essential in plant or mammalian and yeast cell growth and development by controlling cell division or regulating cell expansion. To confirm their interactions, the “pull-down” assay was applied. One of these candidates, TYBP55 (a protein kinase), was found to interact with FW2.2. Further experiments to verify their interactions by *in vivo* binding assay are in progress.

229 Comprehensive interaction map of the Arabidopsis MADS box transcription factors

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Interactions between proteins are essential for their functioning and the biological processes they control. The elucidation of interaction maps based on yeast studies is a first step towards the understanding of molecular networks and provides a framework of proteins that possess the capacity and specificity to interact. Here, we present the first comprehensive plant protein-protein interactome map of nearly all members of the Arabidopsis MADS box transcription factor family. A matrix based yeast 2-hybrid screen between over one hundred members of this family revealed a collection of specific heterodimers and a few homodimers. Clustering of proteins with similar interaction patterns pinpoints proteins involved in the same developmental program and provides valuable information about the participation of uncharacterized proteins in these programs. Furthermore, a model is proposed that integrates the floral induction and floral organ formation networks based on the interactions between the proteins involved. Heterodimers between flower induction and floral organ identity proteins were observed, which point to (auto)regulatory mechanisms that prevent the activity of flower induction proteins in the flower.

230 Towards Understanding Phenotypic Diversity within Brassicaceae

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The plant family Brassicaceae provides an excellent, yet underutilized resource for extending the knowledge base developed in Arabidopsis thaliana and exploring the molecular basis of the striking phenotypic diversity among species. Specifically, this family exhibits extraordinary diversity in morphological, life history, and chemotaxonomic traits, yet the genetic basis of this variation is poorly understood. Previous analyses of inter-species phenotypic variation have shown that some traits, such as branching in teosinte/maize, or floral architecture and color in Mimulus are under the control of major genes. With these observations in mind, we have initiated a project that aims to identify single-gene modifier traits from the Brassicaceae. We are exploiting Arabidopsis as a genetic background into which genomic libraries from diverse Brassicaceae will be introduced by high throughput transformation. To facilitate this approach we have designed a bacteriophage lambda cloning vector (lambda NOEL) that enables construction of plant-transformable genomic libraries. DNA cloned into lambda NOEL can be converted into a binary-plasmid using a cre-lox recombination system; the recombinant binary plasmid contains a BAR marker that enables high-throughput soil-selection of transgenic plants. At present, genomic libraries from several Brassicaceae have been constructed and are being transformed en masse into Arabidopsis with the ultimate goal of screening for dominant traits that modify Arabidopsis leaf, trichome, flower and silique morphology (all of which are highly variable within Brassicaceae). Additionally, we have been systematically surveying the susceptibility of several dozen Brassicaceae to transformation by the floral-dip method. Results from both sets of experiments will be presented. Ultimately we aim to combine shotgun-transformation based methods and conventional genetic methods to gain a deeper understanding of the phenotypic diversity present in the Brassicaceae.

231 The nuclear actin-related protein ARP6 is a developmental regulator required for the maintenance of *FLC* expression and repression of flowering in *Arabidopsis*

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Members of the actin-related protein (ARP) family perform a wide variety of functions both within the cytoplasm and the nucleus of eukaryotic cells. The cytoplasmic ARPs play well-defined accessory roles within the actin and tubulin cytoskeletal systems, while the functions of the nuclear ARPs are generally understood only on the basis of their presence in various yeast and animal chromatin-modifying complexes. *Arabidopsis thaliana* ARP6 is a clear ortholog of ARP6s from other eukaryotes including *S. cerevisiae* ARP6, which was identified as a component of the Swi2/Snf2-type SWR1 chromatin remodeling complex. In order to address the function of ARP6 in *Arabidopsis*, we have examined the subcellular localization, expression patterns and loss-of-function phenotype for this protein. We find that *Arabidopsis* ARP6 is localized to the nucleus and is expressed most strongly in vascular and above-ground vegetative tissues, as well as in a subset of reproductive tissues. Null mutations in ARP6 result in a multitude of defects including altered development of the leaf, inflorescence, and flower, as well as reduced fertility and early flowering in both long and short day photoperiods. The early flowering of *arp6* mutants is associated with reduced expression of the floral repressor gene *FLOWERING LOCUS C* (*FLC*), and these mutations repress the *FLC*-mediated late flowering of a *FRIGIDA* (*FRI*) expressing line, indicating that ARP6 is required for the activation of *FLC* expression. We propose that ARP6 regulates multiple aspects of plant growth and development through its role in the modulation of chromatin structure and the control of gene expression.

232 Leaf polarity and growth factors play important roles in patterning the fruit in Arabidopsis

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Seed dispersal facilitated by the fruit is dependent on the formation of a specialized stripe of tissue termed the valve margin, which enables the valves to detach from the fruit and disclose the seeds. Valve margin development is dependent upon the *SHATTERPROOF* (*SHP*) genes and is limited to a stripe of cells through the repressive activities of *FRUITFULL* (*FUL*) in the valves and *REPLUMLESS* (*RPL*) in the replum. A critical aspect of fruit development that is not well understood is how the patterns of gene activities that control valve margin formation are initially established. We have found that *FILAMENTOUS FLOWER* (*FIL*) and *YABBY3* (*YAB3*), genes which regulate the polarity of tissues in lateral organs, are required to promote the expression of *FUL* and *SHP*. The unrelated gene, *JAGGED* (*JAG*), which promotes the growth of tissues in lateral organs, acts redundantly with *FIL* and *YAB3* to promote the expression of *FUL* and *SHP*, with *jag fil yab3* triple mutants lacking *FUL* and *SHP* expression in the valves or valve margins. We also provide insight into the mechanism by which *RPL* regulates valve margin development by showing that *RPL* negatively regulates *JAG/FIL* activity in the replum. Our work describes the first functional link between patterning systems in plants that define organ polarity and growth and those that control tissue identity and provides a genetic framework for the patterning of the three tissue types composing the fruit.

233 Genetic Analysis of the ubiquitin-specific proteases and ubiquitin conjugating enzymes in *Arabidopsis thaliana*

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The ubquitin 26S proteasome pathway is a major pathway of selective protein degradation operative in the cytoplasm and nucleus of eukaryotic organisms including the plant *Arabidopsis thaliana*. It has become increasingly evident that this pathway is important in numerous physiological and developmental processes including embryogenesis, hormone and light responses, cell division, and senescence. We are investigating the roles of two enzyme classes using a reverse genetic approach: 1) the ubiquitin-specific proteases (UBPs) and 2) the ubiquitin conjugating enzymes (UBCs). UBPs serve to remove ubiquitin monomers from the primary translation products of ubiquitin genes, from free multi-ubiquitin chains, and from ubiquitinated proteins. UBCs serve in the attachment of ubiquitins onto target proteins. We have assembled a collection of T-DNA insertion mutants and are currently analyzing the effects of homozygous single gene mutations. We determined previously that one particular UBP, UBP14, is essential during early embryo development. Many UBPs and UBCs are members of a subfamily of related proteins; therefore, it is not surprising that many homozygous, single gene mutants do not display any obvious phenotypic abnormalities. We are performing genetic crosses to obtain plants in which all genes encoding a particular subfamily have been interrupted by T-DNA insertion. We have directed our attention to the phenotypic characterization of individuals defective in the UBP3/4 subfamily of ubiquitin-specific proteases. We will present evidence that UBP3/4 is essential for pollen transmission and report our progress on the genetic characterization of other UBP and UBC subfamilies.

234 Brassinosteroid receptor BRI1 regulates floral timing

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Transition from vegetative to reproductive growth is a critical phase change in the development of a flowering plant. In *Arabidopsis thaliana*, timing of this transition is controlled by at least four genetic pathways: the photoperiodic, the autonomous, the vernalization, and the gibberellin pathways. To identify additional signaling components, we mutagenized the autonomous-pathway mutant *luminidependens* (*ld*) and performed a genetic screen to isolate genes that enhances the late-flowering phenotype of *ld*. Two alleles of *bri1* were found in this screen. *BRI1* encodes for a leucine-rich-repeats receptor kinase that binds to the plant hormone brassinosteroid. The strong enhancing activity of *bri1* onto *ld* is largely abolished in the presence of a functional *LD* gene, implicating *BRI1* as a floral-timing modifier. To assess the specificity of the modifying activity of *bri1*, a series of double mutants between *bri1* and other flowering-time mutants was generated, and their flowering time was examined. We found that *bri1* was merely additive to the phenotype of *gi*, implying that *BRI1* does not interact with the photoperiod pathway. Interestingly, combining *bri1* with elements of the autonomous pathway leads to similar extremely late-flowering phenotype as with *ld*, under both long- and short-day condition. Thus, *BRI1* surprisingly functions in parallel to the autonomous pathway. That raised a question as to whether similar molecular targets are involved, including *FLC*, whose expression is repressed by genes of the autonomous pathway. The levels of *FLC* transcript in the double *bri1 ld* mutant are several fold higher than in *ld* and remained very high even in *ca*. 100-days-old plants. Since *FLC* is a strong repressor of flowering, we hypothesize that the high levels of *FLC* expression fully defines the molecular basis of the *BRI1* modifying activity on flowering time. Based on these results, we propose that *BRI1* would not act directly to promote flowering, but would assist the autonomous pathway to repress the expression of *FLC*. Surprisingly, *BRI1* also seems to function in the vernalization response. The role of *BRI1* in the control of flowering time and in the vernalization response will be discussed in detail.

235 Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility

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Arabidopsis contains a family of 12 putative callose synthase genes (*GSL1-12*). Their role in plant development is still largely uncertain. We used TILLING and T-DNA insertion mutants (*gsl1-1*, *gsl5-2* and *gsl5-3*) to study the role of two closely related and linked genes, *GSL1* and *GSL5*, in sporophytic development and in reproduction. Both genes are expressed in all parts of the plant. Sporophytic development was nearly normal in *gsl1-1* homozygotes and only moderately defective in homozygotes for either of the two *gsl5* alleles. On the other hand, plants that were *gsl1-1/+ gsl5/gsl5* were severely defective, with smaller leaves, shorter roots and bolts and smaller flowers. Plants were fertile when the sporophytes had either two wild-type *GSL1* alleles, or one *GSL5* allele in a *gsl1-1* background, but *gsl1-1/+ gsl5/gsl5* plants produced very few viable seeds. A chromosome with mutations in both *GSL1* and *GSL5* rendered pollen infertile, although such a chromosome could be transmitted via the egg. As a result, it was not possible to obtain plants that were homozygous for mutations in both the *GSL* genes. Many pollen grains were collapsed and inviable in the *gsl1-1/gsl1-1 gsl5/+* and *gsl1-1/+ gsl5/gsl5* plants. In addition, *gsl1-1/+ gsl5/gsl5* plants produced abnormally large pollen with unusual pore structures, and had problems with tetrad dissociation. In this particular genotype, while the callose wall formed around the pollen mother cells, no callose wall separated the resulting tetrads. We conclude that GSL1 and GSL5 play important, but at least partially redundant roles in both sporophytic development and in the development of pollen. They are responsible for the formation of the callose wall that separates the microspores of the tetrad, and also play a gametophytic role later in pollen grain maturation.

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236 Linking Polycomb activity to morphogenesis via the Arabidopsis formin, *AtFH5*

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The Polycomb group (Pc-G) proteins are widely conserved and well studied modular complexes that inhibit their target genes epigenetically through chromatin remodeling. However, less is known about how their patterning activity in development engineers downstream morphogenic events such as polarity and segmentation.

In Arabidopsis, polarity and cellularization events in the seed endosperm provide a simple model for developmental pattern and timing. Mutations in any of the fertilization independent seed (FIS) Pc-G members result in an aberrant endosperm development, including enlargement of cysts in the posterior pole, an absence of endosperm cellularization and the overexpression of enhancer trap lines that report posterior pole identity. One such line, KS117, reports the expression of an Arabidopsis formin homologue, *AtFH5*. Formins are conserved actin-nucleators involved in cell polarity and cytokinesis, thus reasonable candidates for a Pc-G effector gene in the endosperm. We have recently characterized two *atfh5* insertional mutants [NCB 7(4) 374-380, 2005]. These lines are defective in endosperm cellularization and can also lack posterior pole structures. This result suggests a model where Polycomb activity is needed to restrict *AtFH5* expression to an appropriate level during endosperm development. Using real-time PCR, we find that *AtFH5* is expressed in buds and flowers and that this expression diminishes as siliques develop. In a *fis2* background, *AtFH5* levels are consistent with wt in buds and flowers, but expression after fertilization can increase up to 6 fold. This further supports a temporal regulation by Polycomb. Current studies include the examination of Pc-G *atfh5* double mutant lines to examine if posterior pole reduction in the *atfh5* mutants is epistatic to the enlargement seen in *fis* backgrounds.

To identify additional genes in this pathway, we took advantage of the strong visual phenotype of KS117 overexpression in the *fis2* background for a genetic screen. 5211 γ irradiated *fis2* KS117 plants were screened for reduction of KS117 expression in 50% of M1 seeds. 39 potential gametophytic revertants of the *fis* phenotype were discovered. Initial examinations of backcrossed revertants suggest that these lesions vary in their effect on pattern and cellularization. We envision that these mutants will represent additional components of the *fis* pathways, and parallel activating components similar to the conserved Pc-G antagonist, Trithorax.

237 The *quartet1* tetrad pollen phenotype is caused by mutations in a putative pectin methylesterase gene

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The Arabidopsis *QUARTET (QRT)* genes are required for pollen separation during normal floral development. In *qrt* mutants, the four products of microsporogenesis remain fused, and pollen grains are released as tetrads. In Arabidopsis, tetrad analysis in *qrt* mutants has been used to map all five centromeres, easily distinguish sporophytic from gametophytic mutations, and accurately assess crossover interference. The identification of the genes responsible for the quartet phenotype may enable the transfer of tetrad analysis to other plant species. Using a combination of forward and reverse genetics, we have identified the gene responsible for the *qrt1* phenotype. *QRT1* encodes a putative pectin methylesterase (PME). PMEs constitute a large gene family in Arabidopsis, are involved in cell wall loosening, and have been implicated in various aspects of floral development. Promoter and transcription analysis indicate *QRT1* is expressed in anther tissues. Unexpectedly, the *QRT1* promoter is also active in a variety of developmentally unrelated tissues including developing guard cells, the hypocotyl-root transition zone, areas of lateral root emergence, and anther dehiscence zones. We are currently developing strategies to transfer the *qrt* phenotype to other plant species.

238 ATG6/VPS30, a component of phosphatidylinositol 3-kinase, plays essential roles in pollen germination in Arabidopsis

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ATG6, which was originally isolated as an autophagy-related gene in yeast, is allelic to VPS30, a component of phosphatidylinositol 3-kinase (PI3K) complex. Application of wortmannin, the PI3K inhibitor, interfered with autophagy in Arabidopsis suspension cells. To further reveal roles of ATG6-mediated PI3K signaling in *planta*, we have screened T-DNA insertion mutants of Arabidopsis ATG6. Although no homozygous mutant lines were identified, approximately 50% of the seeds in the progeny of selfed-heterozygous plant were heterozygous (ATG6/*atg6*), suggesting a male gametophytic mutant phenotype. This possibility was confirmed by crossing experiments between AtATG6 heterozygous and wild type plants. Mature pollen grains from ATG6 heterozygous plants were normal in appearance, whereas efficiency of *in vitro* pollen germination in ATG6 heterozygous plants seemed to be reduced compared to wild type. When ATG6 heterozygous plants were crossed to the *quartet* mutant, normal and aborted pollen grains from ATG6*/atg6/qrt/qrt* plants seemed to segregate 2:2 in tetrads: less than 2 pollen grains in each quartet germinated. Microscopic observation of double mutant pollen grains detected no phenotypically aberrant pollen. Results suggested that ATG6 is not necessary at the early stages of pollen development but essential for pollen germination.

239 Mechanisms of INO function in ovules of *Arabidopsis thaliana*

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In *Arabidopsis*, the outer integument develops in an asymmetric manner with cell growth and division occurring primarily along the region of the ovule that faces the base of the gynoecium (gynobasal). This process is altered in the mutant *inner no outer (ino)*, which fails to properly establish the outer integument. INO is a member of the YABBY family of putative transcription factors and its expression is restricted to the gynobasal side of developing ovules. The goal of our research is to determine the mechanisms by which INO supports growth by defining the function of specific regions within the INO protein and by uncovering factors and sequences with which INO interacts to coordinate the proper development of the outer integument. The first approach involves domain swap experiments between INO and either YABBY3 or YABBY5. These domain swaps are being made in an extension of previous domain swap experiments between INO and CRABSCLAW in order to determine the functionality of specific regions within INO. Through analysis of ovule-specific phenotypes in *ino-1* plants that contain these chimeric constructs the role of specific regions of INO will be further elucidated. The second approach involves identification of factors that participate in the INO-mediated pathway of integument growth. Yeast-two-hybrid studies are being conducted using the full-length protein as well as truncations of the INO protein containing putative functional domains in conjunction with a floral cDNA library in order to identify potential interactors. The third approach utilizes purified INO in an *in vitro* selection procedure. This process involves recursive purification methods of an incubation reaction of INO with oligonucleotides containing a central random region. It is through this procedure that the DNA binding activity of INO will be tested and potential downstream targets of transcriptional regulation will be identified. Through these studies, it is our goal to identify those genes that participate in the INO-mediated integument growth in *Arabidopsis thaliana* and to gain an understanding of the functionality of specific regions of the INO protein.

240 Genetic analysis of gametophytic parental effect mutants in Arabidopsis

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Seed development requires a coordinated interplay of embryo, endosperm and the maternal seed coat. What roles gametophytic parental (maternal and paternal) factors play in this process is not clear. We have performed various screens to identify haplo-phase specific genes required in the gametophytic phase, or required in a gametophytic parental effect specific manner for embryo and endosperm development, and an outline of these screens will be presented. In the gametophytic maternal-effect *capulet (cap)* mutants, both embryo and endosperm development is arrested at early stages. The *cap* mutant phenotypes were not rescued by wild-type pollen and removal of silencing barriers from the paternal genome also failed to restore seed development. The mutants displayed no autonomous seed development and were epistatic to *fertilisation-independent-seed1 (fis1)* in both autonomous and sexual endosperm development. Embryo and endosperm specific GUS and GFP markers were ectopically expressed in *cap* mutant endosperms. In addition we explore the use of a paternal effect mutant line, where mutant pollen induce embryo and endosperm developmental arrest after fertilization, to dissect the involvement of maternal and paternal gene programmes in seed development.

241 A molecular basis for TFL1 and FT evolution

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Homologous proteins occurring through gene duplication may give rise to novel functions through mutations and contribute to the evolution of new morphological traits. However, it is often unclear which changes are key to determining new functions. To address these ideas we have studied a system where two homologues have evolved clear and opposite functions in controlling flowering, a major developmental switch in plants. Arabidopsis homologues TFL1 and FT are key controllers of flowering, determining when and where flowers are made, but as opposing functions; TFL1 is a repressor, FT is an activator. We have uncovered a striking molecular basis for how these homologous proteins have diverged. Although less than 60% identical, we have shown that swapping a single amino acid is largely sufficient to convert TFL1 to FT function and vice versa. Further, our results suggest that TFL1 and FT are highly conserved in biochemical function, and that they act as repressors or activators of flowering through discrimination of structurally-related interactors by a single residue. Therefore we suggest that a key residue may have strongly contributed to the selection of these important functions over plant evolution. However, TFL1 and FT show different expression patterns. We are currently testing whether specific expression patterns may also have contributed to the evolution of TFL1 and FT functions, by expressing TFL1 and FT under different promoters.

242 *BAM1* and *BAM2* are members of the LRR-RLK family that are essential for proper anther development in Arabidopsis thaliana

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Leucine-rich repeat receptor-like kinases (LRR-RLK's) are the largest group of RLK's in the Arabidopsis genome. Previous characterization of a few LRR-RLK's has shown that they are important for normal plant development and differentiation, as well as cell identity. *EMS1* is a LRR-RLK that is known to be critical for normal anther development in Arabidopsis; *BAM1* and *BAM2* are *CLV1*-related LRR-RLK that share 81% identity to each other. Disruption of either of these genes does not appear to affect plant development; however, disruption of both shows clear morphological defects during male gamete development. This indicates that *BAM1* and *BAM2* might share redundant functions. Herein the characterization of the *bam1/bam2* male-sterile phenotype will be described.

243 BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in Arabidopsis

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Morphogenesis in multicellular organisms involves an intricate balance of mechanisms that regulate cell division, growth, and differentiation. Here, we describe an important role for two NPR1-like signaling proteins, designated BLADE-ON-PETIOLE1 (BOP1) and BLADE-ON-PETIOLE2 (BOP2) in controlling growth asymmetry, a crucial aspect of patterning during morphogenesis. NPR1 is the founding member of small gene family and is a key regulator of the plant defense response known as systemic acquired resistance. Accumulation of the signal molecule salicylic acid (SA) leads to a change in intracellular redox potential enabling NPR1 to enter the nucleus and interact with TGA transcription factors which in turn bind to SA-responsive elements in the promoters of defense genes. Phenotypes in *bop1 bop2* double mutants are diverse, but include leafy petioles, loss of floral-organ abscission, and asymmetric flowers subtended by a bract. We show that BOP2 is localized to both the nucleus and cytoplasm, but unlike NPR1, is highly expressed in young floral meristems and in yeast interacts preferentially with the TGA transcription factor encoded by *PERIANTHIA (PAN)*. In support of a biological relevance for this interaction, we show that *bop1 bop2* and *pan* mutants share a pentamerous arrangement of first whorl floral organs, a patterning defect that is retained in *bop1 bop2 pan* triple mutants. These data provide evidence that BOP proteins control aspects of patterning via direct interactions with TGA transcription factors and illustrate that a signaling mechanism similar to that formally associated with plant defense is likely used for the control of developmental patterning.

244 Identification and Characterization of Proteins that Interact with AGAMOUS-Like 15 (AGL15), a MADS-Domain Transcription Factor that Preferentially Accumulates in the Plant Embryo

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AGAMOUS-Like 15 (AGL15) encodes a MADS-domain transcription factor that is preferentially expressed in the plant embryo, and is believed to be involved in embryonic developmental programs. Research in our lab has identified a number of downstream targets of AGL15, and while some of these target genes are induced in direct response to AGL15, others are repressed. A number of target genes have been analyzed that bind AGL15 and exhibit strong responses to AGL15 levels *in vivo*, yet *in vitro*, AGL15 binds only weakly. Taken together these data suggest that AGL15 may form hetero-dimers, or ternary complexes with other proteins, thus modulating AGL15's specificity and function in planta.

The yeast two-hybrid system has been used to screen an embryonic expression library to identify putative novel interactors of AGL15, and true positives, as verified in the yeast system, will be presented here. Truncations of AGL15 have been created and used to determine which of its domains are involved in various interactions. Other MADS box genes are expressed in the plant embryo, and some, although not all, are also shown to interact with a few of the "AGL15 partners" that were identified from our yeast two-hybrid screen.

A number of novel partners have been elucidated, including putative nucleic acid binding proteins and proteins that may be involved in chromatin modification, such as *SIN3 Associated Protein of 18 KDa (SAP18)*. Yeast-three-hybrid assays were used to test for complex formation involving AGL15-SAP18, and components of the SIN3 deacetylase complex.

245 Characterisation of an Arabidopsis flowering time mutant

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Flowering time has been studied for many years. Recent molecular studies suggest that plants perceive inductive photoperiods through the coincidence of active CONSTANS (CO) protein expression with light which promotes the expression of *FLOWERING LOCUS T (FT)*, which in turn promotes flowering. A flowering time mutant of Arabidopsis was identified from a Ds insertional mutant population and named *regulator of CONSTANS (roco)*. The roco mutant flowers late in Long days (LD) but at the same time as WT in short days (SD) which suggests that it is defective in a photoperiodic inductive signal normally seen in LD. Quantitative real-time PCR has shown that in the *roco* mutant expression of *FT* is down regulated in LD, explaining its late flowering phenotype. The expression of *CO* is unaffected in the mutant suggesting that the *roco* mutation affects the activation of *FT* by the CO protein. Inverse PCR revealed that the Ds element has inserted into the promoter of a gene (ROCO) encoding a RING-finger protein. In the mutant ROCO is down regulated 10-fold. RING-finger proteins act as ubiquitin ligases, which are components of the ubiquitin proteasome pathway that target specific proteins for degradation via the 26S proteasome. It is hypothesized that ROCO targets a repressor of flowering for degradation in inducing LD conditions. Genetic and physiological characterisation of the mutant will be presented.

246 Arabidopsis endosperm development in WT, *acc1*, and *cac1A* mutant ovules

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Dicotyledonous plants like Arabidopsis contain two structurally distinct acetyl-CoA carboxylases that are localized to the plastids and cytosol. Mutations in either the *ACC1* gene, which codes for the cytosolic isozyme, or in the *CAC1A* gene, which codes for the plastidic isozyme, show embryo-lethal phenotypes by the heart stage. To understand the role of these genes in endosperm formation, we analyzed endosperm development in the *acc1* and *cac1A* mutants as compared to that of WT plants. In wild-type Arabidopsis, after fertilization the primary endosperm nucleus undergoes a series of synchronous nuclear divisions without cytokinesis, yielding free nuclei (coenocyte endosperm). Radial microtubule rearrangements take place prior to endosperm cellularization between sister and non-sister nuclei. Vesicles carried along the microtubules form phragmoplasts, initiating cellularization. Cell wall formation follows. *acc1*-mutant embryos grow slower than WT, and cease growth by the early heart stage of development. In *acc1* mutants, as in *cac1A* mutants, endosperm develops more slowly than in WT. In the *acc1* mutant, free nuclei and cytoplasm appear normal but form few, if any, radial microtubules; cellularization does not occur. Endosperm degenerates by the time the embryo reaches the early heart stage. In contrast, endosperm of *cac1A* mutants forms an amoeboid-like composite of multiple nucleoli and nuclei. Cellularization of the endosperm fails or is only partial. In both *cac1A* and *acc1* mutants, early heart stage embryos may not show obvious morphologic defects, despite being surrounded by non-cellularized endosperm. We conclude that the *ACC1*and *CAC1A* genes are necessary for different aspect of normal endosperm and embryo development. Our results raise the possibility that embryo formation through the early heart stage may not require interaction with the endosperm.

247 The *MS1* gene of *Arabidopsis* regulates pollen wall development

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We have previously identified an *Arabidopsis* male sterile mutant, *ms1-8*, and isolated *MS1* gene (Ito and Shinozaki, Plant Cell Physiol. 2002 43:1285). *MS1* was expressed specifically in tapetums and transiently around tetrad stage. Until microspore separation from tetrads, phenotypic difference was not recognized between wild type and the mutant. After that stage, both microspores and tapetums gradually became vacuolated, and finally empty locules resulted. MS1 protein was a PHD finger-type protein.

We examined here biological functions of the MS1. We showed the PHD motif actually worked as a functional domain by introducing modified *MS1* genes into the *ms1* mutant. This result suggests that MS1 functions as a transcriptional regulator like other PHD-type factors.

To our knowledge, there are some loss-of-function *ms1* alleles in which *MS1* functions were completely lost, but no reduction-of-function alleles in which *MS1* function has been partially repressed. To obtain more information about physiological function of the *MS1* gene, we created transgenic *Arabidopsis* in which MS1 function has been partially repressed by using transcriptional repression domain (SRDX). We introduced a T-DNA cassette expressing MS1-SRDX fusion protein into wild-type *Arabidopsis*. Resultant plants were semi sterile and produced mature pollens. But we observed almost no reticulated exine patterning on their pollen surfaces. In addition, the mature pollen did not keep its rugby ball shape, and collapsed along apertural furrows. These results indicate that MS1 regulates construction of pollen exine.

248 *Arabidopsis* plants having a mutation in the *CDKA* gene show very early developmental lethality

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Regulation of the cell cycle is indispensable for all living organisms. In eukaryotes, cdc2 protein kinase is conserved from yeast to animals, and acts as a key regulator of the cell cycle through cyclin-dependent phosphorylation. In plants, two types of cyclin-dependent kinases (CDKs), CDKA and CDKB, are largely responsible for cell cycle control. CDKA, which includes the PSTAIR motif, can complement the yeast p34*cdc28/cdc2* mutation, while CDKB is thought to be a plant-specific CDK. Here we investigate the phenotype of *Arabidopsis cdka-1* plant, which was obtained from SALK T-DNA insertion lines. Plants containing heterozygous T-DNA were indistinguishable from wild type in the vegetative stage, whereas no homozygous seeds germinated. Heterozygous plants produced mature siliques containing normal and aborted seeds (or ovules) at a 1:1 ratio, suggesting that the insertion is probably gametophytic lethal. Indeed, cross-fertilization experiments suggested that the male gametophyte of *cdka-1* plants was lethal. Fluorescent microscopy revealed that about half of the mature pollen grains obtained from *cdka-1/CDKA* plants contain a single sperm cell nucleus, in contrast to the pollen grains of wild type which contain two sperm cell nuclei. These results suggested that cell division of regenerative cell was arrested due to the loss of CDKA. To establish whether or not these abnormal pollen grains are fertile, we examined embryogenesis of self-fertilized *cdka-1/CDKA* plants. In accordance with the results of cross-fertilization experiments, about half of the seeds were of normal size, but the remainder were significantly smaller than wild type. Although development in the latter embryos ceased by the eight-cell stage, the morphology of embryo cells and suspensor cells was normal. We concluded that, in *cdka-1* plants, cell division is perturbed in very early embryogenesis but cell differentiation is not affected.

249 *Early in short days 6* (*esd6*) and *early in short days 7* (*esd7*), two mutations that accelerate flowering time in Arabidopsis

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Proper control of the floral transition is pivotal for reproductive success in flowering plants. In the past years, several early-flowering genes have been characterized although their interaction with the flowering inductive pathways is almost unknown. In a collection of *Ds*-containing T-DNA lines in Ler background, we have isolated the *esd6* and *esd7* mutations, which cause early flowering independently of photoperiod. These mutants are smaller and appear less vigorous than wild-type plants. The leaves on *esd6* plants appear paler than those in wild-type plants; on the other hand, *esd7* mutants show narrowed leaves and moderate elongation of the primary root. Siliques on both mutants are typically smaller on length than wild-type ones. Analyses of double mutants for the *ESD6* locus and different loci of the flowering inductive pathways suggest that flowering inhibition mediated by *ESD6* is through *FLC*-dependent and independent pathways. On the same way, *ESD7* appears to be involved in multiple flowering pathways. *ESD6* has been cloned and encodes a previously characterized RING-containing protein found to harbour an incomplete RING domain. With respect to *esd7*, the *Ds* element does not cosegregate with the mutant phenotype and therefore it has been delimited by a map-based cloning approach in a 50 Kb genomic region of chromosome I; the gene is being identified by complementation with overlapping cosmids.

250 Arabidopsis HOS15, A Human TBL1-like WD-40 Repeat Protein, Modulates Plant Stress Tolerance and Flowering

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To investigate the essential components mediating stress signaling in plants, we initiated a large scale screen for stress response mutants using Arabidopsis plants that carry the firefly luciferase reporter gene under the control of the stress-responsive *RD29A* promoter. Here we report the identification and characterization of one mutant, *hos15-1* (for high expression of osmotically responsive genes), which displays the super-induction of luminescence by low temperature, exogenous abscisic acid (ABA), or osmotic stress (NaCl). The expression of the endogenous *RD29A* and other stress responsive genes but not the stress-induced transcription regulator, CBF, was super-induced in the *hos15-1* mutant plants compared to wild type plants. The *hos15-1* plants are more sensitive to freezing treatment. The seed germination of *hos15-1* appears to be more sensitive to inhibition by ABA or NaCl. In addition, the *hos15-1* plants flower late. The expression level of key flowering regulatory genes, *SOC* and *FT*, was substantially reduced in *hos15-1* compared to WT. The *HOS15* locus was identified by TAIL-PCR and it encodes a protein with sequence identity to the human WD-40 repeat protein TBL1 which is a component of the chromatin repression complex. It is well documented that the chromatin repression complex interacts with histone protein. In the yeast two hybrid assay we found that HOS15 interacts with H4 and confirmed this interaction with a co-IP assay from protoplasts which are harboring HOS15::GFP and T7::H4 fusion constructs. Introducing the genomic fragment, which contains the wild type *HOS15* gene into *hos15-1* mutant plants complemented *hos15-1* mutant phenotype. HOS15 is localized to the nucleus. Together, these results suggest that HOS15 is an important component of the stress signaling and flowering regulatory system of plants.

251 Arabidopsis SUMO E3 ligase, AtSIZ1 is a negative regulator of flowering through modification of FLD

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Small ubiquitin-like modifier (SUMO) conjugation plays critical roles in many cellular processes. Here we show that *Arabidopsis* SUMO-E3 ligase, AtSIZ1 negatively regulates flowering time through post-translational motification of FLD. *siz1* null mutant plants (*siz1-2* and *siz1-3*) exhibit an extreme early-short-day flowering phenotype. RT-PCR results indicate that transcript abundances of the main flowering-time control genes *FT* and *SOC1* are elevated (*SOC1* greater than *FT*) in *siz1* compared to wild type plants at comparable stages of development. The *siz1* mutation does not alter circadian rhythm or *GI* and *CO* mRNA abundances. Quantitative PCR revealed that *FLC* mRNA levels are substantially lower in *siz1* plants compared to wild type. However, the mRNA levels of other *FLC* homologs *FLM*, and *MAF2* are equivalent in *siz1* and wild type plants. These results indicate that up-regulation of *FT* and *SOC1* expression may be the result of reduced FLC activity in *siz1* plants. *FLC* mRNA abundance can regulated by *FLD*, which is one of six genes in the autonomous pathway, that deacetylate histones in *FLC* chromatin. In vitro sumoylation assays revealed that FLD is sumoylated by yeast Siz1. We hypothesize sumoylation represses FLD activity to regulate flowering.

252 Structure and function of the PETAL LOSS protein, a transcription factor involved in regulating sepal and petal development in Arabidopsis thaliana

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PETAL LOSS (PTL) is a member of the trihelix transcription factor family known only in plants (Brewer et al. 2004 Development 131, 4035-4045). In *Arabidopsis* there are 28 genes in this family, and all members characterized so far except for PTL (GT-1, GT-2, GTL-1 and DF1) are involved in light signaling pathways.

Loss of PTL function results in fused sepals and loss of petals, defects that increase progressively in later forming buds. The *PTL* gene is expressed during the early stages of flower development, especially in four limited zones between newly arising sepals. Mis-expression of *PTL* in a range of plant tissues results in suppression of their growth. These results suggest that PTL acts as a growth suppressor in the inter-sepal zone, resulting in sepal separation and allowing space for the later initiation of petals.

In this study we are interested in the mode of action of the PTL protein at the molecular level. The protein has two trihelix DNA binding domains, and a central domain predicted to form a coiled-coil. In all three domains a putative nuclear localization signal (NLS) is present.

First we have identified which of the putative signals are required for nuclear localization. Alanine scanning mutagenesis of each of the three NLSs in a full length PTL-GFP fusion protein revealed that the C-terminal trihelix NLS is, by itself, sufficient for nuclear localization, but, in its absence, both NLSs in the N-terminal trihelix and the central domain are required.

The second question asked was whether PTL carries a region that activates the basal transcription machinery equivalent to those identified in the other trihelix proteins GT-1 and GT-2. This was tested in yeast using fusions of PTL with the GAL4 DNA binding domain, and a GAL4 binding site in the promoter of either the *HIS3* or *β-GAL* reporter genes. The results showed that PTL does carry a transactivation domain, although its activation is relatively weak (3.3%) compared with the strong GAL4 control. We are now testing if transactivation activity also occurs in planta.

Finally, other members of this transcription factor family act as multimers. However, we have found that PTL can function as a monomer, at least in yeast, to activate the basal transcription machinery. Yeast two hybrid screening of an inflorescence cDNA library is now in progress to establish if PTL interacts with other transcription factors, and with co-activators.

253 Regulatory control of PETAL LOSS, a flower development gene of Arabidopsis

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PETAL LOSS (*PTL*) is a regulatory gene that controls the architecture of the perianth (non-reproductive) whorls of the Arabidopsis flower. In *ptl* mutants, second whorl petals are mostly absent, a phenomenon accentuated in later forming flowers, and petals that do initiate may show an abnormal orientation within the flower. These effects are not constrained to petals but are rather specific to second whorl organs, as revealed by double mutant analysis with organ identity genes. First whorl sepals are also defective in appearance and adjacent sepals are sometimes fused along their margins – an effect that is exacerbated in double mutants with *cup-shaped cotyledon* (*cuc*) organ boundary genes. *PTL* encodes a trihelix transcription factor, one of a 28 member family specific to plants. *PTL* is expressed in various tissues, most importantly in early flower buds at the intersepal zones (ISZ – defined as the regions between adjacent sepals from whence petals develop).Expression also occurs in the marginal tissue of developing leaves, sepals, petals and stamens (Brewer et al. 2004 Development 131, 4035-4045).

Here I show, by reporter gene analysis and comparative genomics, that a specific 50 bp region within the intron of *PTL* activates ISZ expression, and that this region is evolutionarily conserved. Expression in organ margins is controlled in a combinatorial manner by regulatory elements in both the 5' upstream region and the intron. Various known binding sites of transcription factors are shown to influence expression of *PTL*. I also found that exon sequences are required to repress expression of *PTL* in certain tissues.

To identify genes acting with *PTL*, an enhancer screen following EMS mutagenesis of ptl-3 plants was conducted. One enhancer mutant completely lacks second whorl petals and shows a reduction in size and numbers of nectaries (normally found internal to the petals). This phenotype is apparent only in the *ptl* background. Presumably the enhancer acts in conjunction with PTL to specify initiation of second whorl organs. Additionally, I am investigating the role of *SISTER OF PETAL LOSS*, the closest relative of *PTL*, and show that its expression is also localised to the ISZ.

254 Construction of Arabidopsis Transcription Factor ORFeome For Genome Wide Study of Protein-Protein Interactions of Plant Transcription Factors

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Transcription factors (TFs) play a key role in various growth and developmental process of plant. The understanding the complex mechanism of transcription regulation requires genome-wide analysis of large number of transcription factor families. To verify the genome annotation and to create resource to functionally characterize the transcription factor open reading frames we attempted to clone all predicted protein-encoding reading frames of Arabidopsis thaliana transcription factor. We successfully cloned 1,057 Arabidopsis TF ORFs predicted by computer analysis. Recently Gong et al.(2004)) reported the isolation of 1,282 Arabidopsis TF clones. The combined ORFeome collection resulted in the isolation of 1,503 Arabidopsis TF ORFs altogether. These transcription factor ORFeome clones have been cloned in the Gateway entry vector (donor vector), a powerful recombinational cloning (RC) system. We have constructed expression library for yeast two-hybrid screen carrying about 1,400 transcription factor ORFeome in correct reading frame. Our study shows that the newly generated yeast AD-ORFeome library will be a valuable tool in isolating many novel interacting proteins to the regulatory protein of interest (Supported by BK21 program and the grant from Crop Functional Genomic Center of 21C Frontier Research Program).

255 Sub-domains for intercellular transport via plasmodesmata are established coincident with the apical-basal pattern during late embryogenesis of Arabidopsis

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Embryogenesis is a critical stage in development where the basic body pattern is established. The axial pattern is recognizable in early stages of Arabidopsis embryogenesis and is determined by different factors such as auxin signaling and differential gene expression. Here we elucidate that another pathway, cell-to-cell communication, is regulated during apical-basal pattern formation. Plant cells communicate through dynamic intercellular channels, plasmodesmata (PD), that span cell walls and connect the cytoplasm between neighboring cells to establish symplastic networks throughout the plant body. The *SHOOT MERISTEMLESS (STM)* promoter was used to drive expression of various sized soluble green fluorescent proteins (sGFPs), in the shoot apical meristem (SAM) and the hypocotyl of transgenic Arabidopsis embryos. In the early heart stage, 2XsGFP (54 kDa, besides 27 kDa 1XsGFP) move throughout the whole embryo, while 3XsGFP (81 kDa) shows restricted movement near the SAM. Overall PD apertures are down regulated as the embryo develops revealing distinct size exclusion limits in local sub-domains. Cotyledons and roots stop transporting 2XsGFP and 3XsGFP, respectively, by the torpedo stage. The SAM and hypocotyl allow more extensive movement of all tracers. Differential movement of the three different sized sGFP tracers demonstrates that, by the torpedo stage, the embryo establishes four distinct symplastic sub-domains along its apical-basal axis: shoot apex, cotyledons, hypocotyl, and root. Additional studies to monitor movement of sGFP fused to the P30 movement protein of *Tobacco mosaic virus* (TMV) reinforce the distinction between embryonic symplastic sub-domains. While P30 targets embryo cell walls as punctae (diagnostic for functional localization of P30 to PD in adult plants), P30 cannot gate embryonic PD to overcome the barriers for transport between symplastic sub-domains. Thus, P30-1XGFP (57 kDa) cannot move into cotyledons at the torpedo stage, just as similarly sized 2XsGFP cannot, and P30-2XGFP (84 kDa) cannot move into roots just as 3XsGFP (81kDa) cannot. The results with P30-GFP fusions reveal that specific boundaries separate symplastic sub-domains of the torpedo embryo. We propose that cell-to-cell communication via plasmodesmata conveys positional information critical to establish axial body pattern during late embryogenesis in Arabidopsis.

256 Insights into cell separation, apical dominance, dehiscence and meristem arrest using dab4-1, a delayed abscission mutant in Arabidopsis. thaliana

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Abscission is a developmental process that includes a series of programmed events rendering detachment of organs. In addition to many developmental and environmental cues, this process is modulated by the balance between ethylene and auxin. We have been characterizing a novel floral abscission mutant *dab4-1* (delayed abscission) in Arabidopsis. This mutant has several unique phenotypes including delayed floral organ abscission, lack of pollen dehiscence, delayed meristem arrest, epinastic leaf growth, and strong apical dominance. Recent observations indicate that this mutant has altered seedling responses to auxin and cytokinins, as seedlings grown on different concentrations of NAA, 6 benzyl adenine, and kinetin displayed altered responses. In order to further understand these hormone responses of *dab4-1*, gene expression of several hormone response genes (*ARF3, IAA7, SPY, EIN2*, and *BRI1*) was examined using RT PCR. In addition, to specifically investigate the role of auxin in *dab4-1*, auxin responsive promoter constructs DR5:GUS and DR5:GFP were introgressed into *dab4-1*. Also, in collaboration with Jerry Cohen's laboratory (University of Minnesota) levels of free auxin in the meristem are being measured. Genetically, *dab4-1* is regulated by a single recessive gene and preliminary map-based cloning indicates it is located on long arm of Chromosome II. We will present phenotypic, physiological, genetic, and molecular analyses of this mutant. We believe that understanding genes such as *dab4-1* will ultimately contribute to our understanding of cell separation, specifically abscission, and the role of auxin in this process.

257 Cytokinin receptors are required for dehiscence and stigmatic functions, and have an important role for production of female gametophytes

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Arabidopsis has 3 genes (*CRE1*, *AHK2*, and *AHK3*) for cytokinin receptors. Recent studies showed that it was possible to isolate triple mutants lacking the 3 genes, indicating that cytokinin receptors in the haploid cells are not required for the development and functions of male and female gametophytes. Although the triple mutants were very small , they could grow to maturity bearing infertile flowers. We investigated what reproductive processes are affected in the triple mutants. Anthers of mutant plants contained decreased numbers of pollen, and did not undergo dehiscence. Pollen in the anthers completed the formation of one vegetative nucleus and two sperm nuclei, but pollen taken out from anthers did not germinate on wild-type stigma, and infrequently germinated in an artificial germination medium. Majority of the ovules were abnormal: about 80% of them had no discernible female gametophyte structures, about 10% carried a female gametophyte that terminated their development before completion, and about 10% appeared to be morphologically normal. Reciprocal crosses between wild-type and the triple mutants indicated that mutant pollen did not germinate on wild-type stigma, and wild-type pollen did not germinate on mutant stigma. These results suggest that cytokinin receptors are indispensable for anther dehiscence, pollen maturation, and stigmatic functions to induce pollen germination and those in a maternal tissue have an important role in female gametophyte development.

258 Stress-induced flowering in *Arabidopsis thaliana*? Effects of mineral nutrition deficiency on *A. thaliana* flowering

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Flowering of *Arabidopsis* is regulated by many environmental and internal cues. The most important flower induction pathways are photoperiodic, vernalization, autonomous, gibberellin, and light quality pathways. However, flowering of many plant species can be also induced by abiotic stresses. It has been recently reported that *Arabidopsis* flowering is accelerated by UV-C irradiation. This prompted us to investigate the effects of other stresses (combined with different levels of photoperiodic induction) on flowering of this species. Preliminary results concerning mineral nutrition deficiency are presented here.

Ecotypes Col and Ler were grown in sand and supplied with 1% or 100% Hoagland solution (1% H or 100% H). In the first experiment, plants were continuously grown at these nutritional levels in 2 daylengths (8 h or 12 h). When compared to 100% H, 1% H plants of the Ler ecotype had generally lower number of leaves at flowering but their times of anthesis and of visible flower bud appearance were delayed. The interval between bud appearance and anthesis was also significantly shortened in 1% H. All these parameters were strongly affected by photoperiod in 100% H but markedly less in 1% H. Similar results were found in Col but the reduction of photoperiodic responsiveness in 1% H was not as pronounced as in Ler.

The second experiment tested the response of Col to a change in nutrient supply. Plants were grown in 100% H in 8-h photoperiod for 5 weeks, then the solution was changed to 1% H and plants transferred to 14-h photoperiod after another 2 weeks when they already exhibited strong symptoms of nutrient deficiency. 1% H plants developed flower buds and opened flowers significantly earlier and after making fewer leaves than control plants kept continuously in 100% H. 1% H also shortened the time between bud appearance and anthesis.

These data indicate that in *Arabidopsis*, continuously poor mineral nutrition delays flowering and may also reduce sensitivity to photoperiodic flower induction. In contrast, flowering is accelerated by the stress associated with a sudden decrease of nutrient levels. Further studies are planned to confirm this latter effect and characterize it in more detail.

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259 RABBIT EARS is a second whorl repressor of AGAMOUS that maintains spatial boundaries in Arabidopsis flowers

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The RABBIT EARS (RBE) gene has been identified as a regulator of petal development in Arabidopsis thaliana. We have isolated an allele of rbe in which second whorl petals are sometimes replaced with staminoid organs, stamens, or filaments. In addition, some rbe flowers have increased numbers of sepals and exhibit fusion of sepals. We show that these rbe defects are due to AG misexpression in the second whorl starting around stage 6 of flower development. Consistent with its role in maintaining the spatial boundary of AG expression, rbe enhanced the second whorl defects present in ap2-1 and lug-1 mutants. In the development of second whorl organs, RBE acts in the same pathway and downstream of UFO. Enhanced first whorl organ fusion in ap2-2 rbe-3, ant-4 rbe-3, and cuc2-1 rbe-3 double mutants supports an additional role for RBE in organ separation. RBE thus acts to maintain two different types of spatial boundaries in young flowers: boundaries between organ primordia within a whorl and boundaries of homeotic gene expression between whorls.

260 *bilfu* Affects Cell Division and Cell Expansion

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Normal plant morphogenesis requires that both cell division and cell expansion are coordinated to give the predictable size and shape of organs. The cell expansion in plant organs is often enhanced by endoreduplication that can lead to massive cells in the final organs. Interestingly, compensatory mechanisms seem to exist, whereby a decrease in cell proliferation may be countered by an increase in cell size, or *vice versa* . We have been trying to identify new mutants that disrupt this process, leading to abnormal expansion or cell division. One mutant, *bilfu*, shows defects in floral organ size as the result of an imbalance between cell number and cell size. *bilfu* petals and sepals are shorter and stouter than the wildtype, and some leaf cells expand in an uncoordinated manner. We will present this mutant phenotype and discuss its potential role in controlling cell expansion and proliferation.

261 Transcriptome analyses of laser-captured cells from flowers and siliques

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Abscission and dehiscence are cell separation events that cause organ shedding and seed shed. We are using laser capture microdissection (LCM) to identify key genes controlling cell separation in reproductive organs of *Arabidopsis*. Although LCM protocols for capture of plant cells have recently been reported, low RNA yield resulted when we applied these procedures to floral organ abscission zone (AZ) cells, which are very small and limited in number. We thus sought to develop an alternative technique to recover higher yields of high-quality RNA. First, we took advantage of the comparatively large size of replum cells in *Arabidopsis* siliques to optimize RNA quality from laser-captured cells. High RNA integrity and yield were achieved by using optimized methods. Affymetrix array analysis using RNA targets derived from replum cells captured at two developmental times revealed significant, reproducible differential gene expression. In addition to replum cells we have successfully captured cells from ovules and from AZs of stamens and petals. High integrity and yield of RNA from all cells have been confirmed and Affymetrix profiling of gene expression during floral organ abscission is in progress. Our cell type-specific genomic approach to understanding *Arabidopsis* abscission is expected to identify novel genes and gene populations whose expression may coordinately control organ shed in multiple plant species.

262 A Novel Dehydrogenase/reductase Gene Promotes Growth in Arabidopsis thaliana

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A novel dehydrogenase-like gene, which is a member of the short-chain dehydrogenase/reductase family, was identified and functionally characterized in Arabidopsis thaliana. This gene encodes a 349-amino acid protein. Transgenic plants overexpressing the dehydrogenase-like gene showed increased growth and seed yield, a reduction in seed dormancy and resistance to stresses relative to wild type. Antisense transgenic plants exhibited dwarfism and very poor fertility. This study demonstrates that the novel gene has an important functional role in plant growth and development.

263 Promoter Analysis of *END1-LIKE* in Arabidopsis

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Arabidopsis *END1-LIKE* is the orthologue of barley endosperm-specific gene *END1*. In barley, *END1* transcript is restricted to a small area over the nucellar projection in the syncitial endosperm, whereas, it is shown in the basal transfer cell layer in the starchy endosperm and the aleurone cells in the early endosperm cellularization stage (Olsen OA, 1996). However, the developmental function of this gene is unknown. Here we examined the spatial and temporal expressions of Arabidopsis *END1-LIKE* gene. The expression of a transcriptional GUS fusion revealed that it was expressed in pollen grains and the embryo sacs shortly before fertilization and only in embryo sacs from pollination until early heart stage of the embryo development. RT-PCR, Northern blot and *in situ* hybridizations were also performed to confirm the expression profile. To identify the elements of *END1-LIKE* promoter directing expression in gametophyte tissues and seeds, five truncated promoter fragments were fused to GUS and the expression patterns have been observed. These data will be presented.

264 Molecular Genetics of Arabidopsis Seed Development and Storage Product Synthesis

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Seeds contain large amounts of storage products and serve as important sources of human diet. The process of nutrient reserve accumulation is an integrated part of the seed differentiation program, but currently the underlying mechanisms are poorly understood. In order to identify novel components and pathways that influence protein or oil deposition in Arabidopsis seeds, we selected Arabidopsis mutants using transgenic plants carrying a luciferase (*LUC*) reporter construct. In this construct, the 5' flanking region of the oleosin (*OLE*) gene is used to drive the expression of an OLE-LUC fusion protein, which is expected to accumulate on the oil body membrane in seeds. The EMS mutagenized M2 population were screened for mutants with reduced or elevated LUC activities. Because the abundance of OLE-LUC is influences by seed development, storage accumulation, as well as oleosin gene expression *per se*, it is not surprising that the identified mutants show variable phenotypes. We will present molecular, genetic, and biochemical characterization of the *shrunken seed 2* (*sse2*) mutant that exhibits reduced amounts of seed oil and other developmental defects.

265 More components in FCA/FY pathway controlling flowering

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Expression of FLC, the main repressor of flowering in Arabidopsis, is repressed by the autonomous floral pathway. Up to now, seven proteins in autonomous pathway have been identified: FCA, FY, FVE, FPA, LD, FLD and FLK. FCA (an RNA binding protein) and FY (a component in complexes required for RNA 3' end formation) work in one genetic pathway and actually interact with each other. Over expressed FCA suppressed the late flowering phenotype of *fve* and *fpa* mutants but could not suppress *fy*. To identify components required for FCA/FY function, we screened for suppressors of FCA over-expression (sof, for suppressor of over expressed FCA). Three complementation groups were identified and we will report on the identification of SOF1.

266 Characterization of a functional knockout in the Arabidopsis thaliana Group 1 LEA ATEM6

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The completion of embryo development among higher plants is commonly associated with desiccation of the seed. During this time, the embryo begins to accumulate various proteins in preparation for developmental arrest. The LATE EMBRYOGENESIS ABUNDANT or LEA proteins are a large and diverse family whose products accumulate to a high titer during this desiccation period. This family of proteins has been linked to desiccation tolerance, possibly by mitigating water loss or maintaining cellular stability within the desiccated seed. The actual mode(s) of function of these genes, however, remains largely unknown. Two genes, *ATEM1* and *ATEM6*, belong to the Group 1 subclass of LEA genes in the model plant *Arabidopsis thaliana*. To better understand the function of these proteins during late embryogenesis, T-DNA insertional libraries are being screened to identify functional knockouts. A BLAST search of an *Agrobacterium tumefaciens* T-DNA insertion library maintained by Syngenta identified a potential T-DNA insertion in the *ATEM6* gene. Plasmid rescue analyses identified the insert location, and RNA gel blot, immunoblot and RT-PCR analysis were used to characterize the molecular phenotype. The plant line discussed was found to be a functional knockout for the ATEM6 protein. Here we report the complete molecular characterization and initial phenotypic analysis of this knockout mutant of ATEM6 in *Arabidopsis thaliana*.

267 REGULATION OF FLOWERING TIME BY NITROGEN, A DEVELOPMENTAL PROCESS SUBJECT TO REGULATION BY A LARGE NUMBER OF INPUTS

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Flowering is a major developmental event in plants since it represents the transition from vegetative growth to reproduction. The timing of flowering is determined by complex interactions between environmental cues and an internal genetic program in order to favour seed production and reproductive success. The control of flowering time in response to photoperiod, temperature and GA has been intensively studied by genetic analyses in several plant species, particularly in Arabidopsis thaliana.

We are interested in the effect of nutrient availability in the transition from vegetative growth to flowering and especially in nitrate as a metabolite that triggers widespread and coordinated changes in metabolism and development. Nutrient availability has a dramatic effect on flowering time, with a marked delay of flowering when nitrate is supplied (Stitt, 1999). Plants were grown on glutamine as a constitutive source of nitrogen, and the nitrate supply varied. Low nitrate led to earlier flowering. Mutants and transgenic plants with lesions in the photoperiod, autonomous and GA floral induction pathways still flower on low nitrate. The response to nitrate is accentuated in short days and in the CONSTANS deficient co2 mutant, whereas long days or overexpression of CONSTANS overrides the nitrate response. These results indicate that nitrate acts downstream of the known flowering signalling pathways for photoperiod, autonomy, vernalization and gibberellic acid. To identify those genes that are affecting flowering time in response to nitrate, Affymetrix transcriptional profiling was carried out with the co2 mutant exposed to low and high nitrate (1mM and 35mM). We will try to answer the question whether nitrate could act as a signalling pathway that controls the transition to the flowering state of the plant.

268 Aminoacyl-tRNA Synthetases are Required for Reproductive Development in *Arabidopsis*

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Aminoacyl-tRNA synthetases (AARSs) are required for translation in three different compartments of the plant cell: chloroplasts, mitochondria, and the cytosol. Although elimination of this basal function should result in lethality early in development, phenotypes of individual knockouts may vary considerably, depending on patterns of gene expression, functional redundancy, allele strength, and protein localization. We describe here a reverse genetic screen of 50 insertion mutants disrupted in 21 of the 45 predicted AARSs in *Arabidopsis*. Our initial goal was to find additional *EMB* genes with a loss-of-function phenotype in the seed. Several different classes of knockouts were discovered, with defects in both gametogenesis and seed development. Three major trends were observed. Disruption of translation in chloroplasts often results in seed abortion at the transition stage of embryogenesis with minimal effects on gametophytes. Disruption of translation in mitochondria often results in ovule abortion before and immediately after fertilization. This early phenotype was missed in prior screens for embryo-defective mutants. Knockout alleles of non-redundant cytosolic AARSs were in general not identified, consistent with the absolute requirement of cytosolic translation for male and female gametophyte development. These results provide a framework for evaluating redundant AARS functions and predicted localizations in *Arabidopsis*, a valuable dataset of phenotypes resulting from multiple disruptions of a basal cell process, and insights into which genes are required for both gametogenesis and embryo development and therefore might escape detection in screens for embryo-defective mutants.

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269 Isolation and functional characterization of an embryo-specific *cis* element from the *FIE* promoter

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The *FIE* (*Fertilization Independent Endosperm*) Polycomb gene in Arabidopsis is crucial to both endosperm and embryo development. Polycomb group proteins form complexes that silence gene transcription. When maternally inherited, a mutant *fie* allele triggers endosperm development in the absence of fertilization, and embryo arrestment at the heart stage when fertilization occurs. Yadegari et al. (2000) have shown that a reporter gene fused to the *FIE* promoter is expressed in two separate phases in the developing Arabidopsis seed: phase I is from pre-fertilization to embryo globular stage, and phase II is from embryo heart stage onward. We have identified an element from the *FIE* 5' UTR that regulates expression specifically in embryos in phase II, and have delimited it to 88 bps. We found that, albeit residing on the 5' UTR, the regulation of this 88 bp box is transcriptional. Moreover, when relocated adjacent to the *FIE* core promoter, the 88 bp box resulted in enhancement of expression in embryos in phase II.

We are currently examining the sufficiency of the 88 bp box from the *FIE* promoter when driving a minimal core promoter to allow phase II embryo specific expression. We are also studying the phenotypes of *fie* mutant plants with a *FIE* transgene that lacks the 88 bp box. In addition, we are further localizing the embryo specific *cis* element within the 88 bp box by analyzing the expression of *FIE* transgenes with small deletions and substitutions in the 88 bp box. This study will provide new insights in the regulation of gene expression and Polycomb group function in the developing Arabidopsis embryo.

270 Generation of transgenic tomato with *Arabidopsis* early flowering *LEAFY (LFY)* and *APETALA1 (AP1)* genes

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Important agronomical traits such as fruit quality, harvesting efficiency or production largely depend on flowering time. We have generated transgenic tomato (*Lycopersicon esculentum* Mill cv Rio Grande) to study the effects of over expression of *LEAFY (LFY)* and *APETALA 1 (Ap1)* genes, which promote flower initiation in *Arabidopsis. Agrobacterium* strain EHA 105 harboring binary vectors containing either *LFY* or *Ap1* genes driven by 35 S promoter has been used for transformation. Both constructs contained a *B-glucuronidase (GUS)* and *neomycin phosphotransferase II (Npt II)* genes. Transient *GUS* expression was observed after 3-4 days of co-culturing of 10 days old explants. Hypocotyl and root showed higher transformation efficiency than cotyledons and epical meristems. The transformed explants were regenerated on shoot regeneration medium (MS medium containing BAP 1 mg/L, kanamycin 100 mg/L and cefotaxime 100 mg/L). The shoots regenerating from transformed hypocotyls upper portions developed quite normal and healthy leaves. Stable *GUS* expression was carried out 20 days after co-cultivation. Expression was uniform in all regenerating shoots. Three weeks old transformed shoots were shifted to root regenerating medium (1/2 MS containing, kanamycin 50 mg/L and cefotaxime 50 mg/L). The molecular analysis of independent transformed plants is being carried out. The expression of early flowering genes in tomato breeding lines could provide advantages for the development of new verities with shorter generation time, determinate growth and reduced pruning requirements.

271 The *Arabidopsis* SeedGenes Project

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We present here an update of the *Arabidopsis* SeedGenes Project. The purpose of this collaborative effort is to provide detailed information on indispensable genes with a knockout phenotype in the developing seed through a web-accessible database (www.seedgenes.org). The current database (January, 2005) presents information on 295 genes and 462 mutants. Included are 269 mutants generated at Syngenta, 32 produced at the Salk Institute, 22 from the Versailles collection of INRA/Genoplante, and 139 contributions from the *Arabidopsis* community. Recent additions to the database include a detailed tutorial on screening for seed phenotypes, flanking sequence files and improved visualization of insertion sites for T-DNA mutants, updated gene models and BLAST results, confidence levels for gene identities, additional Nomarski images of mutant seeds, and links to ABRC seed stocks and outreach activities. Ongoing efforts include the analysis of expression data from developing seeds and the systematic identification of candidate *EMB* genes that represent promising targets for reverse genetics. Emphasis in the future will be placed on the use of reverse genetics to approach saturation for this valuable class of *Arabidopsis* genes with critical functions during plant growth and development. Members of the community are encouraged to utilize the SeedGenes database to learn more about existing collections of embryo-defective mutants and to determine whether a knockout of their favorite gene of interest is known to give a seed phenotype.

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272 Molecular and Genetic Characterization of *B-CLASS MODIFIER* (*BCM*) genes

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The ABC model of flower development postulates that three activities, A, B, and C, specify floral organ identity in a combinatorial manner. Two genes in Arabidopsis comprise B class activity: *APETALA3* (*AP3*) and *PISTILLATA* (*PI*). In strong *ap3* or *pi* mutants, petal to sepal and stamen to carpel floral organ identity transformations are observed. Both *AP3* and *PI* encode MADS transcription factors and an AP3/PI heterodimer is postulated to comprise B class activity. To isolate additional genes that function in *AP3/PI* pathway, we carried out an enhancer/suppressor screen utilizing the unusual *pi-5* allele. In *pi-5* mutants, petals develop as sepals in whorl two, but stamens develop normally in whorl 3. *pi-5* homozygous seeds were mutagenized with EMS. Recessive extragenic enhancers and suppressors were identified in M2. *pi-5* enhancers resemble strong *pi* alleles in that third whorl organs are either absent, rudimentary or carpelloid. *pi-5* suppressors exhibit petalloid organs in the second whorl. We have named these genes as *B-CLASS MODIFIERS* (*BCM*) genes. Here we present our progress in mapping of *pi-5* enhancers *bcm1*, *bcm2*, *bcm3* and *bcm4*. Several lines of evidence suggest that *BCM1* encodes an AP2 domain protein and is expressed in developing petals and stamens. Future work will be aimed at elucidating the role of *BCM* genes in petal and stamen development.

273 Gene trap identification of genes involved in Arabidopsis petal and stamen differentiation

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Although the mechanisms behind identity specification in Arabidopsis floral organs are well known, how the identity translates to specific differentiation events in each type of organs remains relatively unexplored. In order to identify genes involved in petal and stamen differentiation, we have employed a gene trap strategy and screened for genes expressed in specific patterns in these organs. We examined the patterns of reporter expression in inflorescences of 1,765 TRAPPER gene trap lines. 82 lines were recovered in which the reporter gene showed petal and/or stamen-specific expression or lack of expression, or expression in distinct patterns within the petals and/or the stamens. These included lines showing distinct sub-organ domains of expression, such as tissue-specific lines marking epidermis or vasculature, as well as lines demarcating the proximodistal or abaxial/adaxial axes of the organs. In general, reporter gene expression was typically restricted along the proximodistal axis of petals and stamens, indicating the importance of this axis in establishing gene expression domains during lateral organ development. We also identified novel domains of gene expression within the petals and the anthers. Most of the genes tagged by the gene trap insertions in the 82 lines here were identified, and expression of some of these genes depends on the homeotic regulators specifying petal and stamen identity. The gene trap lines reported here represent both useful markers and valuable starting points for reverse genetic analyses of the differentiation pathways in Arabidopsis petals and stamens.

274 Lipid Transfer Proteins enhance cell wall extension. A new function for an old protein

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Radboud University Nijmegen

Plant cells are enclosed by a rigid cell wall that counteracts the internal osmotic pressure of the vacuole and limits rate and direction of cell enlargement. When developmental or physiological cues induce cell extension, plant cells increase the wall plasticity by a process called loosening. It was previously demonstrated that a class of proteins known as expansins are mediators of wall loosening. Here, we report a new type of cell wall loosening protein that does not share any homology with expansins but is a member of the Lipid Transfer Proteins (LTP). LTPs are known to bind a large range of lipid molecules to their hydrophobic cavity and we show here that this cavity is essential for the cell wall loosening activity of LTP. Furthermore, we show that LTP-enhanced wall extension can be described by a logarithmic time function. We hypothesize that LTP associates with hydrophobic wall compounds, causing non-hydrolytic disruption of the cell wall and subsequently facilitating wall extension.

Our results provide new insight on the mechanism of cell wall loosening as such, but also suggest an important role for LTPs in plant growth and development.

275 In-vivo analysis of MADS-box transcription factors involved in ovule development

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Specific combinations of transcription factors control most regulatory networks in a plant lifecycle. The specificity and activity of these "master switches" appeared to be dependent on direct physical interactions between the individual transcription factor proteins and the target DNA in the nucleus. Monitoring the intracellular transport, protein-protein interactions and DNA binding are essential to understand transcription factor functioning. We focus on MADS box transcription factors that form specific hetero- and homodimers. In Petunia hybrida, a key MADS box factor is FBP2, which is required for petal, stamen, carpel and ovule identity specification, floral determinacy and seed formation. It interacts with a dozen other MADS proteins in yeast, but virtually nothing is known about interactions in planta. We studied the interaction with the MADS protein FBP24, which is also involved in ovule and seed formation. Based on yeast studies, we suggest that also the ovule identity protein FBP11 is involved in a transcription complex containing FBP2 and FBP24. We used microspectroscopic techniques to study the interactions of these proteins in living plant cells. Imaging has been perfomed using CLSM (Confocal Laser Scanning Microscopy) and interactions were monitored by Fluorescence Lifetime Imaging Microscopy – FLIM, in order to detect FRET (Fluorescence Ressonance Energy Transfer). It was observed interactions among these ovule-specific MADS box transcription factors

276 TWO-IN-ONE, a new player required for conventional and non-conventional modes of cytokinesis in Arabidopsis

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Cytokinesis is the final step required after mitosis to complete cell division. Whereas the mechanisms underlying mitosis seem conserved, cytokinesis is mediated in distinct ways in plants and animals. In animals daughter cells are separated inwardly by constriction involving an actinomyosin contractile ring, whereas plant cells divide by building a centrifugally expanding cell plate. Plant cells have also adopted cell type-specific modes of cytokinesis (1). Somatic and male gametophytic cell divisions involve 'conventional' modes of cytokinesis where cytokinesis immediately follows mitosis. In contrast, endosperm, meiotic and female gametophytic cell divisions rely on 'non-conventional' modes of cytokinesis that result in cellularisation after more than two cycles of mitosis. In a screen for Arabidopsis pollen cell division mutants we identified gemini pollen1 that affects microspore polarity as well as male and female gametophytic cytokinesis. The encoded microtubule associated protein GEM1/MOR1however has a wider role that is not restricted to cytokinesis (2). In this study, we present new cytokinesis-specific mutants named two-in-one (tio). tio mutations act gametophytically and are not transmitted through pollen, but show limited transmission through the female. Unlike gem1 mutants, tio mutants display normal microspore polarity, but cytokinesis is defective at pollen mitosis I resulting in binucleate pollen. During female gametogenesis tio affects cellularisation following mitosis resulting in multiple nuclei at the micropylar end of the embryo sac. Mapping of tio-1 and tio-2 and sequencing of candidate genes reveals that both mutants contain point mutations in a putative serine/threonine protein kinase. Moreover, the identity of the TIO gene was confirmed by the isolation of a T-DNA insertional allele, tio-3 showing the same phenotype. RT-PCR and microarray analyses show that TIO mRNA is broadly expressed in sporophytic tissues, as well as in male gametophytic cells. Using inducible RNAi constructs we have demonstrated that TIO is also required for cytokinesis in sporophytic cell types. In current work we are attempting to localise the TIO protein in dividing cells and we are screening for interacting proteins. In conclusion, we have identified a new molecular component of the cytokinesis signalling machinery that is broadly required in cell types that utilise both conventional and non-conventional modes of cytokinesis. (1) Otegui, M et al (2000) Curr. Opin. in Plant Biol. 3:493–502. (2) Twell, D. et al (2002) Nat Cell Biol. 4:711-714.

277 Identification of FLOWERING LOCUS H (FLH) Candidate Genes that Respond to Vernalisation in Arabidopsis using DNA Microarray

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Flowering is a mechanism most plants have developed to ensure reproductive success. In Arabidopsis, genes regulating the floral transition involve four major pathways: the autonomous and gibberellin pathways allow plants to monitor their development and physiological status, whereas the photoperiod and vernalisation pathways respond to light and temperature changes associated with the seasonal transition. The isolation of FLOWERING LOCUS C (FLC) and the genes that regulate its expression has provided insight into the molecular mechanisms involved in vernalisation. Here we describe the characterisation of a natural variant affecting vernalisation response that appears to be independent of FLC. The FLH QTL was identified in a RIL population derived from a Cvi x Ler cross with Cvi alleles enhancing the vernalisation response. The progress of map-based cloning of the FLH gene and analysis of the FLH function using Affymetrix 24K ATH1 Arabidopsis GeneChips will be presented.

278 Guidance signals that direct pollen tube entry into micropyle are highly variable across species and species-specific and developmentally regulated

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To directly monitor pollen tube-ovule interactions, we developed an in vitro pollen tube guidance system in *Arabidopsis*. The *in vitro* system uses three components: pollen, stigma and style portion of the pistil, and excised ovules complete with a funiculus. After completing their early stages of growth within the excised pistil tissues, pollen tubes then grow on a simple agarose matrix towards the ovules. This system recapitulates many *in vivo*pollen tube behaviors : i) pollen tubes make a sharp turn toward the ovules, ii) enter the micropyle, iii) cease elongation and iv) burst open to release their cytoplasmic contents and v) do not enter ovules that have already been targeted . To facilitate real-time observation of pollen tube interaction with ovules, we used GFP-tagged tubes. *In vitro*, these tubes release a visible spot of GFP within the ovules, conveniently marking targeted ovules. In these assays, ~ 50% of the ovules are targeted by the pollen tubes and to calculate targeting efficiency, we considered only those ovules that were sufficiently close to a pollen tube (~ 0.1 mm) to provide an opportunity for interaction. Targeting efficiency increases significantly when pollen tubes are first grown through pistil tissues (54%) compared to tubes that are germinated on agarose (3%). These results suggest that growth through an *Arabidopsis thaliana* stigma and style confers competence to pollen tubes, allowing them to respond to subsequent guidance cues from ovules; a similar competence step has been uncovered in *Torenia (Higashiyama et al, 1998)*. We exploited the modular nature of our *in vitro* guidance system to assess the nature of the guidance signals. First, we assessed targeting efficiency by using mature pollen and mature pistils, while sampling ovules from different developmental stages. Results from these experiments demonstrated showed that mature ovules show significantly higher targeting efficiency (54%) than ovules from buds that are 16 hours younger (17% P=0.0007). Second, we used pollen and pistil tissues from *A.thaliana*, while sampling ovules from close relatives of *Arabidopsis* such as *A. arenosa, Olimarabidopsis pumila, Capsella rubella*, and *Sisymbrium orio*. Results from this analysis revealed that the guidance signals that direct tube entry into ovules are highly variable. We are now using this *in vitro* system to isolate and characterize the guidance molecule(s) that direct pollen tube entry into ovules.

279 Functional Analysis of the CCAAT Binding Transcription Complex Containing Arabidopsis LEAFY COTYLEDON1, a Central Regulator of Embryogenesis

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We are investigating mechanisms by which Arabidopsis LEAFY COTYLEDON1 (LEC1) regulates the transcription of genes essential for embryo development. LEC1 is a central regulator of embryogenesis that is required for specification of cotyledon identity, acquisition of desiccation tolerance, maintenance of suspensor cell fate, and inhibition of precocious germination. Moreover ectopic expression of *LEC1* induces embryonic programs and somatic embryo formation from vegetative cells. Thus, LEC1 is sufficient to create a cellular environment that promotes embryo development.

LEC1 encodes a HAP3 subunit of the CCAAT binding factor (CBF), a transcription complex that consists of three core subunits. Although there are at least ten Arabidopsis HAP3 subunits, LEC1 defines a novel subclass. *LEC1* is expressed specifically in developing seeds, and ectopic expression of *LEC1* induces embryonic programs in cells. By contrast, non-LEC1-type *AHAP3* genes are not expressed seed-specifically, and overexpression of these genes does not confer embryonic traits. We hypothesize that CBF complexes containing LEC1 and non-LEC1-type AHAP3 subunits regulate the transcription of different sets of genes and that the LEC1-CBF controls genes essential for embryo development. We have conducted experiments to define functional differences in CBFs containing LEC1 and non-LEC1-type AHAP3 subunits.

To this end, we first showed that Arabidopsis possesses functional CBF complexes. Both LEC1 and non-LEC1-type AHAP3 subunits formed CBF complexes with AHAP2 and AHAP5 subunits *in vitro* and in yeast cells. We next defined the DNA sequence motifs bound by CBF complexes containing either LEC1 or non-LEC1-type AHAP3 subunits *in vitro*. Binding of the two complexes to these DNA sequence motifs was compared in yeast containing the reconstituted Arabidopsis CBF complexes. The results of these studies and potential mechanisms to explain how LEC1 specifically regulates genes involved in embryo development will be discussed.

280 Isolation of AP3/PI protein complexes from floral extracts

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We are interested in understanding the molecular mechanism by which APETALA3 (AP3) and PISTILLATA (PI), two B class MADS proteins, act to control floral organ identity. Several lines of evidence indicate that AP3 and PI function as a DNA binding heterodimer. *In vitro* biochemical data indicate that AP3 and PI function together with other MADS proteins in a higher order complex. However, to date, MADS protein complexes have not been isolated from plant cells. We are attempting to identify the protein complex partners of AP3 and PI using a tandem affinity purification (TAP) tag strategy. A TAP tag has been fused to the C terminal end of AP3 and PI. AP3-TAP and PI-TAP proteins are functional, in that they rescue their respective mutant phenotypes. Efforts are underway to characterize *in planta* binding partners of AP3 and PI.

281 Constructing a regulatory network for the Arabidopsis female gametophyte

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The female gametophyte (FG) is essential for reproduction in flowering plants. At maturity, the *Arabidopsis* FG is composed of one central cell, one egg cell, and two synergid cells. The molecular processes by which the individual cells of the FG acquire their unique functions during cell differentiation are not understood. Few genes expressed in specific cells of the FG have been identified, and none of these are known to encode regulatory molecules controlling cell differentiation during FG development. As a first step toward dissecting the gene regulatory networks controlling cell specification and differentiation during FG development, we identified a collection of genes expressed in the *Arabidopsis* FG using microarray assays. One of the FG-expressed genes we identified, *MYB98*, is a member of the R2R3-MYB family of transcription factors. In plants, R2R3-MYB transcription factors regulate many biological processes, including development and cell differentiation. The *MYB98* gene is expressed specifically in the synergid cells. *myb98* mutants have defects in pollen tube guidance and formation of the filiform apparatus. To identify additional genes involved in synergid cell development and function, we have identified six genes whose expression requires *MYB98*. We hypothesize that these genes are expressed in the synergid cell, and that they include direct transcriptional targets of *MYB98*. We are using transcriptional reporters to determine the expression pattern of these genes and gel-shift assays to determine which of these downstream genes are direct targets. We also are examining insertional alleles of these genes to determine if they play a role in the acquisition of the synergid cell's unique structures and functions during development. These genes may represent part of the synergid cell's regulatory hierarchy, and as such, provide us with a tool by which to begin to dissect this network.

282 Molecular Analysis of Self-compatibility in Arabidopsis allotetraploids

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Outcrossing promotes heterozygosity and prevents inbreeding depression. Therefore, many plants evolve to become self-incompatible. Arabidopsis suecica is a natural self-pollinating allotetraploid that is derived from interspecific hybridization between A. thaliana and A. arenosa ancestors that are self-compatible (SC) and self-incompatible (SI), respectively. To understand the molecular basis for the changes in self-incompatibility, we analyzed candidate genes that control the sporophytic form of self-incompatibility in Brassicaceae. The interaction between a pollen ligand (SCR) and its stigmatic receptor kinase (SRK) triggers pollen rejection, and SCR and SRK are two key players in the incompatible response. Inactivation of either SRK or SCR is sufficient for inducing self-pollination, as demonstrated in A. thaliana that lacks full-length SRK and SCR proteins. Pollen germination assays indicated that pollen failed to germinate in self-incompatible A. arenosa flowers after 3-5 hours of pollination, whereas normal pollen germination and growth were observed in A. thaliana under the same conditions, suggesting that A. arenosa is self-incompatible and contains functional SRK and SCR proteins. We cloned partial SRK cDNA in A. arenosa. Real-time RT-PCR analysis indicated that SRK is expressed 2-fold higher in flowers than leaves in A. arenosa. Experiments are underway to clone full-length cDNAs of SRK and SCR and analyze genomic regions of SRK and SCR loci in self-incompatible A. arenosa. SRK and SCR expression patterns in Arabidopsis synthetic allotetraploids and their progenitors will be analyzed to determine how SRK and SCR expression changes in the early generations of allotetraploid formation.

283 Finding targets for a Subtilisin-like Serine Protease in Arabidopsis thaliana: a proteomic approach

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Plant subtilisin-like serine proteases are supposed to be involved in several processes, such as signaling, general protein turnover or pathogenic defence. Forward genetics has identified two subtilases as highly-specific regulators of plant development. In the Arabidopsis *sdd1* mutant (*stomatal density and distribution 1*) the pattern of stomata formation is disrupted, resulting in clustering of stomata and in a dramatic increase in stomatal density. ALE1 (*abnormal leaf shape 1*) is required for cuticle formation and epidermal differentiation during embryo development in Arabidopsis. Both are well known members of a large gene family in *Arabidopsis thaliana* (subtilases, AtSBTs) that comprises 56 members. Sequence analysis categorised the AtSBT proteins into six distinct subgroups. In a mutant screen we collected and confirmed 144 T-DNA insertion mutants comprising knockouts of 55 out of the 56 AtSBTs. AtSBT1.1 is strongly expressed during embryogenesis and during seed maturation. SBT1.1 protein accumulates in dry seeds. To gain insight into the role of this particular Arabidopsis SBT we analysed the Arabidopsis seed proteome, comparing Col-0 wildtype and two independent *sbt1.1* mutants, applying two dimensional polyacrylamide gel electrophoresis (2-DE) in respect to dormancy, germination and phytohormone treatment. Using 2-DE we separated about 2000 proteins from *Arabidopsis thaliana* dormant, non dormant and phytohormone treated seeds. We identified several differences comparing the wildtype and *sbt1.1* seed proteome. So far we analysed more than 800 proteins including the differentially expressed ones by MALDI-TOF MS. Based on this approach we identified hitherto unknown seed proteins and confirmed known ones. The identification of numerous differences between the wildtype and the sbt1.1 seed proteomes will provide insights into the role of SBT1.1.

284 Restoration of replum development in the *Arabidopsis* fruit by suppressors of *replumless fruitfull*

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The *Arabidopsis* fruit forms a seedpod that dries and opens at maturity. Fruit opening, or dehiscence, occurs at the valve margins, allowing the valves (seedpod walls) to separate from the replum (central ridge) and fall from the plant to disperse the seeds. Valve margin identity is specified by the *SHATTERPROOF*, *INDEHISCENT* and *ALCATRAZ* genes. REPLUMLESS (RPL; also known as PENNYWISE and BELLRINGER) and FRUITFULL (FUL) negatively regulate the valve margin identity genes in the replum and valves respectively such that the valve margin forms precisely between the valves and replum. In the *rpl ful* double mutant, the entire circumference of the fruit is covered with valve margin cells, providing a sensitized background for a mutant screen to identify additional genes involved in valve margin development as well as genes that function with *RPL* and *FUL*. Mutants that affect valve margin development would be expected to suppress the *rpl ful* fruit phenotype and restore replum and/or valve development. Twenty-one suppressor mutants that rescue replum development and three suppressors that rescue valve development have been identified in a screen of EMS mutagenized *rpl ful* plants. Many of these suppressor-mutant fruit are dehiscent suggesting that they may have subtle roles in valve margin development and have not been identified previously in screens for indehiscent mutants. A morphological analysis of the suppressors will be presented.

285 *Arabidopsis thaliana* methyl-CpG-binding domain (MBD) proteins are involved in plant development – including the regulation of flowering time

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The genome of *Arabidopsis thaliana* contains twelve putative genes encoding proteins with domains similar to the methyl-CpG-binding (MBD) domain found in animals and other eukaryotes. In vertebrates, most MBD proteins seem to function at an epigenetic level of regulation, through the participation in transcriptional regulation through interactions with proteins involved in chromatin remodelling, such as histone deacetylases. We have, in our collection of T-DNA mutagenised lines in C24 background, identified a mutant in an *AtMBD* gene showing delayed flowering. Complementation experiments confirm that the phenotype is caused by the mutant *AtMBD* gene. This mutant is still vernalisation responsive, indicating that the AtMBD protein is involved in the autonomous pathway of control of flowering time. RNAi knockdown lines for *AtMBD* display the same late flowering but vernalisation responsive phenotype. Northern hybridisations have shown that the expression of the flowering repressor FLC is increased and prolonged in the mutant plants compared to wild type, and that the expression of the floral promoter SOC1 is reduced. An augmentation of the late flowering phenotype is observed with each generation, consistent with the idea of an epigenetic mode of influence. We are currently in the process of identifying putative interacting partners to AtMBD using the yeast two-hybrid system.

286 *PDX2* a *de novo* vitamin B6 biosynthetic pathway gene, is essential for seed development in Arabidopsis

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Vitamin B6 (pyridoxine) biosynthesis has been well characterized in *E. coli*, but plants, fungi, archaebacteria, and many bacteria contain an alternate pathway for *de novo* synthesis (PNAS 96:9374). The two genes in the pathway, *PDX1* and *PDX2*, encode proteins that form a complex and function as a glutamine amidotransferase and also carry out the ring closure step in the pathway. *Arabidopsis* has three copies of *PDX1* (on chromosomes 2, 3, and 5) and one copy of *PDX2* (on chromosome 5). Previous work in our lab demonstrated that pyridoxine vitamers have antioxidant activity and quench singlet oxygen and superoxide, and that the pathway genes are upregulated in Arabidopsis in response to oxidative and environmental stress. To further test the possible role of this vitamin in plant stress responses, segregating seed for knockout mutants of all four genes were obtained from the Arabidopsis Biological Resource Center (ABRC). For *PDX2*, no homozygous mutants could be identified by PCR screening. Analysis of heterozygous plants demonstrated that 25% of the seeds produced by the plants were shriveled, in agreement with data from the SeedGenes Project, indicating that this mutation is embryo lethal at the heart stage. Total vitamin B6 content was assayed using a yeast bioassay; no differences in B6 levels were identified between the wild type and *PDX2* heterozygous mutants grown in soil. Screening of *PDX1* knockout lines is in progress. Our goal is to modulate the levels of B6 vitamers in the plant to further clarify the importance of this vitamin in stress responses during seed and whole plant development.

287 *FLC PROMOTER 1* is Required for the Winter-Annual Habit in Arabidopsis thaliana

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The winter-annual habit in Arabidopsis is mainly determined by functional expression of two genes *FRIGIDA* and *FLOWERING LOCUS C*. To better understand the winter-annual habit a genetic screen, using T-DNA as a mutagen, was performed to isolate mutants that block the late flowering behavior of a winter-annual strain. We have identified a locus, *FLC PROMOTER 1 (FCP1)*, which is specifically required for the up-regulation of *FLC* observed in the winter-annual strains. Although *fcp1* is a complete suppressor of the winter-annual habit, it is unable to suppress the late flowering phenotype observed in "autonomous pathway" mutants. Using TAIL-PCR flanking sequences were identified in a CCCH zinc finger. *FCP1* does not share significant sequence similarity outside of the zinc finger motif with any other genes. Overexpression of *FCP1* in the Columbia accession *(frigida)* is unable to promote the late flowering behavior observed in winter-annual strains. Epistatic analysis between *FRIGIDA, FCP1*, and another winter-annual suppressor *FRIGIDA LIKE 1* will be presented.

288 Putative lipid/sterol binding domains in homeodomain transcription factors of *Arabidopsis*

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A signaling role for sterols in plant embryogenesis is suggested by the patterning defects of three sterol biosynthesis mutants of *Arabidopsis*: *fackel*(*fk*), *cephalopod*/*sterol methyltransferase 1* (*cph*/*smt1*) and *hydra1* (*hyd1*). Thus far the campesterol-derived brassinosteroids are the only steroids shown to participate in signal transduction, which occurs via a plasma membrane receptor-like kinase. In our work we aim to identify putative intracellular sterol-binding proteins of plants. Candidates are Steroidogenic Acute Regulatory (StAR)-related lipid transfer (START) domains: lipid/sterol-binding modules implicated in lipid/sterol transport, metabolism, and signal transduction. START domains are amplified in a special class of plant-specific homeodomain transcription factors that is conserved across the plant kingdom. GLABRA2 (GL2) is a homeodomain-START transcription factor whose mRNA is expressed in embryogenesis. Here we utilize a translational fusion of GL2 to the fluorescent protein EYFP to reveal the dynamic subcellular expression of GL2 in the developing embryo. Expression is abnormal in the sterol biosynthesis mutant *cph*/*smt1*, suggesting that sterol composition is important for proper subcellular localization of GL2. Our current studies examine expression of GL2 in other mutants that affect sterol or sphingolipid biosynthesis. Mutational analysis of the START domain from GL2 is currently in progress to test the hypothesis that this homeodomain-START protein is regulated by lipid/sterol binding via its START domain.

289 Characterization of flowering time QTL in a new RIL population

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We are utilizing natural variation present in different accessions of Arabidopsis thaliana to identify genes involved in light responsiveness. Plants are capable of responding in a variety of ways to specific light cues. We have created a RIL (Recombinant Inbred Line) population between Est-1 (Estland) and the common lab strain Col (Columbia). We have characterized a population of 280 RILs for light responsiveness by analyzing both hypocotyl elongation and flowering time. Multiple QTL (Quantitative Trait Loci) have been identified in this population for both traits analyzed. Two QTL have been confirmed in HIF (Heterogeneous Inbred Families) and/or NIL (Near Isogenic Lines). Both QTL affect flowering time, however one QTL is specific to a long day photoperiod while the other QTL is specific to a short day photoperiod. The short day QTL locates at the very bottom of chromosome II, and the autonomous pathway gene FPA is a candidate for this QTL. The long day QTL localizes to chromosome I, and FT is a candidate gene. To determine if FT is responsible for the long day QTL on chromosome I, we have used quantitative complementation. The results suggest that FT may be the causative gene for the QTL. Additional experiments to confirm that FT is responsible for the QTL include fine mapping, sequencing, expression studies, and transgenic complementation.

290 Natural variation in Arabidopsis thaliana for the vernalization response

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We are utilizing natural variation present in different accessions of Arabidopsis thaliana to identify genes involved in the vernalization response. Vernalization is defined as an extended cold treatment, which mimics winter and leads to more rapid flowering. We have conducted a survey of over 300 natural accessions of Arabidopsis and have found substantial variation in nature for this trait. The majority (70%) of natural Arabidopsis accessions flower early and have little or no response to vernalization. The remaining accessions display a broad spectrum of vernalization sensitivity and responsiveness. We have categorized the responsive accessions and are focusing on two classes; 1) those that are vernalization extra-sensitive (VES), and 2) those that are vernalization insensitive (VIN). Accessions from the two classes will be crossed and the progeny analyzed for the vernalization response. Those displaying Mendelian segregation can be mapped using traditional techniques. Those populations displaying a continious distribution for the trait, which suggests multiple causative loci, will require more complicated crossing schemes and/or techniques to identify the causative loci.

291 Comparative Analysis of CONSTANS-like B-box Zinc Finger Protein Family of Arabidopsis

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The CONSTANS (CO) of Arabidopsis has an important role in the regulation of flowering in the photoperiod promotion pathway. CO is a member of protein family containing B-box type C2C2 zinc finger motif in the amino terminus of the protein. 32 B-box zinc finger proteins are predicted from the analysis of Arabidopsis genome sequence. The protein family is suggested to be important in at least two important plant developmental processes such as photomorphogenesis (STO) and flowering control (CO). To understand the role of CO in flowering time control we have isolated and compared 32 B-box zinc finger proteins of transcription activity in yeast, protein interactions, pattern of circadian gene expression and tissue specific expression. We found that CO and 16 CO-like (COL) B-box zinc finger proteins contain transactivation domain in yeast. These proteins form homodimer and heterodimers with each other through the B-box zinc finger domain located at the amino terminus. The identification of extensive protein-protein interactions among CO-like B-box zinc finger proteins suggests that photoperiodic control of flowering time maybe much more complex than the one predicted previously. (Supported by BK21 program and the grant from CFGC of 21C Frontier Research Program).

292 Analysis of *IDA-like* (*AtIDL*) genes in *Arabidopsis thaliana*

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A central question in biology is how the cells in multi-cellular organisms communicate. One way cells can communicate is through ligand-receptor interactions. In plants very few ligand- receptor pairs have been identified.

More than 600 Receptor Like Kinase (RLK) have been identified in the *Arabidopsis* genome, this implies that there should be numerous ligands interacting with these putative receptors. However, very few ligands have been identified to date.

Recently a putative new group of ligands in plants, the IDA- like (IDL) proteins, was identified. The five *IDL* genes in *Arabidopsis thaliana* were identified based on their similarity to IDA, a protein involved in floral abscission (Butenko et al, 2003 Plant Cell 15: 2296-2307). These genes contain an N-terminal signal peptide, and are therefore believed to represent a new family of plant ligands. The C terminus of the IDL family is distinct from previously characterized putative ligand families, such as the CLE and SCRL families. A ligand is predicted to be exported through the secretory pathway, to investigate whether the IDL proteins are secreted we will look at the subcellular localization in transient transformed onion cells, and in stable GFP transformants.

In order to find the gene expression pattern of the *IDL* genes, RT-PCR has been performed, and lines transformed with promoter::reporter gene (GUS) constructs have been investigated. Using reverse genetics, we wish to investigate what kind of processes the IDL proteins are involved in. We will utilize T-DNA insertion lines and transgenic lines of *Arabidopsis* expressing RNAi constructs of each of the five genes, to study loss-of-function and knock down phenotypes of the IDL genes. In addition we will investigate if the over-expression of the IDL genes will lead to a mutant phenotype.

Future studies may reveal which, if any, interacting protein partners the *IDL* genes may have, and give us insight into which processes the IDL proteins regulate.

293 TOPLESS is involved in floral organ identity

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TOPLESS (TPL), a putative transcriptional corepressor, has been characterized for its role in the maintenance of apical-basal polarity in the *Arabidopsis* embryo. Here, we present data for the role of TPL in floral organ identity. The floral phenotypes of *tpl-1* include homeotic transformations of floral organs, such as the development of petalloid sepals and stamenoid petals, suggesting the expansion of B and C function genes according to the ABC model of floral development. A T-DNA screen identified a new allele of the APETELA (AP2) (designated At709) as a strong enhancer of the *tpl-1* floral phenotypes. *at709; tpl-1* double mutants display an increased penetrance of petalloid sepals, stamenoid petals, fusion defects in the gynoecium, as well as reduced organ number. Additionally, a yeast two-hybrid screen revealed that TPL physically interacts with AP2. These data suggest that TPL and AP2 act together in the floral meristem to specify floral organ development.

294 *shk1-D*, a dwarf Arabidopsis mutant caused by activation ofthe *CYP72C1* gene, has altered brassinosteroid levels

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Brassinosteroids (BRs) are plant steroidal hormones that regulate plant growth and development. An Arabidopsis dwarf mutant, *shrink1-D* (*shk1-D*), was isolated and the phenotype was shown to be caused by activation of the *CYP72C1* gene. *CYP72C1* is a member of the cytochrome P450 monooxygenase gene family similar to BAS1/CYP734A1 that regulates BR inactivation. *shk1-D* has short hypocotyls in both light and dark, and short petioles and siliques. The seeds are also shortened along the longitudinal axis indicating CYP72C1 controls cell elongation. The expression of *CPD*, *TCH4* and *BAS1* were altered in *CYP72C1* overexpression transgenic lines and endogenous levels of castasterone, 6-deoxocastasterone and 6-deoxotyphasterol were also altered. Unlike *BAS1*/*CYP734A1* the expression of *CYP72C1* was not changed by application of exogenous brassinolide. We propose that CYP72C1 controls BR homeostasis by modulating the concentration of BRs.

295 Transcript Profiling of Jasmonic Acid Modulated Genes in Arabidopsis Stamens Identifies Key Regulators of Male Fertility

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In Arabidopsis, the oxylipin signaling molecule jasmonic acid (JA) has an essential reproductive role in that it is absolutely required for stamen filament elongation, anther dehiscence, and pollen viability. While much is known about JA biosynthesis via the octadecanoid pathway, very little is known about the signal transduction pathways and downstream transcription networks that respond to this hormone. We have taken a genomics approach using Affymetrix ATH1 full genome arrays to help elucidate JA responsive transcription networks in stamens. We have measured gene expression in JA treated stamens beginning at flower developmental stage 12, the stage at which JA is required, and at four subsequent timepoints over the course of 22 hours. A concern with JA treatment of reproductive tissue was that many defense genes might also be represented in our dataset, and therefore our ability to identify those genes with primarily reproductive roles would be limited. In an effort to enrich our dataset with reproductive genes as opposed to defense genes, we have taken advantage of the fact that the JA precursor 12-oxo-phytodienoic acid (12-OPDA) can substantially substitute for JA in defense responses (1) but cannot substitute for JA in fertility. We have used the *opr3* mutant, which synthesizes OPDA but is blocked in production of JA, and we have treated these plants with either MeJA as the experimental or OPDA as a control. The filtered lists of genes significantly regulated by MeJA were divided into early and late genes. Amongst the early genes are 22 transcription factors that we considered candidates for regulators of male fertility. Using the Botany Affymetrix Resource Expression Browser from the University of Toronto (2), we examined AtGenExpress tissue datasets and produced electronic Northerns of these 22 transcription factors in about 80 samples throughout the course of Arabidopsis development. Two closely related MYB family transcription factors, AtMYB21 and AtMYB24, are induced by MeJA in stage 12 stamens and are solely expressed in reproductive tissues according to their electronic Northerns. Furthermore, a T-DNA insertion in *AtMYB21* yields a phenotype similar to that of *opr3*. Current efforts are aimed at characterizing other early transcription factors that are likely to play roles in these processes, identifying late transcription factors with roles in reproduction, and delineating metabolic pathways that have roles in dehiscence and the final stages of pollen maturation.

1) Stintzi A et al. (2001) PNAS 98 (22): 12837-42.
2) http://bbc.botany.utoronto.ca

296 A central role of A. thaliana OVATE family proteins (AtOFPs) in networking and subcellular localization of TALE homeodomain proteins

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Plant development is based on a complex network of regulatory pathways, the integration of external signals and the inter-cellular communication of positional information. Key players in the regulation of many different aspects of plant development are homeodomain proteins of the TALE subclass comprising the KNOX and the BELL subfamilies. Plant TALE proteins are important regulators of meristem identity, organ development and cell proliferation. Despite the fact that in recent years considerable advance has been made in understanding the genetic networks of homeodomain proteins, little is known about the regulation and the actual mechanisms of TALE protein functions on a cellular level.

Systematic analysis of protein interactions of TALE homeodomain proteins revealed a highly connected complex network. The network includes nine members of Arabidopsis thaliana Ovate Family Proteins (AtOFPs), a novel plant specific protein family, indicating a close functional connection to TALE homeodomain proteins. In Arabidopsis this family consists of 18 members sharing a conserved domain with the tomato OVATE protein. Evidence is provided that AtOFP1 is an essential pleiotropic developmental regulator. AtOFP1 and AtOFP5 are shown to associate with the cytoskeleton and to regulate sub-cellular localization of TALE homeodomain proteins suggesting a novel control mechanism in plant development.

297 Microarray analysis of gene expression in the *pop2* mutant

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The *POP2* gene in *Arabidopsis* encodes a transaminase that modulates γ-amino butyric acid (GABA) levels. GABA is elevated up to 100 fold in *pop2* flowers, resulting in pollen tube growth defects in the septum and misguided pollen tubes during the final stages of growth, which ultimately causes sterility. Pistils maintain a concentration gradient of GABA from the stigma to the micropyle—the final target of the pollen tube; *pop2* pistils have a gradient only from the septum to the micropyle, but at much higher overall levels. *in vitro*, high GABA impairs pollen tube growth, while lower levels are stimulatory. Consequently, elevated GABA levels in *pop2* pistils reduce the growth of *pop2* pollen. These results provide evidence of a role for GABA in stimulating growth and directing guidance of pollen tubes. In order to identify components controlling or responding to GABA in *Arabidopsis*, we performed microarray analysis comparing *pop2* flowers to Landsberg erecta flowers. Several genes showed altered expression in *pop2*; most interestingly, three genes showed significantly decreased expression (more than ten fold lower) relative to wild type. They are a multi-copper oxidase, a hydroxyproline-rich protein, and an unknown protein. In order to elucidate the functions of these genes we are performing mutant analysis to see if mutations in these genes recapitulate the reproductive or GABA-level phenotypes of *pop2*.

298 Dimerization and DNA-binding properties of MIKC*-type MADS-domain proteins in *Arabidopsis* pollen

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MIKC*-type MADS-box genes have first been identified in the moss *Physcomitrella patens* (1). Phylogenetic analyses of all MADS-box genes in *Arabidopsis* indicated that six genes, homologous to the *Physcomitrella* MIKC*-type genes, form a distinct cluster, and expression analyses showed that at least four of them (*AGL30*, *AGL65*, *AGL66* and *AGL104*) are pollen-predominant (2, 3). Expression of MADS-box genes in gametophytic tissue is quite unusual, and has only been observed in a few other cases. These *Arabidopsis* MIKC*-type genes are all co-expressed in pollen after the second mitosis, and are therefore likely to control gene expression in the last stages of male gametophyte development.

Because MADS-domain transcription factors usually bind DNA as dimers, we used the Yeast-2-Hybrid technique to investigate the possibility for these pollen-specific MIKC* proteins to form homo- and heterodimeric complexes. We observed that potentially three specific heterodimers can be formed, and two homodimers. With this information, we performed Random Binding Site Selection and Electrophoretic Mobility Shift Assay (EMSA) experiments for some of the complexes, in order to confirm their functionality and to identify their preferred binding motifs.

Our knowledge of the DNA-binding specificity of the MIKC* complexes, in combination with microarray analyses of mutants, will allow us to identify direct target genes of each complex. This way, we aim to elucidate the role of the different MIKC* complexes in late pollen development.

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2. Kofuji et al (2003) *Mol Biol Evol* 20: 1963-1977
3. Parenicova et al (2003) *Plant Cell* 15: 1538-1551

299 Overlapping Roles of SET-Domain Polycomb-Group Proteins in Suppressing Autonomous Endosperm Development in Arabidopsis

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Endosperm initiation is controlled by double fertilization in angiosperms. The uncoupling of these two events is observed in the Arabidopsis *fis/fie* (fertilization independent seed/endosperm) class of mutants defective in Polycomb-group (PcG) function, including *mea*, *fie*, *fis2*, and *msi1*. In Arabidopsis, the SET-domain PcG (MEA family) and Zinc-finger PcG (FIS2 family) proteins are encoded by a few genes with divergent developmental functions that include roles in early seed development, control of flowering time and regulation of vernalization. The divergent roles of PcG proteins suggest that plants utilize distinct PcG complexes to regulate different developmental processes. Using a yeast-2-hybrid screen, we have identified MEA and EZA1 (a.k.a. SWINGER) as distinct SET-domain partners of FIS2 during early seed development. Mapping of the FIS2-interacting regions indicated that the two SET-domain proteins may have diverged functionally due to changes in a region containing five cysteines. Moreover, protein localization experiments indicated a more ubiquitous pattern of expression for *EZA1* as compared to *MEA*. Finally, mutations in the *EZA1* gene enhanced *mea* mutant phenotypes including parthenocarpic development and formation of autonomous endosperm. As EZA1 has been implicated in flowering timing control, our data indicate that EZA1 and MEA perform partially-overlapping yet distinct functions during plant development.

300 Aberrant growth of stamen filament in a dominant *Aux/IAA* mutant, *msg2/iaa19*

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In an autogamous plant, *Arabidopsis thaliana*, self-pollination is accomplished by coordinated elongation of stamen filament and pistil. When the length of stamen exceeds the length of pistil, the anther can deposit pollen grains on the stigma papilla. The pollination occurs when pistil is about 2 mm long. The number of epidermal cell file of stamen filament was 20.3 ± 2.6 (n=17) irrespective to the length of filament in wild-type plants. This indicates that the growth of stamen filament is due to cell elongation and not due to cell division.

An auxin-insensitive dominant mutant, *msg2/iaa19*, is reported as partially infertile (Tatematsu et al., 2004), however artificial pollination recovers fertility. Although the stamen filament of *msg2/iaa19* can grow longer than 2mm in aged flowers, stamen filament is always shorter than pistil. Infertility of *msg2/iaa19* may be explained by slow growth of stamen filament. The expression of *IAA19* promoter:*GUS* gene was detected in elongating stamen filament, suggesting that *IAA19* may be involved in growth of the filament. These observations evoke a coordinated signalling event between the growth of stamen filament and pistil, which might be mediated by auxin.

301 A Mutation in a MicroRNA Target Site Reveals Novel Regulation Mechanisms in Arabidopsis Development

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MicroRNAs (miRNAs) are ~21 nucleotide (nt) RNAs that regulate gene expression in plants and animals. In Arabidopsis, most miRNAs target transcription factors that influence cell fate determination and therefore organ development. To date, the biological functions of miRNA-directed regulation have been reported for several miRNA subfamilies. In a screen of an EMS-mutagenized Arabidopsis population for genes that affect fruit development, we identified a semi-dominant mutant, *syl* for *styleless*, that shows defects in leaf blade margins, internode patterning and fruit morphology. Map-based cloning revealed that there is a single base mutation (C to T) in a transcription factor gene. The mutation, which occurred in the miRNA target site of this transcription factor gene, caused a significant accumulation of the transcripts as detected by RT-PCR and Northern blot analysis. Transgenic plants harboring the genomic version of *syl* recapitulated the phenotypes observed in the original EMS *syl* mutant. Moreover, introduction of a complementary mutation in the miRNA reversed the *syl* mutant phenotypes. In summary, we identified a mutant that has a mutation in the microRNA target site of a transcription factor gene that exhibited pleiotropic phenotypes during Arabidopsis development. The molecular basis of the mutant phenotypes is the increased level of the transcription factor due to loss of its microRNA regulation.

302 Flowering-time genes - from Arabidopsis to grasses

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Molecular-genetic analysis in *Arabidopsis* is revealing a network of genes controlling flowering time. To understand how flowering-time is regulated in agriculturally important grass species, we are isolating and characterising orthologues of these genes from rice and ryegrass.

We have isolated components of the autonomous floral promotion pathway from rice and/or ryegrass, including *FCA*, *FY*, *FVE* and *FPA*. These genes are being introduced into the corresponding *Arabidopsis* flowering-time mutants to determine if they can complement their late flowering phenotype. In collaboration with AgResearch (Palmerston North, NZ), we are also knocking out these genes transgenic ryegrass using RNAi. In *Arabidopsis*, FCA limits its own production by promoting the polyadenylation of *FCA* pre-mRNA within the intron 3 to form a truncated transcripts call *FCA*-β. We have identified *FCA*-β transcripts from rice and ryegrass suggesting that FCA is regulated by an equivalent mechanism in grass species. Despite the fact that the target of the *Arabidopsis* autonomous pathway, *FLC*, has not been identified in grasses, the components of this pathway are present. However, whether they regulating the flowering time of grasses is not yet known.

303 Gene Expression in the Germinating Seeds of Brassica napus

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Seed germination can be arbitrarily defined as the transition of the quiescent embryo into a photosynthetically active plant. Seed germination is a process involving a network of many coordinated cellular and metabolic events. Although germination mechanisms and their control by dormancy have been studied in a wide range of plant species, the regulatory network of the process remains poorly understood. The process of seed germination begins with the uptake of water (imbibition) by the quiescent dry seed and ends with the elongation of the embryonic axis inside the seed. Using RNA extracted from imbibed seeds of Brassica napus DH12075 at various time intervals, gene expression profile was examined on microarray containing 12K Arabidopsis cDNA. In B. napus, the number of genes profiled varied significantly from 108 genes in the early stage to 655 genes in the late stage of germination. Taking advantage of the high degree of sequence homology between Arabidopsis and B. napus, and the efficacy of high throughput sequencing technology, a large number of genes in seed germination of B. napus were examined. At present, we are focusing on a small set of highly expressed genes.

304 Analysis of the *roxy1* mutant reveals a novel function for a plant-specific glutaredoxin during flower development

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Glutaredoxins (GRXs) are a group of small proteins existing in all prokaryotic and eukaryotic organisms examined. The function of these proteins has been extensively investigated in *E.coli* and yeast, showing that they are mainly involved in the response towards oxidative stress. In *Arabidopsis*, 30 genes code for GRXs, however, information on their function is scarce. Here, we report the isolation and characterization of the first plant GRX mutant, named *roxy1*. *ROXY1* belongs to a CC type subgroup of GRXs that are specific for higher plants. Three *roxy1* mutants were obtained from the GABI-KAT T-DNA tagging collection. All initiate a reduced number of petal primordia and exhibit abnormalities during further petal development. Double mutant analysis reveals that the defects are specific to the second whorl of the flower and independent of organ identity. *ROXY1* is predominantly expressed in tissues that give rise to new flower primordia, including petal precursor cells and petal primordia. Occasionally, filamentous organs with stigmatic structures are formed in the second whorl of the *roxy1* mutant, indicative for an ectopic function of the class C gene *AGAMOUS (AG)*. Premature and ectopic *AG* expression was observed in *roxy1-3 ap1-10* double mutants. Similarly, enhanced carpeloidy of first whorl organs in double mutants of *roxy1* with the *AG* repressors *AP2* and *LUG* indicates ectopic *AG* expression and thus supports a function for *ROXY1* in the negative regulation of *AG*. Glutaredoxins are oxidoreductases that reduce target protein disulfides. Mutagenesis of conserved cysteines within the ROXY1 protein demonstrates the importance of cysteine 49 for its function. These data demonstrate that, unexpectedly, a plant GRX is involved in flower development, likely by mediating posttranslational modifications of target proteins required for normal petal organ initiation and morphogenesis. Our work can now serves as a starting point to understand the function of the plant GRX family in more detail and to unravel the intriguing connection between redox regulation and flower development.

305 TWIN SISTER OF FT (TSF), a new member of floral pathway integrators

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Flowering is regulated by multiple environmental and endogenous signals. In Arabidopsis, several genetic pathways that control floral transition are integrated at transcriptional regulation of FT, LFY, and SOC1. TSF is a member of a small gene family in Arabidopsis, which includes FT.

tsf-1 mutation enhanced late-flowering phenotype of ft in both LD and SD conditions. However, with functional FT, tsf-1 did not have significant effect on flowering time in LD conditions. TSF expression was rapidly induced upon activation of CO, suggesting that TSF is a direct regulatory target of CO. mRNA levels of TSF and FT showed similar patterns of diurnal oscillation and response to photoperiods: an evening peak, higher levels in LD than in SD conditions, and immediate up-regulation upon day-length extension. These suggest that TSF promotes flowering redundantly with FT in the photoperiod pathway downstream of CO. Consistent with this, 35S::TSF plants showed precocious-flowering phenotype independent of photoperiods or activity of CO.

TSF and FT also shared similar modes of regulation by FLC, an integrator of autonomous and vernalization pathways. Expression level of TSF was reduced in fca-1 and FRI-Sf2, which accumulate high levels of FLC mRNA. After vernalization treatment, expression of TSF was released from repression by FLC. 35S::TSF plants suppressed extreme late-flowering phenotype conferred by the FRI-Sf2. These support the notion that TSF acts downstream of FLC.

In phyB mutants, amount of TSF and FT mRNA was increased. Light quality monitored by phyB seems to affect transcription of TSF as well as FT. In addition to environmental inputs, EBS regulates both TSF and FT expression to prevent premature flowering, whereas TFL2 represses FT but not TSF. Taken together, TSF is a new member of the floral pathway integrators and acts redundantly with FT.

Interestingly, spatial patterns of TSF and FT expression in seedlings did not overlap, although they are common targets of multiple pathways and expressed in the phloem within the vascular tissues. In addition, tsf-1 flowered later than wild type under SD conditions. These results suggest that there is subtle functional differentiation between the two genes. Among the floral pathway integrators, TSF and FT seem to act independently of each other and partially upstream of SOC1. Our work revealed additional complexity and intricacy of regulatory network of flowering at the pathway integration level which may contribute to fine control of floral transition.

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306 Overexpression of *SOB5* suggests the involvement of a novel class of plant proteins in cytokinin-mediated development

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Cytokinins are a class of phytohormones that play a critical role in plant growth and development. However, relatively few genes that are involved in cytokinin biosynthesis and signaling have been identified. *sob5-D*, an Arabidopsis activation-tagged mutant, showed phenotypes typical of *ipt* (*A. tumefaciens*) - expressing transgenic plants and these mutant phenotypes were found to be caused by the overexpression of a novel gene, *SOB5*. Sequence analysis places SOB5 in a previously uncharacterized family of plant-specific proteins, with two close relatives present in Arabidopsis, SOB5L1 and SOB5L2. Adult *sob5-D* plants accumulated *trans*-zeatin 9-riboside (Z9R) and *trans*-zeatin monophosphate (ZMP) 6.1- and 2.5- fold over the wild type, respectively. Adult *sob5-D* plants also accumulated twice as much N^6- (Δ^2-isopentenyl) adenine 9-glucoside (IP9G) when compared to the wild type. These results suggest a role for SOB5 in modulating specific cytokinin levels. To further explore the role of this gene's function, a SOB5-GUS translational fusion was expressed in Arabidopsis, under the control of the *SOB5* promoter. In these SOB5::SOB5-GUS transgenic plants, GUS activity showed a pattern that nearly overlapped with the expression of type-B response regulators involved in cytokinin signal transduction. GUS activity in these plants was also induced by exogenous cytokinin. Furthermore, *sob5-D* seedlings were less responsive to exogenous cytokinin compared to the wild type in root elongation and callus formation assays. Together these results suggest a complex role for SOB5 in modulating cytokinin-mediated development in plants. This hypothesis is currently being tested via the genetic analysis of hypomorphic mutations in *SOB5, SOB5L1 and SOB5L2*.

307 Control of *Arabidopsis* Anther Cell Fate Determination by the EMS1 Receptor-like Protein Kinase

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In flowering plants, male gametophytes develop in anther where cell differentiation and subsequent degeneration are essential for successful reproduction. The anther contains highly specialized cell types. The reproductive cells, microsporocytes (pollen mother cells), undergo meiosis and eventually develop into pollen grains. The remaining non-reproductive cells (somatic cells), including epidermis, endothecium, middle layer and tapetum, are required for the normal development and release of pollen. However, very little is known about the molecular mechanisms of cell fate determination during anther development. We identified an *Arabidopsis* mutant *excess microsporocytes1* (*ems1*), which produces excess microsporocytes and lacks tapetal cells. The number of excess microsporocytes in the mutant is close to the sum of wild-type microsporocytes and tapetal cells, indicating that the tapetum precursor cells differentiate into microsporocytes in the mutant. The male meiotic nuclear division seems normal in the mutant, but meiotic cytokinesis does not occur, resulting in the failure of microsporogenesis and male sterility. The *EMS1* gene encodes a leucine-rich repeat receptor-like protein kinase (LRR-RLK), and its expression is associated with the differentiation of the microsporocytes and tapetal cells, indicating that EMS1 mediates signals that control cell fate determination during anther development. Our future research will focus on defining the *EMS1* gene function and elucidating the EMS1-dependent signal transduction pathway in anther cell differentiation.

308 Tissue specific expression of members of the family DUF642 in Arabidopsis thaliana

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The study of some multigenic families with conserved genetic functions has contributed to understand some of the crucial processes during plant development. In *A. thaliana* genome, it has been proposed the presence of more than 1000 multigenic families some of them are plant specific. Many protein families contain domains with an amino-acid sequence that indicates an involvement in the interaction of proteins with molecular components. We have focused our studies on the DUF642 family; proteins included in this family present a domain with a tertiary structure similar to that described for Galactose binding domain. Previous studies in our laboratory suggest that proteins of this family could interact *in vitro* with members of the MADS-box family. The analysis of the expression of 10 members of this family revealed that some of them are tissue specific. At2g41810 is expressed in flowers and inflorescences At5g14150 is expressed only in flowers, inflorescences and roots. In order to determine a protein-protein interaction function of the DUF642 family, we used an affinity column with the recombinant proteins of this family to isolate and identify of putative protein interactor partners. The identification of these partners will provide an insight in the role of this protein family in plant development.

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309 Histochemical and Gene Expression Analysis of Tissue-reunion Process in the Arabidopsis Cut Flowering Stem

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We have previously shown that the cortex of cut hypocotyls completely unites in 7 days, and that leaf gibberellin (GA) is required for this process in cucumber and tomato seedlings. To better understand how the tissue-reunion takes place at the molecular level, histochemical and gene expression analyses were performed using *Arabidopsis* cut flowering stem. The lowest internode of the main flowering stem was cut through half of its diameter transversely with a micro-surgical knife, and the reunion process was analyzed. As in cucumber and tomato hypocotyls, cell division was initially observed 3 days after the cut and the tissue-reunion almost completed in 10-14 days in *Arabidopsis* cut flowering stems. Tissue-reunion of the *Arabidopsis* flowering cut stem was inhibited by the removal of flower shoot apex and cauline leaf, which is similar to the observation that tissue-reunion did not occur in cucumber and tomato hypocotyls when cotyledons were removed. Histochemical analysis using a cyclin B-promoter::GUS reporter gene was performed to examine the spatio-temporal changes in mitotic activity in the process of tissue-reunion. In cyclin B-promoter::GUS plants, GUS activity was detected in the pith and vascular tissues in both upper and lower regions of the cut position at 3-7 days after cutting. In the apex- and cauline leaf-eliminated plants, GUS activity is too low to be detected in the reunion region of the cut stem. To identify genes involved in the tissue-reunion process, RNA samples from 2-5 mm segment of cut flowering stem around the cut surface and non-cut region in the same internode at various reunion stage were subjected to oligonucleotide-based microarray analysis. A number of genes involved in signal transduction, cell division and cell wall modification were determined to be up-regulated during the reunion process. We also found that a subset of these up-regulated genes were previously determined to be GA- responsive.

310 Characterization of proteins that interact with the LATERAL ORGAN BOUNDARIES protein

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The *LATERAL ORGAN BOUNDARIES (LOB)* gene in Arabidopsis is expressed in a band of cells at the adaxial base of all lateral organs formed from the shoot apical meristem and at the base of lateral roots. LOB contains a domain that is conserved in 42 other Arabidopsis proteins (the LBD family), and is known to be nuclear localized. Loss-of-function mutations in *LOB* do not result in a conspicuous phenotype, while plants that express *LOB* ectopically are stunted and resemble brassinosteroid-deficient mutants. Thus, the biological role of *LOB* remains unclear. In order to gain insight into the function of LOB, we have begun identifying LOB interacting proteins. A yeast two-hybrid screen has identified a transcription factor of the basic helix loop helix (bHLH) family to be a binding partner of LOB. This transcription factor belongs to a subclade of *bHLH* genes of which several members are regulated by phytohormones, in particular brassinosteroids. Interestingly, recent microarray data indicate that several *LBD* genes are also regulated by phytohormones. *In vitro* pull-down experiments have revealed that closely related bHLH family members also interact specifically with LOB and the closely related LBD25. We are currently investigating the relationship between LOB function and brassinosteroid signaling.

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311 Intercellular Communication During Root Epidermis Development Mediated by the GL3 and EGL3 bHLH Proteins

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The specification of the hair and non-hair cells in the Arabidopsis root epidermis provides a useful model for the study of cell fate determination in plants. A network of putative transcriptional regulators, including the related bHLH proteins GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3), is known to influence the patterning of these cell types. The GL3 and EGL3 genes act in a partially redundant manner to help specify the non-hair and hair cells by regulating the expression of the GL2 and CPC genes. Unexpectedly, the GL3 and EGL3 genes were found to be expressed preferentially in the developing hair cells, as assessed by promoter activity and RNA accumulation. By analyzing the expression of GL3::GUS and EGL3::GUS reporter fusions in various mutant and overexpression lines, we discovered that the expression of both genes is negatively regulated by WER, GL3, and EGL3 in the developing non-hair cells and positively regulated by the CPC and TRY proteins in the developing hair cells. Further, a GL3-YFP translational fusion, expressed under the GL3 promoter, accumulates in the nuclei of non-hair cells, indicating that the GL3 protein moves from the hair cells to the non-hair cells. These results suggest that GL3/EGL3 accumulation in the N cells is dependent on specification of the hair cell fate, which itself is known to be influenced (via CPC-mediated lateral inhibition) by the non-hair cells. This bi-directional signaling mechanism defines a new regulatory circuit for intercellular communication to specify the distinct epidermal cell types.

312 Transcription factor HAIRY acts upstream of GL1 and GL3 to regulate trichome initiation and branching in Arabidopsis

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The development of Arabidopsis trichomes from undifferentiated epidermal cells is an excellent model for studying differentiation and cell fate determination in plants. For this reason, trichome initiation, growth and branching have been the object of keen interest from plant developmental biologists. Arabidopsis trichome development consists of three genetically separable phases. In the induction phase, developmental or environmental signals are transmitted to epidermal cells to trigger initiation. In the initiation phase, epidermal cells commit to a trichome fate and start differentiating. Concurrently, a lateral inhibition mechanism prevents cells neighboring trichome initials to become trichome cells. In the growth and branching phase, trichomes undergo a number of differentiation events that lead to their final size and morphology. Although there is a clear link in Arabidopsis between developmental and hormonal signals and trichome initiation in Arabidopsis, no molecular association has yet been found between these signals and the trichome initiation complex that is centered around transcription factors GL1, GL3/EGL3 and TTG1.

We report here the identification of a transcription factor, HAIRY, which is necessary for normal trichome initiation and branching in Arabidopsis. We will show that HAIRY acts downstream of gibberellin signalling but upstream of the trichome initiation complex, and is involved in the regulation of *GL1* and *GL3* expression.

313 ADF5 and ADF9 are involved in the regulation of key growth processes in Arabidopsis

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The plant actin-based cytoskeleton involves hundreds of genes encoding both diverse actins and actin binding proteins. Actin Depolymerizing Factor (ADF) comprises one of the 16 diverse families of actin binding proteins in Arabidopsis. ADFs modulate rates of actin polymerization and depolymerization by severing actin filaments and enhancing actin filament turnover. Higher plant genomes contain at least four ancient classes of *ADF* genes, which may be hundreds of millions of years diverged from common ancestral *ADF* sequences. These *ADF* genes are differentially expressed in various organs and tissues. Microarray data and phylogenetic analysis suggest that *ADF5* and *ADF9* share similar expression patterns and sequence homology. In order to dissect gene function, knockout mutants were isolated for these two genes. Analysis of *adf9-1* and *adf9-2* mutants showed delayed seed germination and loss of apical dominance in the inflorescence. The *adf5-1* and *adf5-2* mutants are in the process of being characterized. Because of potential redundancy between ADF5 and ADF9, the mutant studies are being supplemented with RNA interference (RNAi) targeting both genes. The ADF5/9 RNAi transgenic plants exhibit various phenotypes including smaller organs, delayed bolting, fused stems, and loss of apical dominance in the inflorescence. These phenotypes, along with those exhibited by the knockout mutants, suggest that ADF5 and ADF9 are required for normal meristem function. Future studies will examine *ADF* overexpression lines to further characterize the functions of these two genes.

314 RAP2.6L is involved in a transcription factor network during shoot development in Arabidopsis

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Regenerating tissues in Arabidopsis can acquire different developmental fates in tissue culture leading to the formation of shoots, callus or new roots. We compared gene expression profiles on the three different developmental pathways and focused on genes that were specifically upregulated during shoot development. One gene that was highly upregulated during early shoot development was Rap2.6L (At5g13330), a member of the AP2/ERBP transcription factor gene family. Rap2.6L is important in shoot development because T-DNA knock-down mutations reduced the efficiency of shoot regeneration. Rap2.6L promoter GUS fusions demonstrated that the upregulation during shoot regeneration is, at least in part, a transcriptional phenomenon. Rap2.6L is upregulated following transfer of root explants onto a cytokinin-rich shoot induction medium, and we found that a transcriptional effector of cytokinin signaling, *ARABIDOPSIS RESPONSE REGULATOR2 (ARR2)*, a B-type response regulator, is a direct upstream activator of Rap2.6L. Downstream targets of Rap2.6L were identified by profiling gene expression patterns of T-DNA knock-down mutations and in estradiol-treated roots expressing Rap2.6L-estradiol receptor fusion constructs. Fusions of Rap2.6L with the transcriptional repressor EAR motif demonstrated the role of Rap2.6L in many different developmental processes – cotyledon, leaf, inflorescence, flower and silique development. Thus, Rap2.6L is a pivotal player in a transcriptional network transmitting cytokinin signals from B-type response regulators to downstream genes expressed during shoot development in tissue culture. (Supported by a grant from the National Science Foundation IBN-0236060)

315 Functional Interdependence between SHR and SCR in Radial Patterning in Arabidopsis Roots

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SHR and SCR are key regulators of endodermis differentiation and stem cell maintenance in the *Arabidopsis* roots. Previous studies showed that SHR controls the expression of SCR, whereas SCR acts downstream to effect asymmetric cell division that separates the endodermis from the ground tissue. There are also indications that SCR expression is autoregulated and SCR restricts SHR movement. Here we present evidence that not only provides an insight into the underlying mechanisms, but also uncovers a transcriptional regulatory module for endodermis specification and stem cell maintenance. To dissect the transcriptional network that governs radial patterning in the Arabidopsis roots, we first have developed a chromatin immunoprecipitation (ChIP) method for GFP-tagged transcription factors. Using this method and gene-specific PCR, we show that SHR binds to the *SCR* promoter; we further show that SCR binds to its own gene promoter. Intriguingly, we find that SCR also binds to a number of other SHR target genes. To explore the relationship between SHR and SCR, we have performed protein immunoprecipitation with plant extracts and find that SHR and SCR are reciprocally pulled down, suggesting that SHR and SCR are in a protein complex. Using the yeast two-hybrid system we demonstrate that the two proteins interact directly and preferentially form heterodimers. Importantly, this heterodimerization appears to be essential for both SHR and SCR to function properly. In scr mutants, SHR does not bind to the promoters of its target genes including SCR; RT-PCR analyses show that the expression levels of the common targets by SHR and SCR are significantly reduced in both shr and scr mutants. Taken together, our present studies have revealed a functional interdependence between SHR and SCR. Our findings support a model whereby SHR initiates the transcription of SCR, which in turn reinforces its own expression through a positive feedback mechanism that requires the SHR/SCR complex. Since no SHR protein is expressed in the ground tissue, the rapidly accumulating SCR protein in the endodermis will retain the SHR protein in the nucleus, thus restricting its further movement and preventing reiterative generation of the endodermis cell layer. The importance of this combinatorial feedback mechanism for radial patterning will also be discussed from an evolutionary perspective.

316 Osmotic regulation of root system architecture

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Root system development involves the continuous production of lateral roots from parent roots. This developmental process is strongly influenced by environmental conditions. We have little information about the physiological and molecular mechanisms that regulate root system development, or its modification in response to environmental cues. To address these questions, we have examined Arabidopsis (var. Columbia) seedlings growing in the presence of increasing amounts of osmotica. We have found that even small increases in osmotica, at levels that impose only mild osmotic stress, reduce the formation of lateral roots. This repressive mechanisms acts primarily by blocking or delaying the formation of lateral roots from lateral root primordia. We took advantage of the near-complete repression of lateral root formation in the presence of 60mM mannitol to identify mutants that have increased lateral root formation and/or fail to respond to repressive cues. These studies lead to the identification of two mutants in lateral root development, lrd1 and lrd2. Lateral root formation is increased in both mutants under all osmotic conditions tested. However, lrd2 also has an altered ability to respond to increases in osmotica. Our studies also implicated ABA and auxin as key regulators of lateral root formation under all conditions, and in the response of the root system to osmotica. Characterization of the mutants, identification of the LRD genes, and a functional model of root system development will be presented.

317 Genetic regulation of gene expression during shoot development in Arabidopsis

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The genetic control of gene expression during shoot development in Arabidopsis thaliana was analyzed by combining quantitative trait loci (QTL) and microarray analysis. Using microarray data from thirty recombinant inbred lines derived from a cross of Columbia and Landsberg erecta ecotypes, the Arabidopsis genome was scanned for marker-by-gene linkages or so-called eQTLs. The data were purged of single feature polymorphisms (SFPs), which may alter the hybridization efficiency between cDNAs from one ecotype with probes of another ecotype. In genome scans, five hot spots were found with significant marker-by-gene linkages, two of which coincided with classical QTLs conditioning shoot regeneration. The most significant eQTLs tended to show "cis-effects" as described in other systems, whereby polymorphisms in a gene result in a change in that gene's own expression. However, in the case of Arabidopsis many of these genes did not have obvious polymorphisms suggesting that epigenetic mechanisms might be prominently involved in regulating gene expression during shoot development. Further down the list of significant linkages were many eQTLS showing "trans-effects", whereby a marker affects the expression of a target gene located elsewhere on the genome. Some of these eQTLS were significantly linked to numerous genes throughout the genome suggesting the occurrence of a large group of coregulated genes controlled by a single marker.

318 Arabidopsis homologs of the TOR (target of rapamycin) and RAPTOR proteins regulate plant growth and are essential for embryo development

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The TOR (target of rapamycin) kinase, a large protein containing many HEAT repeats, was originally identified by mutations in *Saccharomyces cerevisiae* that confer resistance to the macrolide antibiotic rapamycin. The TOR proteins were subsequently characterized in yeast, mammals and Drosophila as central controllers of cell growth in reponse to environmental signals and in particular to the availability of nutrients. Recent studies have also shown that the TOR kinase is found in large complexes with several other protein partners, like the KOG1/Raptor, Rictor and LST8 proteins. These complexes probably serve to recruit the various TOR substrates.

The Arabidopsis genome contains a single Tor (AtTor, At1g50030) and two Raptor (AtRaptor1, At3g08850 and AtRaptor2, At5g01770) genes. But, in contrast to other organisms, plants appear to be resistant to rapamycin. Disruption of the AtRaptor1 gene leads to a very early arrest of embryo development (pre-globular stage) whereas disruption of the AtRaptor2 gene, which is expressed at a lower level than AtRaptor1, has no visible effects on embryo and plant development (1). The AtTOR protein is also essential for embryo development since tor null mutants are blocked at the globular stage (2). To further investigate the TOR function in plant, an extensive search for Arabidopsis insertion mutants affected in the AtTor gene was performed. As the TOR protein is essential for embryo development, we tried to find leaky tor alleles by analysing mutants with T-DNA insertions in the promoter. We isolated such mutants in both the Salk and Gabi collections. The analysis of these mutants showed that modification of the AtTor gene expression led to changes in root and shoot growth. Moreover these lines showed also alterations in responses to nutritional signals. In parallel, we are also generating transgenic Arabidopsis plants that are conditionally affected in Tor expression by the RNAi technique. We thus hope to be able to phenocopy in plants the action of rapamycin.

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319 Arabidopsis extra-large G-Proteins (XLGs) are negative regulators of root growth and development in darkness

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Signaling mediated by heterotrimeric G proteins, which are composed of three subunits: Gα, Gβ, and Gγ, may represent the most combinatorially diverse signal transduction pathways in mammalian systems. Heterotrimeric G proteins also exist in plants and are involved in regulation of plant cell signaling (Ma, 1994; Assmann, 2002; Jones and Assmann, 2004). In addition to *GPA1*, the sole prototypical G protein α subunit gene, the *Arabidopsis* genome has three extra-large GTP-binding protein (XLG)-encoding genes: *XLG1* (At2g23460), *XLG2* (At4g34390) and *XLG3* (At1g31930) (Lee and Assmann, 1999; Assmann, 2002). The carboxy-termini of conceptually translated XLGs are Gα domains that are homologous to *Arabidopsis* GPA1, while their amino-termini each contain a putative nuclear localization signal (NLS) and a cysteine-rich region. We are evaluating the hypothesis that XLG proteins may function as Gα subunits in *Arabidopsis*. Gene expression pattern analysis indicates that, like *GPA1*, all three *XLG* genes are expressed in almost all plant organs and mainly in vascular tissues as well as in primary root and lateral root meristems. Results from biolistic bombardment of 35S::GFP-XLGs indicate that all three XLGs localize to the nucleus, implying that XLGs, unlike GPA1, may function in the nucleus. Despite lack of phenotype in any single loss-of-function mutant, dark-grown *xlg1 xlg2 xlg3* triple mutants show significantly increased primary root growth compared with wild type plants, whereas *gpa1* mutants have similar primary root length as wild type. The increase in primary root growth in the triple mutants can be reversed by introducing the wild type *XLG3* gene, confirming that the primary root phenotype is caused at least partially by *XLG3*. There is only one Gβ subunit (AGB1) in *Arabidopsis*. However, no correlation has been found as yet between XLGs and AGB1 in terms of mutant phenotype, physical interaction, and localization. These data suggest that XLGs, unlike GPA1, may function independently of AGB1. As plant specific proteins, regulatory mechanisms of XLGs may differ from those of conventional Gαs.

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320 Tropic growth responses are proceeded by the formation of a spatial gradient of morphogen-dependent transcription

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The establishment of normal phototropic and gravitropic curvatures in plant stems requires the function of the auxin-responsive transcriptional activator NPH4/ARF7. In addition to providing genetic support for the long held notion that tropic responses require the formation of, and response to, localized changes in auxin concentration, the dependence of tropic responses on NPH4/ARF7 further suggest that auxin-dependent changes in gene expression are critical to these responses. In an attempt to identify "tropic stimulus-responsive" (TSR) genes we used mRNAs derived from phototropically- and gravitropically-stimulated Brassica oleracea seedlings to probe Arabidopsis thaliana whole genome microarrays. Specifically, we isolated mRNA from "lit" and "shaded" stem flanks of unilaterally irradiated Brassica seedlings, and "top" and the "bottom" stem flanks of seedlings whose growth axis was re-oriented 90° to stimulate a gravitropic response. cRNAs were generated from these mRNAs and used to probe Arabidopsis Affymetrix array chips. By comparing the hybridization signals between opposing flanks within and between tropic conditions (as compared to un-stimulated control samples) we expected to be able to identify TSR genes that are shared between phototropic and gravitropic responses and might represent target genes for regulation by NPH4/ARF7. Our results indicate that a small suite of TSR genes are differentially expressed between opposing flanks, including two indole-3-acetic acid-amido synthetases (*DFL1* and *GH3-5*), three cell wall-modifying enzymes (*EXP1*, *EXP8* and *SKS1*), two transcription factors (*bHLH134* and *HAT2*), and one gene of unknown function (*SAUR50*). We have used semi-quantitative RT-PCR to confirm that each of the genes of interest is more abundant in the stem flank farthest from the incident tropic stimulation, where auxin levels increase in response to tropic stimuation. Moreover, the differential accumulation of these transcripts occurs coincident with, or prior to, the first noticeable tropic growth. We further show that application of an auxin transport inhibitor (NPA) during tropic stimulation abolishes differential accumulation of these transcripts, and that application of auxin on one stem flank can phenocopy the differential expression patterns generated via tropic stimulation. Examination of both loss- and gain-of-function mutants for each of the identified genes will allow us to determine whether the encoded proteins play a direct role in tropic responsiveness .

321 Compensatory system: Coordination of cell proliferation and cell expansion during leaf morphogenesis

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In recent years mechanisms of development have been the subject of hot investigations; however, one fundamental question remains unanswered: how organisms acquire their final size. In leaves of Arabidopsis the number and the size of cells is almost constant under defined growth conditions, suggesting that number and size of cells within leaf lamina are strictly regulated and that such regulation determines the final size of leaves. Cell number depends on number of cell cycling and cell size depends on degree of cell expansion. However, our recent knowledge has suggested that leaf size is not a simple sum of cell number and cell size. In addition, several recent studies mentioned that in leaves of several Arabidopsis mutants, a decrease in cell number triggers an increase in the final cell volume. As a result, the decrease in leaf size is less pronounced than that of cell number. In such mutants loss in cell number seems to be compensated by cell expansion, therefore we called this phenomenon compensation. Although a number of examples of compensation have been reported to date, far less is known about its molecular mechanism and its contribution in the regulation of organ size. In order to unveil the molecular mechanism of compensation, we screened several mutants of Arabidopsis with altered leaf size and/or shape and we could identify 5 mutants, namely 163, 218, 219, 697, and 617, that exhibited typical compensation (i.e., decreased cell number associated with increased cell size). The newly identified mutants were subjected to anatomical analysis altogether with *angustifolia 3-4*, *er102* and *KRP2* overexpressor , previously reported to exhibit compensation. As expected compensation mutants had a lower activity of cell cycling than that of wild type. As a result, the total number of cells in the first leaf of each compensation mutant was significantly decreased compared to that of wilt type. Coincidently, palisade cells were much larger in the mutants than in wild type. Besides foliage leaves, strong compensation was also observed in palisade cells of cotyledons of all mutants. We also found that the stage at which compensation was induced in leaves was different among compensation mutants. The identification of genes that trigger compensation will deepen the understanding of this phenomenon and further reveal its importance for size determination and growth coordination in leaves and in organs of muticellular organisms, in general.

322 Genetic analysis of compensation system(s) in leaf development

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It has been a matter of debate that how the size of plant organs is determined. Recent studies have shown that leaf size of *Arabidopsis thaliana* is determined by number and size of cells within the leaf. However, recent reports on phenotypes of several mutants with defective leaf shape or size have suggested the existence of a systems that controls leaf size through coordination between cell number and cell size. For example, a narrow leaf mutant, *angustifolia3* (*an3*), has decreased cell number within the leaf whereas the size of each cell is larger than that of wild-type plants as if reduction of cell number is compensated in terms of final leaf size by the increase in cell volume. Similar phenomena have been reported to occur in leaves of a number of mutants and transgenic plants. These observations suggest that intrinsic mechanism(s) appear to monitor the number of cells in leaf primordia and coordinate cell proliferation and cell expansion in leaf development. We call this phenomenon as “compensatory system or compensation” (Tsukaya 2002, 2003). Mechanisms of compensation, however, are not yet known at all. To understand the mechanisms of compensation, we divided it into two distinct processes, based on the idea that decreased cell number may trigger the increased cell volume. Namely, (1) an induction: the reduction of cell proliferation in leaf primordia and (2) a response: the activation of cell expansion process. To examine the above hypothesis, we used mutants with specific defects on either cell proliferation or expansion in leaves as well as the *an3* mutant as a representative that exhibits compensation.Our analyses of various mutants with defects on number of leaf cells suggested that triggering of compensation is roughly dependent on the severity of the decrease in cell number.Thus, it is likely that cell number has to be decreased below a threshold level to induce the compensation.On the other hand, weproduced double mutants between *an3* and mutants with altered cell size to find genetic pathway(s) required for the “response” of compensation, namely, accelerated cell expansion. Detailed characterizations of these mutants are in progress andpossible mechanisms of compensation will be discussed based on the data obtained from the above analyses.

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323 CKH2/PICKLE negatively regulates a set of cytokinin responses

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Cytokinins promote cell division and chloroplast development in tissue culture. We have previously identified an Arabidopsis mutant, *cytokinin-hypersensitive 2* (*ckh2*), which exhibits cytokinin hypersensitivity for growth and greening of calli. This suggests that *CKH2* is a negative regulator of a set of cytokinin responses. Here, we report that the causal gene for *ckh2* is *PICKLE*, which encodes a protein similar to human Mi-2 (also known as yeast CHD3) of the SNF2 family. Because Mi-2 is a component of the nucleosome remodeling and histone deacetylase (NuRD) complex, CKH2 is possibly involved in deacetylation of histones. To know the molecular mechanisms of *CKH2*-regulation of cytokinin responses, we carried out a microarray analysis for *ckh2* calli, wild-type calli treated with cytokinin, wild-type calli treated with trichostatin A (TSA, a histone deacetylase inhibitor). A significant number of genes, most notably photosynthesis-related genes, were commonly activated in three possible pairs of the above samples. This suggests that *CKH2* and cytokinins may regulate overlapping sets of genes that are normally repressed through deacetylation of histones. Most of the known cytokinin-primary-response genes were unaffected by *ckh2* mutation or TSA treatment, suggesting that target genes of *CKH2/PICKLE* function in late steps of cytokinin responses.

324 The Transcription Factor ATMYB2 Regulates Whole Plant Senescence through Cytokinin Anabolic Pathway

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The initiation and progress of senescence are controlled by many external and internal factors. Plant hormones ethylene, jasmonic acid, salicylic acid and ABA are believed to promote senescence while other hormones such as cytokinins are retardants of senescence. Signal transduction initiated by plant hormones and other senescence-related signals usually leads to changes in gene expression, which is an important characteristic of plant senescence. In a senescing plant, majority of the genes being expressed in green tissues, including genes involved in photosynthesis, are down-regulated, while expression levels of a subset of genes, namely, senescence-associated genes (*SAGs*), increase. Our study on a senescence-specific EST database showed that there are about 2,500 genes expressed in senescent leaves of Arabidopsis, among which are 134 transcription factor genes. Transcription factors often act as switches to cause differential gene expression and play important regulatory roles. Functional analysis of one of the transcription factor gene, *AtMYB2*, showed that this gene plays an important role in whole plant senescence as well as several other aspects of plant development including development of side shoots. Expression of *AtMYB2* is strongly induced by leaf senescence. We obtained two Arabidopsis T-DNA knockout lines of this gene. Both null mutant lines exhibited a phenotype of significantly delayed senescence at the whole plant level, remarkably increased number of shoots, and reduced fertility. A genomic DNA fragment containing *AtMYB2* restored the null mutants to wild type. Fruit removal experiment suggests that the mutant phenotype is not caused by reduced fertility. Grafting experiments showed that this phenotype is not caused by signals from roots. Expression of the two cytokinin-specific genes, *IBC6* and *IBC7*, was strongly enhanced in the *AtMYB2* knock-out plants. Transformation of the *atmyb2* plants with a cytokinin oxidase gene driven by the *AtMYB2* promoter restores the wild type phenotype. Cytokinin oxidase catalyzes the cleavage of side chains from cytokinin molecules, which causes inactivation of this group of plant hormones. These suggested that the transcription factor AtMYB2 regulates whole plant senescence and shoot development by suppressing the cytokinin production pathway.

325 Identification of downstream targets of *KANADI 1*

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Most leaves display significant differences between the upper (adaxial) side and the lower (abaxial) side in morphology and function. In *Arabidopsis thaliana*, the abaxial side of the leaf is light green and is specialized for gas exchange whereas the upper side is dark green and performs photosynthesis. *KANADI 1* (*KAN1*) is one of the key regulators of abaxial identity. A *kan1* mutation leads to a partial loss of abaxial identity whereas its ectopic expression results in radially symmetric abaxialized embryonic leaves and suppression of the shoot apical meristem (Kerstetter *et al.* 2001). The current model explaining leaf polarity establishment proposes an antagonistic relationship between genes promoting abaxial fate, *KANADI* genes, and those promoting adaxial fate, Class III HD Zip genes such as *PHABULOSA, PHAVOLUTA*, and *REVOLUTA*. In order to determine the mechanisms involved in this relationship and in the determination of the abaxial identity in leaves, we looked for downstream targets of *KAN 1* using a global expression analysis approach. Using Affymetrix ATH1 microarrays, we have identified several genes that show differential expression in loss of function mutants as well as in response to ectopic expression of a post transcriptionally activatable *KAN1*. Microarray data indicate that *KAN1* may act primarily as a repressor and is involved in the regulation of various signal transduction pathways and hormone responses.

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326 Some pathways that regulate flowering also influence the rate of vegetative shoot maturation

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As the vegetative meristem matures, the morphology of the leaves produced changes. During the early, "juvenile" stage of development leaves are round and lack abaxial trichomes. Later, "adult" leaves are elliptical and have abaxial trichomes. Here we expand on earlier studies showing that genetic and environmental factors that influence the timing of flowering also influence the juvenile-adult vegetative transition. For example, the juvenile-adult transition is delayed in lines with a strong allele of the flowering regulator, *FLC*. The juvenile-adult transition is furthered delayed by *FRIGIDA (FRI)*, which promotes *FLC* expression. *FLC* expression levels are also elevated by mutations affecting genes in the autonomous pathway and some of these (*fpa* and *fve*) delay the juvenile-adult transition. Vernalization, which induces epigenetic silencing of *FLC*, promotes the juvenile-adult transition. While there is regulatory overlap between vegetative maturation and flowering, this overlap is not complete. Two autonomous pathway mutants, *ld* and *fca*, delay flowering but do not appear to delay the juvenile-adult transition.

327 Maintenance of apical-basal polarity in *Arabidopsis thaliana* transition stage embryos by TOPLESS and HAG1

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One of the first crucial steps in the development of multicellular organisms is the establishment of polarity along an embryonic axis. In *Arabidopsis*, establishment of embryo polarity results in the formation of an apical shoot pole and a basal root pole. The *topless-1 (tpl-1)* mutation causes transformation of the shoot pole into a second, apical root pole at the transition stage. The activity of the GCN5-like histone acetyl transferase HAG1 is necessary for this transformation, as mutations in *HAG1* rescue the *tpl-1* phenotype. TPL shows structural similarities to known transcriptional corepressors. As GCN5-like proteins from other organisms have been shown to act as transcriptional co-activators, one function of HAG1 could be the activation root-specific genes that are misexpressed in *tpl-1*. This leads to a model in which TPL and HAG1 act antagonistically on common downstream target genes. To gain further understanding of the process of polarity maintenance during Arabidopsis embryogenesis and the mode of action of TPL and HAG1, we have started a mutagenesis screen to identify other factors that play a role in this process. Although the embryonic phenotype of *tpl-1* is rescued by the *hag1-3* mutation, the flowers of these double mutant plants show reduced fertility. This can be circumvented by the use of a transgenic line carrying a dexamethasone inducible version of HAG1 that rescues the floral phenotypes of *hag1*, but does not affect the *hag1-3/tpl-1* embryo phenotype. About 15.000 seeds have been mutagenized and 2000 M2 lines have been screened for seedling phenotypes. Three of the M2-Lines analysed so far segregate seedlings that display the original *tpl-1* mutant phenotype. We are currently in the process of characterizing and mapping these mutations and our initial results will be presented.

328 Characterization of the *BLADE-ON-PETIOLE* genes in *Arabidopsis*

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We have characterised two new regulators of lateral organ development *BOP1* and *BOP2*. We show that the *BOP* genes are expressed in the proximal parts of plant lateral organs where they might control the growth of these organs through repression of the transcription of class 1 *knox* genes and genes like *JAGGED* and *JAGGED-LIKE*. Double *bop1 bop2* mutants display ectopic growth of the leaf lamina, leading to the production of elongated leaves lacking petioles. Furthermore, the mutant flowers are often subtended by bracts, indicating a role for *BOP1* and *BOP2* in the suppression of bract development. The leaf phenotypes are also associated with an ectopic expression of the *knox*-genes *KNAT1* and *KNAT2* resulting in ectopic meristem formation at the leaf base. The *BOP* genes encode two new members of a gene family of six genes containing ankyrin repeats and a BTB/POZ domain suggesting a role in protein-protein interaction. A member of the gene family is *NPR1*, a key regulator of systemic required resistance. We discuss possible functions for the *BOP*-genes in the regulation of leaf and flower development.

329 The novel mutant *uni* altered axillary meristem formation in *Arabidopsis*

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Lateral shoot from axillary meristem (AM) on axil of leaves is an important organ to construct diverse bodies adapting to various environmental conditions. To make clear the molecular mechanism of AM-formation, we analyzed novel *Arabidopsis* semi-dominant mutant *uni-1d*. The *uni-1d* heterozygote looked like a sea urchin, named U-NI in Japanese, due to many short lateral shoots from some AMs in an axil of vegetative leaves and cotyledons. *UNI* encoded a novel NBS-LRR family protein and *uni-1d* has missense mutation causing a gain-of-function. Protein of this family has been understood as signaling molecule involving only disease resistance. Indeed, *pathogen related genes* are expressed strongly in *uni-1d*, suggesting *uni-1d* showed constitutive defense response. Interestingly, expression analysis showed that *UNI* has tissue specific expression in some organs like axil of leaves observed abnormality in *uni-1d*. These results indicate that the mutant UNI protein of *uni-1d* effects both AM-formation and defense response.

330 Role of class 1 *knox* genes in *asymmetric leaves1* mutant in *Arabidopsis thaliana*

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The genome sequence of *Arabidopsis* has revealed 4 members of class 1 *knox* genes, *SHOOT MERISTEMLESS* (*STM*), *BREVIPEDICELLUS* (*BP*)/*KNOTTED*-like from *Arabidopsis thaliana* 1 (*KNAT1*), *KNAT2* and *KNAT6*. Though it is proposed that these genes might have important functions in the maintenance of the shoot apical meristem (SAM), our understanding of their functions are limited. Leaves are developed from SAM as symmetrical and flat organs. To understand the process of the development of leaves at higher plants, we have analyzed phenotypes of *Arabidopsis* mutant, *asymmetric leaves1* (*as1*). *as1* mutant generates asymmetrical and downwardly curled leaves with aberrant mid veins and short petioles. Leaves of *as1* can regenerate ectopic shoot on the phytohormone-free medium. Furthermore the class 1 *knox* genes are ectopicaly expressed in *as1* leaves. These suggest that *AS1* regulates the expression of class 1 *knox* genes negatively in leaves (1, 2). The relationship between misexpression of class 1 *knox* and phenotypes of *as1* leaves remains to be unknown largely. To investigate the functions of class 1 *knox* genes in *as1* mutant, we analyzed the phenotypes of the mutants that had various combinations of mutations in class 1 *knox* genes in addition to *AS1* gene. Genetic interactions between *AS1* and class 1 *knox* genes will be discussed.

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331 Identification and functional analysis of QTL that regulate root system architecture

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The architecture of the plant root system is an important factor in plant survival, particularly under stress conditions. Two traits are known to be adaptive in several plant species: an intrinsically large root system and the ability to modify root growth under stress conditions. However, little is known about how either of these traits is regulated. We have observed that root system architecture (RSA) differs intrinsically between the Columbia (Col) and Landsberg *erecta* (Ler) accessions of *Arabidopsis thaliana*: the total lateral root length (TOT) of Col is less than that of Ler in both the presence and absence of mild osmotic stress. We have also observed that these accessions show differential sensitivity to osmotic stress: lateral root formation from lateral root primordia in Col, but not in Ler, is almost completely inhibited by the addition of 60 mM mannitol to the growth media. We have taken advantage of this natural variation to identify loci involved in the regulation of RSA. Through quantitative trait locus (QTL) mapping in a Col X Ler recombinant inbred population, we have identified two major effect QTL that determine TOT under mild osmotic stress conditions. These QTL have been named EDG1 and 2 for Elicitor of Drought Growth. Our analysis predicts that the Ler allele of EDG1 and the Col allele of EDG2 promote TOT under the mapping condition. To test the biological relevance of this prediction, we have created near isogenic lines (NILs) by introgressing genomic regions predicted to contain the promotive EDG alleles into the opposite parental background. Both NILs show an increase in TOT values compared to parental controls, firmly supporting the statistical predictions. Further analysis of the NILs demonstrates that EDG1 has only a slight effect on the initiation of lateral root primordia, but strongly influences the frequency with which these primordia develop into lateral roots. Furthermore, EDG1 appears to be involved in modifying RSA in response to osmotic stress. A similar analysis of the function of EDG2 is currently underway. These results will be presented, along with a model for the role of EDG1 and EDG2 in regulating RSA.

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ML and PI contributed equally to this work.

332 BOBBER encodes a NudC domain protein that affects multiple aspects of *Arabidopsis* development

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The Arabidopsis *BOBBER* (*BOB*) gene encodes a protein containing a NudC domain. *bob* mutants affect multiple aspects of development. *BOB* is an essential gene, as null alleles disrupt embryogenesis resulting in embryonic lethality and patterning defects. A viable partial loss of function *bob* allele isolated using TILLING[1] uncovers a wide range of phenotypes including serrated leaves, delayed leaf initiation, inflorescence phyllotaxy and floral defects, and short roots. Additional phenotypes include pin-like meristems and increased branching which are similar to the defects observed upon disruption of auxin signalling. Ectopic over-expression of *BOB* results in defects in leaf development.

BOB appears to be distributed throughout the cytoplasm, in contrast to NudC domain containing proteins in other organisms which have been shown to be localized to specific cellular locations. In order to define a cellular function for BOB, an analysis of BOB protein structural homology and gene expression was performed. NudC domains have structural homology to the alpha-crystalin/HSP20 family of small heat shock proteins[2]. NudC domain containing proteins in other organisms contain structurally conserved N-terminal domains which have homology to HSP90 heat shock proteins. *BOB* mRNA levels increase in response to heat shock. This change in expression and the structural similarity to heat shock proteins suggests a possible role for BOB in heat shock responses.

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333 Isolation and Characterization of *siamese* Phenotypic Modifiers

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Arabidopsis trichomes (leaf hairs) are cells that extend from the epidermis of leaves and sepals. They are a well established model for the study of cell differentiation because they are large and easily visible, making it easy to identify mutant phenotypes. During development, wild type (WT) *Arabidopsis* trichomes exit the mitotic cycle and enter an endoreplication cycle. Here DNA replication occurs without nuclear or cellular division, resulting in cells with DNA content that is more than 2C. This mitosis-endoreplication switch is an integral part of the trichome differentiation program.

The *siamese* (*sim*) mutation is a recessive mutation that produces multicellular trichomes. The *sim* mutation appears to trigger extra rounds of cell division in mutant trichomes that is not observed in WT trichomes, suggesting that the gene encodes a cell cycle regulator. It is likely that the SIAMESE (SIM) gene product interacts with other cell cycle components.

Phenotypic modifiers have been used to identify genes functioning together in the same pathway or to indicate that gene products interact. We mutagenized homozygous *sim* seeds with ethyl methane sulfonate (EMS) and examined seedlings from M2 seeds for alteration of the *sim* multicellular paired trichome phenotype by new mutations. In particular, several alleles have been recovered at a locus, *enhancer of siamese 1* (*ens1*) that, when mutant, strongly enhances the multicellularity of *sim* homozygotes three- to four-fold. Homozygous *ens1* single mutants have reduced trichome branching. The ENS1 locus maps to the lower arm of Chromosome 1. Progress towards positional cloning of ENS1 will be presented.

334 Functional analysis of GCN proteins in Arabidopsis

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In order to establish a system for characterizing gene function by utilizing the genome sequence information, we have generated a large number of T-DNA insertion lines using a newly constructed binary vector according to the vacuum infiltration transformation method. The vector carries a *uidA* [β-glucuronidase (GUS)] reporter gene which allows monitoring of promoter activity of the inserted genes, a transposable element *Ds* for generation of knock-out, and the *cis* sequences required for *Agrobacterium*-mediated transformation. We have generated a total of 57,000 transgenic lines. We screened the transgenic lines showing GUS staining in root tips and found that one line reduced root growth compared with that of wild type plants. The tagged gene was a soluble ABC protein gene related to the yeast GCN20 gene. To assess the root growth in other knockout lines of the ABC protein gene, we have screened and isolated 3 additional lines. Reduced root growth rate was also observed in these lines. GCN20 is known to interact with GCN1 in yeast, so we screened knockout lines of GCN1 orthologue from the Tag-lines. One out of seven knockout lines exhibited GUS staining in root tips, shoot apical meristem and carpels. Three knockout lines showed reduced root growth and sterile phenotype. The ABC protein interacted with the Arabidopsis GCN1 in the yeast two-hybrid system. These results indicated that the two proteins played an important role in growth and development in Arabidopsis.

335 Translational regulation in the specification of abaxial leaf identity

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Normal leaf morphogenesis requires the proper specification of adaxial-abaxial polarity. Three classes of transcriptional regulators, *KANADI* (*KAN*), *YABBY*, and *HD-ZIPIII*, have been demonstrated to play essential roles in the specification of abaxial and adaxial fate in lateral organs. Combining loss of function mutations in members of the *KAN* family leads to progressive loss of abaxial identity. In order to identify additional factors that contribute to leaf polarity, we have screened for enhancers of the *kan1 kan2* double mutant phenotype. We have recently identified three novel mutants that have similar effects on leaf polarity as single mutants and yield similar phenotypes in combination with *kan1 kan2*. One of the genes encodes a *Pumilio*-related RNA-binding protein. *Drosophila Pum* functions as a sequence-specific RNA-binding repressor of translation in anterior-posterior patterning of the embryo and in stem cell maintenance in neurogenesis. Another of the genes encodes a specific ribosomal protein indicating a potential role for translational regulation in leaf polarity. Here, we present the characterization and cloning of these new polarity genes and discuss a model for their roles in leaf morphogenesis.

336 sku11 mutant affects directional cell expansion and trichome development

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Plants control organ shape and position by regulating the orientation and extent of cell expansion and division. To learn more about the mechanisms underlying these processes, we screened for EMS mutants with altered patterns of root growth, identifying the sku11 mutant. sku11 roots meander and skew in a rightward direction on tilted agar medium plates instead of waving downward like wild type. sku11 roots also twist abnormally about their longitudinal axes and exhibit slower bending kinetics upon gravistimulation. In addition, sku11 plants have other cell expansion defects including unbranched trichomes and misshapen cotyledons, leaves and flower petals. We mapped the sku11 mutation to the upper arm of chromosome 1 and identified a nonsense mutation in the ANGUSTIFOLIA (AN) gene. ANGUSTIFOLIA encodes a homologue of the C-terminal binding protein (CtBP) family of proteins, which act as transcriptional repressors. Microarray analysis suggests that AN is involved in regulating the expression of cell wall forming genes including the xyloglucan endotransglycosylase MERI5. While we confirmed by complementation analysis that the sku11 mutant is allelic to an-1, we found these two mutants exhibit significantly different trichome phenotypes. The implications of these and other data will be discussed.

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337 Overexpression of COG1 represses photoperiodic flowering irrespectively on circadian clock in Arabidopsis

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Higher plants use day-length or photoperiod as an environmental cue to regulate many aspects of development including the transition from vegetative to reproductive stage. The recognition of day-length in plants can be achieved by fine molecular interactions between light perception and clock components. Recently, some interactions between circadian clock and light signalling were reported, but understanding of molecular mechanisms are largely unknown. *COG1*, cogwheel1 in light signaling, was reported to be a phytochrome signaling component that acts as a light repressor of photomorphogensis in Arabidopsis. *cog1-D* and overexpressors of *COG1* (COG1-OXs) also exhibited an extremely late flowering phenotype in long-day condition, not in short-day condition. In agreement with flowering phenotypes, the rhythmic expression of *CO* and *FT* in long-day condition was decreased and the peak level of *GI* was shifted earlier in *cog1-D* and COG1-OXs. Several genetic and physiological analyses in *cog1-D* supported that the late flowering phenotype of *cog1-D* in long day was not due to the aberrant clock function, but due to repression of photoperiodic genes such as *CO* and *FT*. Although the gain-of-function mutants showed distinct photomorphogenic and flowering phenotypes, intragenic suppressors of *cog1-D* did not show any alterations in both phenotypes compared with wild type, which might be caused by the redundant functions between *COG1* and *COG2* in photomorphogensis and control of flowering.

Now, we will focus on the molecular interactions between *COG1* and other photoperiodic flowering genes and their results will be discussed in detail.

338 Regulatory genes of leaf senescence in Arabidopsis

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Leaf development ends with senescence, a process consisting of deterioration events that ultimately led to death. Since leaf senescence is well coordinated developmental process, it is well expected there are many genes that influence leaf senescence. However, in spite of the biological and practical importance, the genetic mechanisms of senescence are largely unknown. Through forward and reverse genetic approaches, we are trying to unveil the molecular and genetic mechanisms for leaf senescence. Using forward genetic approach, we have identified several delayed leaf senescence mutant, *ore12-1* from chemical-mutagenized lines of Arabidopsis. However, upstream or downstream components functioning with *ORE12* in leaf senescence have less been described. To answer the question why leaf senescence is delayed in the *ore12-1* mutant and to define novel components important for *ORE12* pathway, we screened EMS-mutagenized suppressors of the *ore12-1* mutant. The recent progress on characterization of mutants and identification of the mutated genes will be presented. We have also screened delayed leaf senescence mutants from activation tagging lines to identify the negative regulators of leaf senescence. From reverse genetic approach, a putative transcription factor that is up-regulated in an early senescence stage was isolated. The overexpressor lines exhibit early senescence symptoms, revealing a possible positive regulator of leaf senescence. Some of our results suggest unique mechanisms for leaf senescence that have not been described in any other organisms in controlling longevity by cytokinin signaling. Although we are still seeing only a glimpse of the regulatory genes occurring during leaf senescence, we anticipate that we will be able to reconcile or organize general mechanisms relevant to leaf senescence in near future.

339 Functional Analysis of *bpg1*-D (bushy and pale green) mutant

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The multidrug and toxin efflux (MATE) family proteins play diverse roles such as iron homeostasis, toxin efflux, and flavonoid transport in Arabidopsis. The Arabidopsis genome has at least 54 members of this family of which functions are largely unknown. *bpg1*-D mutant, in which a MATE gene is highly overexpressed, is isolated by activation tagging. The initial growth of *bpg1*-D is almost normal but after 2-3 weeks, especially after flowering, it is severely delayed compared with that of wild type. Finally *bpg1*-D becomes short and bushy. In addition, *bpg1*-D showed early flowering and accelerated senescence, suggesting that *bpg1*-D has short life span. On the contrary to delayed growth of above-ground tissues, roots of *bpg1*-D grow faster than those of wild type. *bpg1*-D showed normal responses to BL and cytokinin but hypersensitive responses to paclobutrazol and ABA. By characterization of *bpg1*-D and knock-out mutant the new functions of MATE genes will be revealed.

340 Isolation of genetic targets of *ASYMMETRIC LEAVES 1*

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The shoot apical meristem (SAM) is a tightly regulated structure composed of naiive stem cells from which the aerial parts of the plant are produced. Leaf primordia arise from the flanks of the SAM and the differentiation of these cells is controlled by the interactions of several genes. One gene with a crucial role in the development of leaf primordia is *ASYMMETRIC LEAVES 1* (*AS1*). *AS1* is a MYB transcription factor which is expressed in organ initials and primordia. AS1 forms heterodimers with AS2 , a leucine-zipper LOB domain protein and together they are required to repress *KNOX* gene expression in developing organs. *AS1/2* expression is repressed in the SAM by the *KNOX* gene *STM*. The *stm* phenotype is a result of *AS1* misexpression in the SAM causing the SAM cells to switch to organ fate, thus terminating shoot growth. Although, *AS1* represses *KNOX* genes in developing leaves these are unlikely to be the only targets as the *as1-1* phenotype is unaffected by loss of *KNOX* activity. This suggests that *AS1* is also required to regulate other genes whose misexpression is responsible for the *as1-1* phenotype. In order to identify target genes, we have carried out screens for enhancers and suppressers of the *as1-1* phenotype. Several candidate mutants have been isolated and analysed to elucidate their relationship with *AS1*.

341 Global comparative transcriptome analysis identifies gene network regulating secondary growth in Arabidopsis thaliana

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Our knowledge on the genetic control of wood formation (i.e., secondary growth) is limited. Here, we present a novel approach to unraveling the gene network regulating secondary growth in Arabidopsis, which incorporates complementary platforms of comparative-transcriptome analyses such as 'secondary growth', 'digital northern' and 'digital in situ' analysis. This approach effectively eliminated any genes that are expressed in either non-stem tissues/organs ('digital northern') or phloem and non-vascular regions ('digital in situ'), identifying 52 genes that are upregulated only in the xylem cells of secondary growth tissues as potential regulators. The proteins encoded by these genes participate in signal transduction, transcriptional regulation, cell wall metabolism, and unknown functions. Five of the seven signal transduction-related genes represented in the final gene set encode the essential components of ROP (Rho-related GTPase from plants) signaling cascade, suggesting that xylogenesis may be regulated through small GTPase signaling. Furthermore, the analysis of promoter sequences of the candidate genes identified a novel cis-regulatory element, ACAAAGAA. The validity of this approach was verified by several independent analysis methods.

342 The *ASYMMETRIC LEAVES2* is involved in the formation of a flat leaf lamina in the presence of the *ASYMMETRIC LEAVES1* gene

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The *ASYMMETRIC LEAVES1* (*AS1*) and the *ASYMMETRIC LEAVES2* (*AS2*) genes of *Arabidopsis thaliana* are involved in the establishment of the leaf venation system, which includes the prominent midvein, as well as in the development of a symmetric flat lamina. The genes might also function in maintaining leaf cells in a developmentally determinate state, probably by repressing expression of class 1 *KNOX* homeobox genes. The *AS1* encodes a myb-like transcription factor that is an *Arabidopsis* homolog of *PHANTASTICA* of *Antirrhinum majus*. The *AS2* gene encodes a protein with a novel domain (AS2/LOB domain) that contains cysteine repeats (C-motif), conserved glycine, and a leucine-zipper-like sequence. We examined expression patterns of *AS1* and *AS2* genes in vegetative and reproductive stages. *AS2* transcripts were accumulated in whole cells in shoot apical meristem and leaf primordia and at a slightly higher level in adaxial domain of leaf primordia, although the accumulation of *AS1* transcripts was detected in the central domain that is boundary between adaxial and abaxial sides in early stage of leaf primordia. The loss-of-function mutations in *AS1* or *AS2* result in downward curling of leaves and cotyledons. Overexpression of *AS2* resulted in the repression of cell proliferation of adaxial side in the presence of the *AS1*. Appropriate level of the expression of *AS2* may be important for formation of flat leaf lamina. When *AS2* under the control of *AS1* promoter was transformed into the *as2* mutant plants, the phenotype of *as2* mutation was recovered. Our results suggest that *AS2* functions in formation of a flat leaf lamina in *Arabidopsis* in the presence of the *AS1* gene. Recent results of a genetic screen for the enhancer of *as2* will also be presented.

343 Direct regulation of *CAPRICE* and *GLABRA2* transcription by WEREWOLF protein to determine the cell fate in root epidermis

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In *Arabidopsis* roots, epidermal cells differentiate either into root hair cells or hairless cells. The mechanism of epidermis differentiation is studied as a model system for understanding cell-fate specification. Several transcription factors have been reported to be involved in this pathway. One of these, *CAPRICE* (*CPC*) is a positive regulator of hair cell differentiation, encodes an R3-type MYB protein, and is preferentially transcribed in hairless cells. Genetic analyses revealed that *CPC* expression was promoted by an R2R3-type MYB protein WEREWOLF (WER) but repressed by CPC itself. To assess how these MYB proteins regulate *CPC* transcription, we examined the promoter of the *CPC* gene and identified the sixty-nine bp region, termed as CWB region, that is required for epidermis-specific transcription of *CPC*. Tandem repeat of this region combined to a minimal promoter was sufficient for its expression in epidermis. The CWB region includes two putative MYB-binding sites, CPCMBS1 and CPCMBS2, and the epidermis-specific transcription of *CPC* was abolished when base substitutions were introduced in these sites. By gel mobility shift assay, WER was shown to be able to bind the CWB region in the *CPC* promoter. The CPC protein was, however, not able to bind to the CWB region. Previous study showed that WER promotes *GLABRA2* (*GL2*) transcription which is responsible for hairless-cell differentiation and that *GL2* promoter includes two putative-MYB-binding sites, GL2MBS1 and GL2MBS2. Therefore, we examined and found that WER directly bound to these sites. These results indicate that WER protein has DNA-binding activity and regulates the transcription of *CPC* and *GL2* through direct binding to the promoter regions of these genes. The CPC protein seems to interfere the binding of WER protein to the CWB region indirectly. We discuss the role of the transcriptional network in epidermis differentiation.

344 Molecular and genetic interactions associated with functions of *BP*, *AS1* and *AS2* in *Arabidopsis*

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Arabidopsis *BP (BREVIPEDICELLUS)* gene, a TALE homeodomain transcription factor is one of the key regulators of meristem function that affect plant architecture when mutated or ectopically expressed. The *AS1 (ASYMMETRIC LEAVES1)* and *AS2 (ASYMMETRIC LEAVES 2)* genes suppress *BP* and other class I *KNOX* genes in the leaf founder cells and maintain normal leaf development. The *as1* and/or *as2* mutants produce lobed leaf phenotypes reminiscent of *BP* ectopic expression. Previous studies using constitutive promoter that perturb *AS2* expression and our recent work by mis-expression of *AS1* and *AS2* through regulated or constitutive promoters produced altered inflorescence/shoot architecture that include downward pointing siliques similar to *bp* mutant phenotype. However the molecular mechanisms underlying the genetic interactions of these and other associated genes are largely unknown. In vitro DNA binding studies indicate that AS1 protein binds to *BP* promoter sequences. This suggests that the suppression of *BP* in wild type leaves and in gain of function lines with altered "*bp*" like phenotypes are likely mediated through direct interaction of AS1 with *BP* regulatory sequences. Further to identify other key genes that function in *BP*, *AS1*, and *AS2* regulatory networks, we have compared global gene expression profiles in the corresponding loss and gain of function lines with the wild type plants using whole genome Arabidopsis oligonucleotide gene chips. We will present the results from these microarray studies and discuss the implications of the findings to the functions of *BP*, *AS1* and *AS2* in the meristem and leaf primordia specifications.

345 Positional Signaling Mediated by a Receptor-Like Kinase

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The position-dependent specification of root epidermal cells in Arabidopsis provides an elegant system to study cell patterning during development. Developing epidermal cells overlying two cortical cells differentiate as root-hair cells, whereas non-hair cells arise over a single cortical cell. A regulatory network including TRANSPARENT TESTA GLABRA (TTG), WEREWOLF (WER), GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3), CAPRICE (CPC), TRIPTYCHON (TRY), and GLABRA2 (GL2), is known to specify epidermal cell fate through bi-directional signaling mechanism between adjacent epidermal cells. Although much of this lateral regulation mechanism is uncovered, the nature of positional signaling that regulates this transcriptional regulation is unknown. Here, we describe a new gene, *SCRAMBLED* (*SCM*), required for cells to appropriately interpret their location within the developing root epidermis. *SCM* encodes a receptor-like kinase with a predicted extracellular domain of six leucine-rich repeats and an intracellular serine-threonine kinase domain. SCM regulates the expression of the *GL2, CPC, WER*, and *EGL3*. Further, the *SCM* gene is expressed throughout the developing root apical meristem. Therefore, SCM likely enables developing epidermal cells to detect positional cues and establish an appropriate cell-type pattern.

346 The LATERAL ORGAN FUSION MYB transcription factors regulate lateral organ separation in Arabidopsis

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In an effort to understand boundary formation, our lab has isolated several boundary-expressed genes using enhancer trap lines. The Arabidopsis *LATERAL ORGAN FUSION* genes *LOF1* and *LOF2* encode R2/R3-type MYB domain proteins. LOF1 and LOF2 belong to the same sub-clade of Arabidopsis MYB proteins. To understand the functions of these genes, T-DNA insertion mutants have been isolated and characterized. *lof2-1* mutants are phenotypically normal, while *lof1-1* mutants produce secondary inflorescence stems that are partially fused to the subtending cauline leaves. In addition, *lof1-1* mutants do not produce accessory shoots. *lof1-1 lof2-1* double mutants have novel phenotypes: fusion of the flower pedicel to the inflorescence stem and the presence of decurrent strands of leaf tissue on the stem. In addition, the primary stem of double mutants shows anatomical defects in the vasculature, resembling that seen in polarity defect mutants. The additional phenotypes seen in the double mutants suggest that the two genes have partially overlapping functions and indicate that *LOF1* and *LOF2* are important for lateral organ separation. Consistent with this proposed role, *LOF1* expression is restricted to adaxial domains between the shoot apical meristem and leaf primordia during vegetative development, and adaxial junctions between both the primary and secondary stem, and the primary stem and pedicel. In addition, *LOF1* is expressed in the junction between secondary stems and cauline leaves, and at the base of floral organs. This expression pattern suggests that *LOF1* may function to establish a boundary between the meristem and initiating organs, or to regulate some aspect of communication between the meristem and lateral organs.

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347 Paralogous R2R3 MYB proteins, FLP and MYB88 control Arabidopsis stomatal patterning

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Genes that form stomata were critical for the evolution of land plants. *FOUR LIPS (FLP)*, which encodes an R2R3 MYB transcription factor, regulates stomatal patterning by limiting symmetric divisions in guard mother cells. Of the more than 126 known Arabidopsis R2R3 MYB genes, *MYB88* is most closely related to *FLP*. FLP and MYB88 proteins share 72% of identical amino acid residues overall and 92% of identity in their MYB domain. We studied the functional relationship of these two paralogs. In order to study the function of *MYB88* gene, we have screened the available T-DNA insertion SALK lines. Two alleles of *myb88* mutant were isolated. Neither allele showed an abnormal stomatal phenotype despite reduced MYB88 expression revealed by RT-PCR. To test whether MYB88 function in stomatal patterning is masked by genetic redundancy of FLP, we created *myb88-1* doubl e mutants with both a strong and weak alleles of flp. Double mutant plants showed an exaggerated and synergistic *flp*-like defect. We showed that extra copies of genomic *MYB88* can rescue *flp-1* phenotype. These data imply that MYB88 function might overlap with or be redundant to that of FLP in stomatal patterning.

348 Genome-wide survey of cell- or tissue-type enriched transcription factors (TFs) and regulation of their expression in Arabidopsis roots

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Unraveling gene expression patterns at the cellular level can facilitate linking the role of a gene to the differentiation of the certain cell or tissue types. To understand how transcription factors (TF's) regulate the developmental processes in Arabidopsis roots at genome level, transcripts of 22,000 genes were measured in six representative cell or tissue types of Arabidopsis roots using Affymetrix Arabidopsis genome array and RNA isolation technique in specific cell types (1). From this first-stage gene expression data including stele, endodermis, endodermis and cortex, atrichoblast, lateral root cap, and quiescent center, putative cell-type specific or enriched transcription factors were searched by relative two-fold enrichment of expression level in target cell types. For cross validation and survey of gene regulatory regions, 66 of them were further examined in vivo by generating transgenic Arabidopsis which carries transcriptionally or translationally fused GFP reporter to at most 3kb long 5' upstream intergenic region. About 80% of constructs with transcriptionally fused GFP generated detectable GFP expression in transgenic roots under confocal laser scanning microscope. Among them, 90% generated GFP expression patterns fitting to the most updated expression profiling data, suggesting ~3kb upstream regions of the most genes contain proper spatial regulatory elements. With translationally fused GFP to cDNA of TFs, we found several cases of protein movement, control of nuclear localization, and possible post-transcriptional regulation by microRNA. This study illustrates the dynamic gene regulations in Arabidopsis roots and the importance of incorporating protein localization data for modeling gene regulations in multi-cellular organisms.

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349 Two *MDR* genes with different effects on auxin transport and the control of root growth in Arabidopsis

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Polar auxin transport is facilitated by the asymmetric localization of an efflux carrier complex containing PIN membrane proteins. In roots, auxin moves toward the apex (acropetally) in central cells and towards the base (basipetally) in outer cells. Confocal analysis of PIN1-GFP fusions shows that PIN1 is asymmetrically localized to the apical end of cells in the central cylinder, consistent with the acropetal transport of auxin in this tissue. A T-DNA insertional mutation in an ABC transporter gene, *MDR1* reduces acropetal auxin transport by 80% without affecting basipetal transport. PIN1-GFP signal in *mdr1* roots is typically more diffuse, less constrained to the plasma membrane at the apical ends of cells than in the wild type root. Mislocalization of PIN1 appears to explain the large reduction in polar auxin transport measured in *mdr1* roots. Phenotypes linked to this reduced transport include a randomly wavy root axis caused by sporadic deviations from the vertical taken by the tip as the root elongates, and a reduced number of lateral roots that is rescued by exogenous auxin. An *AtMDR1*:GFP construct rescues the mutant lateral root phenotype. Microscopy shows that MDR1-GFP is localized mainly in the vascular cylinder and cortex of the root apex and elongation zone. The GFP signal is also detectable in lateral root primordia at all stages of development. Like PIN1, MDR1 concentrates in a large endosomal compartment following treatment with Brefeldin A but quickly resumes plasma membrane localization after washout of the BFA. Custom morphometric software was used to examine root phenotypes with high spatial and temporal resolution. Surprisingly, gravitropism and the rate of root elongation are not affected by the greatly reduced acropetal auxin transport in *mdr1*. Analysis of a T-DNA insertion mutation in *MDR4*, a separate member of the gene family, showed that loss of *MDR4* reduced basipetal auxin transport by 50% without an effect on acropetal transport. In contrast to reduced acropetal transport, the reduced basipetal transport in *mdr4* roots was associated with greater and faster gravitropism compared to wild type. Not only do *mdr4* roots begin to curve in response to re-orientation sooner than wild type, the curvature is distributed along a greater portion of the root axis. Thus, these two mutations allow the influences of acropetal and basipetal auxin flows to be separated. The cell biological basis of the *mdr1* phenotype is being investigated using GFP-MDR1 fusions.

350 Functional Characterization of *LATERAL ORGAN BOUNDARIES* in Arabidopsis

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Lateral organs arise from the shoot apical meristem (SAM), a group of undifferentiated cells at the shoot apex. The formation of lateral organs from the SAM requires the simultaneous activation of organ-specific genes and repression of meristem-specific genes. The boundary between the SAM and organ primordia is likely important for communication between the two domains and for proper organ formation. *LATERAL ORGAN BOUNDARIES* (*LOB*), the founding member of the *LOB DOMAIN* (*LBD*) gene family, is expressed at organ boundaries, and is thought to play a role in early leaf development. *LOB* is expressed at the base of lateral organs, on the adaxial side. *LOB* expression is positively regulated by the *KNOX* genes *BREVIPEDICELLUS* and *SHOOT MERISTEMLESS*, which are expressed in the SAM. *LOB* expression is also regulated by *ASYMMETRIC LEAVES1* and *ASYMMETRIC LEAVES2*, which are expressed in organ primordia. Functional characterization of *LOB* and related *LBD* genes will be presented.

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351 NRT2.1, a high-affinity nitrate transporter, plays a role in determining root architechture independent of its nitrate transport activity

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The developmental plasticity of the *Arabidopsis* root sytem is an excellent model for understanding how plants regulate organogenesis in response to environmental signals. The architecture of the *Arabidopsis* root system is determined by the pattern of initiation and elongation of lateral root branches. Both of these physiological events have been shown to be responsive to various environmental signals. We showed that *Arabidopsis* lateral root initiation is repressed by conditions of high sucrose and low nitrate. The mutant *lateral root initiation 1* (*lin1*) was isolated for its ability to overcome this repression. We have identified *lin1* as a missense mutation in *NRT2.1*, a high-affinity nitrate transporter. Indeed, several allelic mutants of *NRT2.1* show similar phenotypes and fail to complement *lin1*. Since the repression of lateral root initiation can be relieved by increasing nitrate, it was surprising to find that a defect in nitrate transport could also relieve this repression. Direct measurements of nitrate uptake and nitrate content in *lin1* seedlings has established that both are indeed reduced in comparison to wild type, suggesting that the mutant phenotype cannot be explained by a compensatory up-regulation of nitrate uptake. Surprisingly, lateral root initiation in *lin1* is de-repressed relative to wild type even when grown on nitrate-free media, suggesting that the mutant phenotype is nitrate-independent. These results suggest that NRT2.1 has a dual role, acting independently in nitrate transport and in lateral root initiation. To begin investigating the mechanisms through which NRT2.1 affects this process, we analyzed the distribution of active auxin with a DR5::GUS reporter line. Under some conditions that repress lateral root initiation, auxin accumulated in the hypocotyl of wild type plants but not in the *lin1* mutants. This suggests that NRT2.1 signaling may work by influencing auxin localization.

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352 The role of subtilases in shoot development

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Subtilisin-like serine proteases (subtilases) are encoded by a large gene family (56 members) in Arabidopsis. The function of only a few subtilase genes, such as SDD1, are known from forward genetics in plants. However, in animal systems, subtilases play interesting roles in receptor processing and in producing bioactive peptides. As part of the NSF 2010 program and in collaboration with The Arabidopsis Subtilase Consortium (TASC), we seek to understand the function of ten subtilase genes that appear to have roles and/or are differentially expressed during shoot development in Arabidopsis. We report here on the progress of studies to determine the subcellular localization, substrate specificity and protein-protein interactions of five subtilases (At1g01900, At1g20160, At1g32940, At1g32960 and At5g59090). For subcellular localization studies, translational fusions of subtilases with YFP tags have been generated and introduced into Arabidopsis. Most subtilases have a preprodomain structure and are expected to be located in the secretory pathway, plasma membrane or apoplastic space. To identify substrates and other proteins with which the subtilases associate, three approaches are being taken. One is a general proteomics survey to identify substrates and products utilizing differential 2-D gel electrophoresis (DIGE) analysis of lines bearing T-DNA mutations or overexpressing subtilase genes. Second is the development of TAP-tagged subtilase constructs and their expression in Arabidopsis. The TAP-tagged constructs will also be used to purify subtilases in native form to test for substrate specificity against a bank of candidate substrates. Third is the use of the yeast two-hybrid system to identify proteins that interact with the holoenzyme or with C-terminal subtilase tail. In both the yeast two-hybrid and TAP-tagged system, subtilases in which the catalytic site has been inactivated will be used in attempts to capture substrates.

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353 Temperature dependent isolation of enhancers and suppressors of topless-1

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The correct specification and maintenance of the root and shoot pole are an important developmental process during embryogenesis. The topless-1 mutation disrupts this process in a temperature dependent manner. At the nonpermissive temperature (29C), most tpl-1 embryos display a transformation of the shoot pole into a second root pole. At lower temperatures (21C) most tpl-1 embryos can develop a shoot system, although with a disrupted pattern. We have taken advantage of this temperature sensitive phenotype to perform both high temperature suppressor screens and low temperature enhancer screens. We have screened roughly 3,000 EMS mutagenized lines and have isolated 10 mutants that can suppress tpl-1 at high temperatures. We have also isolated 4 enhancers from the low temperature screen. Initial characterization, complementation testing, and mapping of these mutants will be presented.

354 ZBF2, a bZIP Transcription Factor, Plays Differential Regulatory Role in Hypocotyl and Cotyledon Growth during Photomorphogenesis

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Several regulatory components of light signaling pathways have been demonstrated to play key roles in Arabidopsis seedling development. However, the regulatory co-ordination between hypocotyl elongation and cotyledon expansion during early seedling development is largely unknown. We report the identification of ZBF2 (Z-box binding factor 2), encodes GBF1, a bZIP transcription factor, and its functional characterization in blue light signaling. Our DNA-protein interaction studies show that although ZBF2 interacts with both Z- and G-box LREs (Light Responsive Elements), the protein has higher affinity for the G-box as compared to the Z-box. Genetic analyses of zbf2 mutants suggest that whereas ZBF2 acts as a repressor of blue light mediated inhibition in hypocotyl elongation, it acts as a positive regulator of cotyledon expansion. We further demonstrate that ZBF2 differentially regulates the expression of light inducible genes. Whereas ZBF2 is required for the proper activation of CAB gene expression, it negatively regulates the expression of RBCS. These results together demonstrate that ZBF2 is a unique transcription factor in light signaling pathways that plays a dual but opposite regulatory role in hypocotyl and cotyledon growth in photomorphogenesis.

355 Functional characterization of the *LATERAL ORGAN BOUNDARIES DOMAIN* GENE *LBD25*

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Members of the *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)* gene family encode proteins containing the conserved, plant-specific *LATERAL ORGAN BOUNDARIES* (LOB) domain. In Arabidopsis, the *LBD* gene family is composed of 43 members. Thus far, two *LBD* genes have been reasonably well characterized: *LOB* and *ASYMMETRIC LEAVES2 (AS2)*. *LOB* is expressed in the boundaries of lateral organs, but its biological function remains unknown. *AS2* has been implicated in *KNOX* gene repression and adaxial-abaxial polarity specification. To gain a better understanding of the function of *LBD* genes, we have begun functional characterization of *LBD25*, the most similar *LBD* gene to *LOB*. A comparison of the expression patterns of *LOB* and *LBD25* revealed that the two genes are not expressed in similar patterns, indicating that they are unlikely to have overlapping genetic roles. *LBD25* is primarily expressed in vasculature, and its expression is down-regulated by the application of exogenous auxin or by the absence of light. To gain an insight into the function of *LBD25*, a T-DNA insertion-mutant, *lbd25-1*, has been characterized. *lbd25-1* homozygotes produce a reduced level of *LBD25* mRNA, and transcript levels are inappropriately regulated in response to auxin or dark. *lbd25-1* mutants produce fewer lateral roots and display moderate resistance to exogenous auxin. *lbd25-1* mutants also show reduced hypocotyl elongation in the dark, suggesting they undergo constitutive photomorphogenesis. These data suggest that *LBD25* represses auxin signaling and promotes photomorphogenesis. Experiments to further understand the role of *LBD25* in these processes are ongoing.

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356 Functional diversity and redundancy among members of the XTH gene family: A apecific role of *AtXTH27* in vascular development in rosette leaves of Arabidopsis

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The xyloglucan endotransglucosylase/hydrolase (XTH) gene family encodes a set of enzymes capable of catalyzing molecular grafting between xyloglucans or endo-type hydrolysis of a xyloglucan molecule. These enzymes are implicated in the construction and restructuring of the cellulose/xyloglucan framework of plant cell walls, which determine cell shape and hence plant morphology. Our previous analysis of mRNA expression profiles of all members of the Arabidopsis thaliana XTH gene family (33 genes) showed that most members exhibit distinct expression profiles in terms of organ specificity and responses to hormonal signals, with some members exhibiting similar expression patterns. This raises the question of whether each of these genes plays a particular physiological role or whether they are functionally redundant. To address this question, we undertook loss-of-function studies using T-DNA insertion lines and RNA interference technology, to analyze 26 members of the Arabidopsis XTH gene family. Loss-of-function mutants of *AtXTH12*, *AtXTH18*, and *AtXTH27* genes showed individual morphological phenotypes, whereas the disruption of other *XTH* genes did not cause significant phenotypic changes. Further analysis of two loss-of-function mutants for the *AtXTH27* gene, *xth27-1* and *xth27-2*, exhibited short-shaped tracheary elements in tertiary veins, and reduced the number of tertiary veins in the first leaf. In mature rosette leaves of the mutant, yellow lesion-mimic spots were also observed. Upon genetic complementation by introducing the wild-type *XTH27* gene into *xth27-1* mutant plants, the number of tertiary veins was restored, and the lesions disappeared completely. Extensive expression of the p*XTH27::GUS* fusion gene was observed in immature tracheary elements in the rosette leaves. The highest level of *AtXTH27* mRNA expression in the rosette leaves was observed during leaf expansion, when the tracheary elements were elongating. These findings indicate that *AtXTH27* plays an essential role during the generation of tracheary elements in the rosette leaves of Arabidopsis.

Matsui, A. et al. *AtXTH27* plays an essential role in cell wall modification during teh development of tracheary elements. Plant J. (in press).

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357 D-type Cyclins link developmental cues to the cell cycle

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Despite the importance of cell division, the interface between the plant cell cycle and development is rather poorly understood. At the molecular level, core cell cycle genes have been identified by both homology and function, and it is clear that plants contain both conserved and distinctive cell cycle regulators. In common with all eukaryotes, cell cycle progression is controlled by cyclin-dependent kinases (CDKs), which are activated by the binding of their regulatory cyclin subunits. The mechanisms that determine whether or not cells enter into the division cycle are of primary importance in the execution of developmental programmes and in the responses of plants to the environment. The molecular pathway controlling the activation of cell division appears to be conserved, at least in outline, between plants and mammals, and a primary event in the stimulation of cell division is activation of cyclin D transcription by external signals. Cell cycle components can be ideally studied using synchronizable cell suspension cultures because large populations of cells at the same stage of the division cycle can be collected and analysed. Highly synchronizable Arabidopsis cell suspension cultures have been developed in our laboratory which we used to study the effect of external cues, such as auxin, on the expression of core cell cycle genes. We show that cell cycle components, such as D-type cyclins and KRPs are responsive to external signals. These data provide new insight on how different signalling pathways mediate cell cycle progression and hence how they regulate developmental processes.

358 Role of Protein Phosphorylation In Polar Auxin Transport

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The plant hormone auxin plays a crucial role in plant development by regulating basic cellular processes such as cell division, cell elongation and differentiation. Auxin also functions as signal between cells, tissues and organs. Auxin is synthesized in young shoot apices from where it is polarly transported down towards the root tip. Polar auxin transport through plant cells and tissues is mediated by at least two biochemically and structurally distinct classes of specific plasma membrane associated carriers, namely auxin influx and efflux carriers. The candidate proteins for these carriers are encoded by AUX1 and PIN gene families respectively. The direction of auxin transport is proposed to be determined by polar localization of the efflux carriers within cells. Physiological and genetic evidence indicates that phosphorylation/ dephosphorylation processes play an important role in auxin transport and the localization of PIN auxin efflux facilitators. Overexpression of the PINOID protein kinase induces a basal-to-apical shift in PIN polar localization. This leads to the defects in both auxin gradients and embryo and seedling root development. Conversely, pid loss of function induces an apical-to-basal shift in PIN1 polar targeting at the inflorescence apex, accompanied by defective apical organogenesis. Besides the PINOID kinase, a protein phosphatase termed RCN1 also seems to be related to auxin transport. Reduced RCN1 activity increases basipetal auxin transport in Arabidopsis roots and leads to corresponding defects in root gravitropism. Further reduction in the phosphatase activity as observed in multiple mutants, leads to defects in cotyledon positioning resembling phenotypes of pin1 and pinoid mutants.

Even though the molecular mechanism of RCN1 function in auxin transport is not fully understood, it is possible that antagonistic RCN and PINOID activities are involved in the regulation of PIN polar targeting and hence they may contribute to decisions about the direction of auxin flow.

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359 ZBF1, a bHLH Transcription Factor, Acts as a Repressor of Blue Light Signaling in Arabidopsis

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Light is arguably the most important factor for plant growth and development. The cross talk of light signaling pathways with other signaling cascades has recently started to be unraveled. We demonstrate the identification and functional characterization of a Z-box binding factor (ZBF1) in blue light signaling. Arabidopsis ZBF1 encodes AtMYC2/JIN1, a bHLH transcription factor, which has recently been shown to be involved in abscisic acid (ABA) and jasmonic acid (JA) signaling pathways. We have demonstrated by DNA-protein interaction studies that ZBF1 interacts with the Z- and the G-box LREs (Light Responsive Elements) of minimal light regulated promoters. ZBF1 is expressed in various light grown seedlings including red, far red and blue lights, and the protein is constitutively present in the nucleus in dark or in light grown conditions. Genetic analyses using T-DNA tagged zbf1 mutant lines suggest that ZBF1 acts as a negative regulator of blue light mediated photomorphogenic growth, and blue and far red light regulated gene expression. However, our studies reveal that ZBF1 functions as a positive regulator of lateral root formation, and flowering under long day conditions. Our results further demonstrate that zbf1 mutants have compromised sensitivity to ABA and JA mediated responses. Taken together, these results suggest that ZBF1 is a common transcription factor of light, ABA and JA signaling pathways in Arabidopsis thaliana.

360 Inhibition of brassinosteroid biosynthesis rescues *nph4* defects in tropistic responses of hypocotyl

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nph4/arf7 of Arabidopsis is insensitive to auxin and has defects in hypocotyl tropism, hook formation, differential growth of leaf and lateral root formation. To analyze auxin-signaling pathway in detail, we carried out screening of suppressor mutants of *nph4* from an EMS-mutagenized population of *nph4-103*. In the first screening, we selected mutants which did not show hyponastic growth of cotyledons and rosette leaves. Subsequently, we chose suppressor candidates that restored gravitropic defects observed in *nph4* hypocotyls. Finally we obtained a recessive suppressor mutant, *snp2* (*suppressor of nph4*). *snp2* had short hypocotyls in dark condition, dark green and round leaves, short petioles and more lateral shoots than wild type due to impaired apical dominance. It also showed a dwarf phenotype. These characteristics suggested that *snp2* may be a brassinosteroid (BR)-related mutants. In fact, *snp2* phenotypes were rescued by application of brassinolide (BL) in both light and dark conditions. Genetic mapping indicated that *SNP2* locus was in the lower part of chromosome 3. In this region, there is *DWF4* gene which encodes C-22 α hydroxylase in the BR biosynthetic pathway. Sequence analysis of the *snp2* mutant revealed that a single base substitution occurred in *DWF4* gene, by which the conserved Gly125 was substituted to Glu in domain A which was believed to bind to dioxygen. In tropism of hypocotyl, *snp2 nph4* displayed stronger curvature than wild type up to 4 hr from starts of gravity and light stimuli. After 16 hr *snp2* partially suppressed defects in gravi- and phototropism of *nph4-103*. To investigate a role of BR in gravitropism, we examined effects of BL and brassinazole, an inhibitor of BR biosynthesis, on gravitropism. An addition of BL to agar medium up to 10 nM had no effects on gravitropism of wild-type hypocotyl. But 1 μM brassinazole partially rescued gravitropic defects of *nph4-102* and *103*. The present observation that inhibition of elongation growth either by *dwf4/snp2* mutation or brassinazole partially restores tropic defects of *nph4* suggests that normal tropic responses require coordinated growth between axial and lateral directions.

361 *TERMINAL FLOWER2*, the Arabidopsis *HP1* homologue, a gene involved in several processes during plant development

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The *Arabidopsis thaliana* gene *TERMINAL FLOWER2 (TFL2*) encodes a protein containing a chromo (chromatin organization modifier) domain and a chromo shadow domain that characterizes the metazoan HETEROCHROMATIN PROTEIN1 (HP1) proteins. The Arabidopsis gene encodes a 445 amino acid protein compared to animal proteins of approximately 200 amino acids. These proteins are found to function in the regulation of gene expression as one of the components of chromatin structure.

The *tfl2* mutation gives rise to dwarfed plants that still show the wt proportions in all of the above ground parts of the plant. The rosette leaves are smaller and the lengths of the internodes are shorter, even though organisation and cell size of the vegetative meristem shows normal proportions. The mutated plant shows no differences from wild type phyllotaxy and root development and after producing approximately 20 flowers there is a termination of the main inflorescence axis which leads to a reduction in the apical dominance. Analysis have shown that the *TFL2* mRNA is weakly expressed in all above ground tissues and at higher levels in shoot apical meristems, floral meristems and in young proliferating tissues.

The pleiotropic phenotype of the mutant and the homology of the gene to mammalian genes suggest that the gene product is involved in the regulation of a wide range of processes during plant development. Regarding regulation at the level of chromatin we have found genetic interaction with factors earlier described to function in this process such as FASCIATA and TOPOISOMERASE1. With respect to the mutants early flowering phenotype it is shown that TFL2 is involved in the regulation of the transition to flowering in at least the autonomous and the long day pathway. We also show that TFL2 is involved in processes regulating photomorphogenesis.

362 Genetic characterization of the *blade-on-petiole* mutants

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Here we describe the *blade-on-petiole* (*bop*) mutants and their genetic interaction with other genes involved in leaf development and bract suppression. Both *BOP1* and *BOP2* are expressed in the proximal parts of lateral organs to repress both *knox* gene expression and also *JAGGED* (*JAG*), a gene involved in the proximo-distal development of the leaf. In *bop1 bop2* double mutants *JAG* and *knox* genes are misexpressed in the proximal parts of the leaves resulting in extended leaf lamina growth and production of ectopic tissue. *bop1 bop2* mutants also produce bracts (leaves) at the base of the flower pedicels, a phenotype not seen in wild-type *Arabidopsis*. Bract formation also occur in other mutants, including *JAG* overexpressors and *leafy* (*lfy*) mutants. The suppression of bract formation in *Arabidopsis* is partly caused by *LFY*, which has two roles, to give floral identity to the primordia and to suppress the development of the bract. It is well known how *LFY* induces floral identity but less is known about how bract development is suppressed. Here we present genetic interaction data of *bop1 bop2* mutants and other well known mutants, such as *brevipedicellus*, *asymmetric leaves1* and *2*, *jag* and *lfy*. We will discuss the role of *BOP1* and *BOP2* and their interaction with these genes during leaf development and bract suppression.

363 Genetic Screen to Identify Components of SCARECROW-Controlled Pathways in Arabidopsis thaliana

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SCARECROW (SCR) is a putative transcription factor that plays important roles in establishing root and shoot radial pattern and in shoot gravitropism. At least some of its functions appear to be conserved in other plant species. In Arabidopsis, SCR is involved in specifying several key asymmetric cell divisions throughout the development and in the maintenance of root meristematic activity. As a result, scr mutants display a number of root and shoot phenotypes. These mutants have short roots that have a determinate growth mode. Hypocotyls and inflorescence stems lack starch sheath cell layer and as a consequence do not respond to gravity. In this project, we aim to identify and characterize other components in the SCR pathways through a scr suppressor screen. We set out to identify scr suppressors that have either normal radial pattern, increased root length or hypocotyls or inflorescence stems that respond to gravity. Seeds were collected in bulk from homozygous scr1 plants, and mutagenized (approximately 14,000 seeds) with EMS in several batches of 1000 seeds. Progeny of mutagenized seeds were raised to maturity and seeds were collected in pools from 70-100 plants in each pool. These seeds were later screened and seedlings with longer roots, larger leaves and with hypocotyls that responded to gravity were selected as potential suppressors. Currently, we have isolated 242 potential suppressors and rescreened 63 of those. Eight of the potential primary isolates have been confirmed. Five of the scr suppressors display an enhanced root growth and three are hypocotyl gravitropic. PCR on genomic DNAs from all eight suppressors was performed and we confirmed that all of the suppressors are homozygous for scr mutation. Cross sections of roots of the five root-growth suppressors show similar radial pattern to scr roots with one internal cell layer missing. The phenotypes of all currently identified root-length scr suppressors indicate that the root meristematic activity was improved without the restoration of the normal radial pattern.

364 Cytokinin Regulated Transcription Factors are Involved in Leaf and Cotyledon Development

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Recent work in studying the function of the plant hormone cytokinin has focused on understanding the cytokinin signaling pathway, including the cytokinin receptors (AHKs), phosphotransfer proteins (AHPs) and the type-A and type-B cytokinin response regulators (ARRs). We have focused on examining cytokinin function by examining cytokinin induced transcription factors. Three AP2/ERF transcription factors were identified in global expression analyses on seedlings as being up-regulated after application of exogenous cytokinin for various times. These three cytokinin regulated transcription factors (CRFs) are all members of a single subclade of six genes of unknown function. We have examined the phenotypes of single, double and triple *crf* mutants which primarily show alterations the normal development of leaves and cotyledons. The single mutants display various phenotypes with a low penetrance that is increased under stress conditions and in higher order mutants, similar to other leaf and cotyledon mutants, such as *asymmetric leaves1* and *2* (*as1, as2*) and *cup shaped cotyledons1* and *2* (*cuc1, cuc2*). Measurements of the expansion rates of leaves and cotyledons in these mutants and in a CRF5 overexpressor line, suggests that these genes are likely to be negative regulators of cytokinin, functioning in leaf and cotyledon expansion. We have additionally, begun to characterize in greater detail the induction of the six CRF genes in response to cytokinin. Only three members of the CRF subgroup are cytokinin inducible as seedlings and each of these three genes follows different kinetics of induction and regulation by the addition of exogenous cytokinin. Together this work indicates direct link between cytokinin and these transcription factors and a role for cytokinin in leaf and cotyledon development.

365 Enhancer trap line WG335 identifies a TCP domain protein of Arabidopsis that is essential for post-embryonic development

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We are interested in identifying and characterizing factors which play roles in plant architecture, and to this end we screened a collection of enhancer trap lines for GFP signatures in meristems and at nodes. Of five lines that met these criteria, we chose one (WG335) for further analysis and cloned the adjacent gene by employing suppression PCR. Sequence analysis revealed that it is a member of the TCP family, which derives its name from the founding members of the group: teosinte branched from maize, cycloidea from snapdragon, and the PCF genes of rice. Across these species, the proteins possess a highly conserved central domain (the TCP domain), but amino and carboxy termini which are very divergent. The available evidence suggests that these proteins are bHLH transcription factors that play roles in meristem activity and morphogenesis. WG335 displays intense expression in young primordia which dissipates as development proceeds. Interestingly, in expanding leaves, expression mimics that of the DR5::GUS reporter, with high level expression at the tip, at hydathodes and along vascular traces. In flowers, WG335 is expressed along valve margins, at the receptacle, and at the boundaries of the stigma/style. In mature plants, expression along the primary plant axis is confined to nodes. We crossed WG335 to a *brevipedicellus* mutant, in which nodal identity is shifted into subtending internodes, and witnessed expansion of the WG335 domain into the internodes. This finding suggests that the TCP protein identified by WG335 plays a role in establishing or maintaining a boundary between different tissues.

We identified a T-DNA insertion line for the TCP gene and found that knockouts are lethal. Selfing of heterozygotes produce apparently normal seeds but nulls fail to germinate. Cytological analyses are being conducted to examine arrest in more detail. The effects of constitutive expression are also under investigation and will be reported at the meeting.

It has been postulated that it is the interaction of NAC domain and TCP proteins that promote boundary formation. The Arabidopsis TCP protein is most closely related to an Antirrhinum protein known to interact with the *CUPULIFORMIS* protein. Based on these facts we conducted pairwise yeast two hybrid screens, using the TCP protein as a bait and the CUC proteins as their prey targets. Surprisingly, interactions were negligible to weak, which prompted a full library two hybrid screen. We are now characterizing primary positives from this screen and will report the results at the meeting.

366 The WEREWOLF MYB protein directly regulates *CAPRICE* transcription during cell fate specification in the *Arabidopsis* root epidermis

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The *Arabidopsis* root epidermis is composed of two types of cells, hair cells and non-hair cells, and their fate is determined in a position-dependent manner. WEREWOLF (WER), a R2R3 MYB protein, has been shown genetically to function as a master regulator to control both of the epidermal cell fates. To directly test the proposed role of WER in this system, we examined its subcellular localization and defined its transcriptional activation properties. We show that a WER-GFP fusion protein is functional and accumulates in the nucleus of plant cells, as expected for a transcriptional regulator. Also, we used the glucocorticoid receptor (GR) inducible system to show that *CPC* transcription is regulated directly by WER. Using EMSA, we found two WER binding sites (WBS‘! and WBSa!) in the *CPC* promoter. WER-WBS‘! binding was confirmed in vivo using the yeast one-hybrid assay, and the binding between the WER protein and both WBSs (WBS‘! and WBSa!) was confirmed in *Arabidopsis* cells. These results provide experimental support for the proposed role of WER as an activator of gene transcription during the specification of both epidermal cell fates.

367 LMI1 acts as a meristem identity regulator downstream of LEAFY

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Studies indicate that the plant-specific transcription factor LEAFY (LFY) controls the transition between vegetative and reproductive phases of plant development via direct activation of APETALA1 (AP1) and its closest homolog CAULIFLOWER (CAL). Microarray and chromatin immunoprecipitation (ChIP) procedures have also identified several other direct putative LFY targets including LMI1, a member of the class I homeodomain leucine zipper (HD-Zip) transcription factors. Using T-DNA alleles in homozygous and heterozygous weak *lfy* mutant backgrounds, we have demonstrated that LMI1 acts downstream of LFY in the meristem identity pathway. In-situ hybridization shows spatial and temporal overlap between LFY and LMI1 in early flower primordia, and RT-PCR suggests that LFY acts together with LMI1 in a feed-forward loop that upregulates CAL expression. Furthermore, T-DNA alleles have also implicated LMI1 protein in leaf morphogenesis defects, including the production of leaflets on rosette leaf petioles and at the base of rosette leaf blades.

368 TORTIFOLIA1, a plant-specific, microtubule-associated protein gives direction to plant organ expansion

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Back in 1947 Erna Reinholz[1] in the lab of Gerhard Röbbelen described a mutant that she named *tortifolia* because it displayed right-handed, torsional growth of the leaves due to the twisting of petioles. This torsion develops during cell expansion mainly in hypocotyls and petioles. Thus, the mutation disrupts the ability to control straight growth and to orient organs in space.

TORTIFOLIA1 was cloned by a map-based approach[2]. All *tor1* alleles (including the independently identified *spiral2* allele[3]) lead to premature termination of translation. Expression from the *TOR1* gene is low but ubiquitous. It encodes a protein of 864 amino acids with no obvious homologues in the animal kingdom. The deduced TOR1 protein reveals a novel family of proteins exclusively found in plants combining coiled coils and HEAT repeats as potential protein binding domains. In Arabidopsis, five members of the TOR1 family are present. Transient expression of fluorescently labeled fusion proteins in Arabidopsis protoplasts showed the association of TOR1 with cortical microtubuli. Furthermore, TOR1 co-localizes with cortical microtubules in planta and the recombinant protein binds directly to microtubules in vitro. These findings classify TOR1 as a novel microtubule-associated protein (MAP). Cortical microtubules are believed to direct the deposition of cellulose microfibrils, thereby controlling the direction of cell expansion. Indeed, a shift in the orientation of cortical microtubules has been observed in tor1 mutants. This shift is preceding the right-handed twisting of hypocotyls in the mutant. Thus, TOR1 is a novel cytoskeletal player required for wild-type cortical microtubule orientation. TOR1 functions as a MAP to regulate the orientation of cortical microtubules and the direction of organ growth.

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2. Buschmann et al. (2004) Curr. Biol. 14, 1515–1521;
3. Shoji et al. (2004) Plant Physiol. 136, 3933–3944.

369 Members of the *MIR164* microRNA family are redundantly required for normal meristem function

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MicroRNAs (miRNAs) are small approximately 20-25 nucleotide non-protein coding RNAs, which negatively regulate expression of genes in many organisms ranging from plants to humans. We have previously described the characterization of the *early extra petals1 (eep1)* mutant of Arabidopsis, whose predominant phenotype is the formation of extra petals in early-arising flowers. *eep1* represents a loss-of-function allele of the miRNA *MIR164c*. As one of three members of the *MIR164* family, miR164c provides most if not all of the negative regulation for CUP-SHAPED COTYLEDON1 (CUC1) and CUC2 in early flowers. Plants homozygous for the putative null allele *mir164b-1*do not show any obvious alterations from wild type development and *mir164b-1 eep1-1* double mutant plants are indistinguishable from *eep1-1* single mutants. We have now isolated a mutant from the GABI-kat collection (Rosso et al., 2003), which carries a T-DNA insertion in the *MIR164a* foldback. Plants homozygous for the *mir164a-1* allele resemble the wild-type. To test whether miR164a functions redundantly with miR164b and c, we crossed this line to the *mir164b eep1* double mutant in order to generate the *mir164abc* triple mutant. Amongst the F2 progeny of this cross, we have identified plants, which form flowers with a strong carpel fusion defect and an increased number of petals. These plants also show a highly distored pattern of flower initiation, both with respect to phyllotaxis and internode length. The phenotype cosegregates with the *mir164abc* triple mutant genotype, but also with the allelic combination *mir164aabBcc*. This result suggests that the three highly similar miRNAs, miR164a, b and c act redundantly in the inflorescence meristem in order to guarantee normal growth and second it reveales a threshold level for miR164b in a background that is compromised for miR164 function. Besides a phenotypic characterization of the *mir164abc* triple mutant, we will address the impact on targets and tissue specific functions for each individual miR164 miRNA, as well as their respective contribution to plant development.

Rosso, M. G., Li, Y., Strizhov, N., Reiss, B., Dekker, K. and Weisshaar, B. (2003). An *Arabidopsis thaliana* T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. *Plant Mol Biol* 53, 247-259.

370 Role of the CAPRICE family of myb genes in root epidermal development

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The pattern of cell fate specification in the root epidermis is regulated in a position dependent manner. Hair cells develop when an epidermal cell overlies the cleft of two cortical cells, and non-hair cells develop when an epidermal cell overlies only a single cortical cell. Molecular genetic studies have identified several genes that regulate root epidermal cell fate; including a homeodomain protein *GLABRA2* (*GL2*), a WD40 repeat protein *TRANSPARENT TESTA GLABRA1* (*TTG1*), basic helix loop helix proteins *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA3* (*EGL3*), an R2R3 myb protein *WEREWOLF* (*WER*), and the single repeat myb proteins *CAPRICE* (*CPC*) and *TRIPTYCHON* (*TRY*). Cell patterning appears to be established through a mechanism of lateral inhibition. *TTG1*, *WER* and *GL3/EGL3* positively regulate both *GL2* and *CPC* in the N cell. *GL2* acts to negatively regulate the hair cell fate in the non-hair cell position. The *CPC* gene product acts to to laterally inhibit the non-hair cell fate in neighboring cells, presumably by direct movement of the CPC protein. We have begun to characterize the role of *TRY* and three additional *CPC*-like genes, identified as *ENHANCER OF TRIPTYCHON AND CAPRICE 1* and *2* (*ETC1* and *ETC2*) and At4g01060, in root epidermal specification. We have shown that *ETC1* acts in a partially redundant manner with *CPC* and *TRY* in hair fate specification, whereas we could not detect a role of *ETC2* in root epidermal patterning. We are investigating the role of the *CPC* family of genes in the regulation of root epidermal cell fate.

371 A Connection Between Apical-Basal and Adaxial-Abaxial Polarity in Arabidopsis Embryogenesis

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The *TOPLESS* (*TPL*) gene product is necessary for correct apical patterning of the *Arabidopsis* embryo. The *tpl-1* mutation causes shoot to root homeotic transformation that results in a second root in place of the hypocotyl and cotyledon structures. The *tpl-1* allele provides an excellent tool to investigate the events involved in apical-basal polarity determination in the *Arabidopsis* embryo.

In a genetic screen we have identified a semi-dominant, gain of function allele of *PHABULOSA* (*phb-14D*), that suppresses the homeotic shoot to root transformation in the *tpl-1* background. *phb-14D* contains a mis-sense mutation in the miRNA binding site of *PHB*. Mutations at other positions in the microRNA binding site of *PHB* have been shown *in vitro* to increase the amount of *PHB* transcript by decreasing miRNA directed cleavage (Mallory et al., 2004) and *in situ* experiments on *phb-1D* show *PHB* mis-expression outside its normal adaxial and vascular domains of expression in the embryo (McConnell et al., 1999). *In situ* hybridization experiments show that markers for adaxial and meristem domains, including *PHB*, are either absent or significantly reduced in *tpl-1*, while abaxial markers are dramatically mis-expressed. Expression of these markers are restored to near wild type in the *tpl-1;phb-14D* double mutant. These results suggest a connection between apical-basal and abaxial-adaxial polarity determination events in the embryo. With this new connection in mind, we are further investigating genetic interactions of *tpl-1* and *phb-14D* with other known radial polarity and meristem mutants.

372 Functional analysis of CLE40 in *Arabidopsis* root meristem development

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Stem cell activity of the initial cells of the root meristem is controlled directly by the adjacent cells of the quiescent centre. They maintain cells in their neighbourhood, comparable with *WUSCHEL*-expressing cells in shoot- and floral meristems. CLE40, a member of the CLE protein family, encodes a potentially secreted protein that is distantly related to CLV3. While *CLV3* transcripts are confined to stem cells of the shoot meristem, *CLE40* is expressed at low levels in all tissues, including roots. Although different in their expression patterns, *CLV3* and *CLE40* show functional redundancy, as already shown by promoter swap and misexpression experiments. High level expression of *CLV3* or *CLE40* results in the premature loss of root meristem activity and differentiation of meristem cells, indicating that activation of a CLV-like signalling pathway may restrict cell fate also in roots. Overexpression of CLE40 and CLV3 protein with different tags showed that protein processing seems to be involved. Root-specific overexpression of *CLE40* leads to root meristem arrest. CLE40 loss-of-function mutants show developmental defects that are probably due to the premature loss of stem cell activity in the root. Our aim is to find CLE40 interaction partners and target genes that are regulated by *CLE40*.

373 Is a CLV-like signaling pathway operating during *Arabidopsis* root development?

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In plants, continuous growth and development is controlled through the activity of the meristems that harbor pools of stem cells. Stem cell division and differentiation of its progeny is coordinated by receptor kinase signaling in the *Arabidopsis* shoot apical meristem. Stem cells secrete CLAVATA3 (CLV3), a member of the CLE protein family that activates the CLV1-CLV2 receptor complex in underlying cells to restrict the size of the stem cell population. Localized ectopic expression of CLE19 in roots results in a premature loss of root meristem activity, suggesting also here activation of a CLV-like signaling pathway regulates meristem size. Interestingly, the stem cell population is not primarily affected in the differentiating meristem.

We performed genetic screens for suppressors to identify downstream targets of this signaling pathway. Firstly, an EMS mutagenesis screen was performed in the root expressed CLE19 background identifying a suppressor mutated in a carboxypeptidase. New additional suppressors have been identified and are being analyzed. Secondly, an activation-tagging mutagenesis was performed to identify dominant suppressors. Current progress of these experiments will be presented. In a reverse genetics approach we are investigating CLV1-like receptor kinases required for root meristem size control. More than 600 receptor like kinases (RLKs) have been identified in *Arabidopsis*, but so far the function of many of these genes has remained elusive as the system appears to operate largely redundantly. Based on an *in silico* expression database of the *Arabidopsis* root (Birnbaum et al., 2003) we selected around 80 LRR RLKs that are expressed in the different tissues of the root meristem. So far, none of the single knockouts revealed obvious phenotypes. Multiple combinations of loss-of-function alleles of these genes are therefore necessary to ultimately uncover their function.

Our data suggest that a CLV-like pathway operates in roots, but in a somewhat different way than in shoots. Finding the components that perform this function in the root can help us to understand the similarities and differences between root and shoot meristem organization.

374 Regulation of LATERAL SUPPRESSOR – a gene involved in the formation of axillary meristems

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The architecture of plants is determined by the number, arrangement and growth intensity of their sideshoots. Sideshoots are initiated by the formation of axillary meristems in the axils of leaves. We are interested to understand at the molecular level the mechanisms that control the formation of axillary meristems.

The las-4 mutant of Arabidopsis fails to initiate axillary meristems during vegetative development, whereas in the reproductive phase sideshoots are formed in the axils of cauline leaves. The LAS gene belongs to the GRAS gene family and encodes a putative transcription factor. RNA in situ hybridization experiments have demonstrated that LAS is expressed in the axils of all primordia originating from the SAM. The aim of our work is to identify and characterize upstream regulators that delimit the LAS expression domain.

To understand the control of LAS expression, we analysed the LAS promoter. Complementation experiments with deletion constructs demonstrated that at least 820 bp upstream of the ATG are necessary for a restoration of the wildtype phenotype. In the region around -820, a cluster of CArG boxes was found. These motives are being tested for interaction with MADS box proteins. In addition, T-DNA insertion lines were characterized to identify regulatory elements in the promoter region. One out of ten lines showed an lateral suppressor phenotype. The sequences flanking this insertion site were used to screen for trans-acting factors. Furthermore, las-4 plants were transformed with constructs lacking putative cis-regulatory elements, to test whether or not these elements are required in vivo for LAS function. In addition to the promoter region of the LAS gene, a 3.5 kb downstream DNA fragment was found to be necessary for complementation. A comparable regulation was demonstrated for the tomato Lateral suppressor (Ls) gene, which requires a 3'-region of nearly 3 kb for full complementation of the mutant phenotype. Transcomplementation experiment will reveal if the Ls downstream region of tomato is also functional in Arabidopsis.

375 Control of organ growth and epidermal patterning by synergistic interactions of receptor-like kinases

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In higher plants, organogenesis occurs in the absence of cell migration or removal of overproduced cells. As such, signals that coordinate proliferation, expansion, and differentiation of the neighboring cells are critical for proper organ growth and development. Our research is aimed at understanding the molecular mechanisms of cell-cell communication during plant growth and development. The presence of a large number of receptor-like kinase (RLK) genes implies that they play multiple and diverse roles in cell-cell signaling in plants. However, only a handful of RLKs have defined biological functions, due in part to intricate redundancy among RLK genes. Arabidopsis RLK ERECTA and its two functional paralogs, ERL1 and ERL2, together regulate cell proliferation and differentiation in aboveground organs. First, three *ERECTA*-family genes interact synergistically in promoting cell proliferation and organ growth, with *ERECTA* being the major player and *ERLs* being redundant. Consistently, loss of the entire *ERECTA*-family genes led to striking dwarfism, reduced organ size, and abnormal flower development, including defects in petal polar expansion, carpel elongation, and anther and ovule differentiation. Second, the three ERECTA-family genes play overlapping but distinct roles during stomatal stem cell fate specification and differentiation. How can the same receptor family regulate organ growth and epidermal patterning, which are two distinct developmental events? Our recent approach in unraveling the molecular basis of the multi-faceted functions of ERECTA-family RLKs will be presented.

376 Genetic characterization of the *bps1* root-derived mobile signal

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We have previously shown that *BYPASS1 (BPS1)* is required to negatively regulate production of a novel graft-transmissible signal that is sufficient to inhibit leaf development and shoot apical meristem activity. *BPS1* encodes a plant specific protein of unknown function and contains no functionally characterized domains. To characterize the *bps1* root derived signal, we carried out hormone supplementation, hormone inhibitor, and hormone response mutant analyses. We found that the *bps1* mutant phenotype is partially rescued by treatment with the carotenoid biosynthetic inhibitor, fluridone, indicating that *bps1* signal production required carotenoid biosynthesis[1]. We will present genetic and inhibitor studies to characterize the *bps1* root derived signal.

There are two possible explanations for the requirement of carotenoid biosynthesis in production of the *bps1* root-derived signal. First because fluridone disrupts an early step of carotenoid biosynthesis, the *bps1* signal could be a carotenoid derived molecule such as abscisic acid (ABA). Alternatively, loss of carotenoid pigments leads to the photooxidation of chloroplasts which disrupts the plastid-to-nucleus signaling that regulates expression of nuclear encoded plastid genes. Thus the *bps1* mobile signal could result from altered gene expression. We will present double mutant analyses using *genome uncoupled (gun1, gun2, gun5)* mutants[2] to distinguish between these possibilities.

To determine which carotenoid(s) must be made for *bps1* signal production, we are characterizing double mutants between *bps1* and carotenoid biosynthetic and response mutants and analyzing the effects of carotenoid biosynthesis inhibitors on the *bps1* phenotype. Our data rule out the two known apocarotenoid signaling molecules, ABA and the MAX-dependant hormone [3]. We will present additional double mutant analyses that further define the carotenoid biosynthesis requirements for production of the *bps1* mobile root-derived signal.

1. Van Norman, et al. (2004) Current Biology, 14:1739-1746.
2. Strand, et al. (2005) Nature, 421:79-82.
3. Booker, et al. (2005) Dev. Cell, 8:443-449.

377 *FLAVODENTATA*: genetic interactions and positional cloning

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Leaves and other lateral organs in plants have an inherent positional relationship with the shoot apical meristem. This relationship defines the adaxial-abaxial axis of the leaf. The proper establishment of adaxial-abaxial polarity within organ primordia is critical for the polarized growth and differentiation of the primordium and contributes to both the external morphology and internal anatomy of the mature leaf. The *PHABULOSA* (*PHB*) gene plays a key role in the adaxial identity in lateral organ development. The *KANADI1* (*KAN1*) gene is one of the determinants of abaxial identity in leaf development. Little is currently known whether other genes are involved in the specification of adaxial-abaxial polarity during leaf development.

The *flavodentata* (*flv*) mutant, donated to the Arabidopsis Information Service mutant collection by G. Redei, was originally described as a plant with serrated pale leaf margins. Mutant plants have pale green adaxial and reddish abaxial leaf surfaces at the earliest stages of seedling development and greening defects throughout development. In the Columbia and Landsberg *erecta* backgrounds, the *flv* mutants occasionally form radialized leaves. To investigate a possible role of the *FLV* gene in leaf polarity, we analyzed the genetic interactions with *KAN1* and *ARGONAUTE1* (*AGO1*) genes. *AGO1* mediates microRNA activity and represses *PHB* expression. Using a map-based strategy, we have localized the *FLV* gene to chromosome 4 and shown that it encodes a pentatricopeptide repeats (PPR) containing protein. A model of the *FLV* function in leaf polarity will be discussed.

378 The UGF protein family: Analysis of gene expression and protein interactions

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The genome of *Arabidopsis thaliana* encodes more than 26,000 proteins, many of which are completely uncharacterized. Analysis of the function of these genes is one of the major challenges in the field. Given the large number of genes, application of genomic techniques such as expression profiling is a good way to pre-select genes of interest to a particular pathway. Based on expression profiling of flowering time mutants (1), a small gene family with four members in *Arabidopsis* was chosen for further investigation. Expression of these genes in the apical region changes throughout the transition from vegetative to reproductive development.

RNA *in situ* analysis demonstrates that the UGF genes are extensively regulated throughout development. This is further confirmed by transcriptional fusion of 2.5kb of the region upstream of the UGF coding sequences to the β-glucuronidase (GUS) reporter in planta. All UGFs are detected strongly in the vasculature, including the pro-vasculature, but individual UGFs are present in specific domains that overlap only partially.

To gain insight into the function of the UGF gene family during *Arabidopsis* development we have carried out extensive yeast-2-hybrid screens and have identified potentially interacting proteins. Most of the proteins found encode for putative transcription factors, but we also found two proteins containing BTP/POZ/MATH domains.

Among the transcription factors identified were VOZ1 and VOZ2, two proteins supposedly involved in vasculature development. Interaction with these and other transcription factors hints at a role of the UGF proteins in transcription control. This possibility is investigated in a yeast-transactivation assay using a binding site in the regulatory region of a confirmed VOZ target, *AtAVP1* (2), as a reporter. BTP/POZ/MATH containing proteins on the other hand participate in Cullin-3a/b-mediated ubiquitination (3-6), suggesting, that the UGF proteins themselves are regulated by protein degradation.

UGF expression overlaps at least partially with the expression of the VOZ and BTP/POZ/MATH proteins identified in the Y2H screens, indicating that those interactions might take place in planta as well.

(1) Schmid *et al.*, 2003. Development 130, 6001; (2) Mitsuda *et al.*, 2004. Plant Cell Physiol. 45, 845;
(3) Weber *et al.*, 2004. Plant Physiol. 137, 83;
(4) Dieterle *et al.*, 2005. Plant J. 41, 386;et al., 2005. J Biol Chem.;
(6) Figueroa *et al.*, 2005. Plant Cell 17, 1180

379 Functional analysis of a laccase-like multicopper oxidase (LMCO) in Arabidopsis thaliana

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Laccase and related laccase-like multicopper oxidases (LMCOs) have been studied in plants for more than a century, yet our understanding of their physiological function(s) remains limited. The Arabidopsis genome contains 17 LMCO genes that exhibit a wide variety of expression patterns in different tissues at various stages of development, as determined using transcriptional profiling techniques, including qPCR, MPSS and DNA microarrays. This study analyzed in greater detail the products of At2g30210, a member of LMCO family that is predominately expressed in young root tissues. To determine the spatial expression pattern of At2g30210 transcripts, a promoter-reporter gene fusion was introduced into Arabidopsis. Histochemical analyses of the transgenic, GUS-expressing plants revealed that At2g30210 transcripts were preferentially expressed in developing endodermis. Low levels of expression were also detected in hypocotyls, as well as major veins of some cotyledons. A chimeric fusion of the At2g30210 coding sequence with modified green-fluorescent protein (YFP) was used to examine subcellular localization of the protein in transgenic plants. Confocal microscopy indicated that the At2g30210-YFP fusion product was localized to the cell periphery, most likely the cell wall. A genetrap insertion (GT7855) mutant of Atg30210 was confirmed by sequence analysis and PCR to have a transposon in the second exon of the gene. RT-PCR of seedling cDNA confirmed loss of transcript expression from the locus. Plants homozygous for the knockout were grown under various culture conditions for comparison with wild-type plants. On solid MS medium containing 1% sucrose, the GT7855 mutant showed more lateral root production than wild-type plants. When grown in soil or MS media without sucrose, growth of the mutant was severely repressed. Quantitative PCR revealed that At2g30210 transcripts were strongly up-regulated in wild-type plants grown on MS media without sucrose. The At2g30210 knockout mutants were also much more sensitive to salt stress than wild-type plants, as all mutant plants were dead after 3 weeks of exposure to solid MS medium containing 40mM NaCl. Enzyme assays of the At2g30210 LMCO showed the protein to be an active phenoloxidase, but found no evidence of ferroxidase activity. The results suggest involvement of the At2g30210 LMCO gene product in formation of the Casparian strip.

380 Signaling cascade controls stomata development and patterning in Arabidopsis thaliana

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Stomata are specialized epidermal cell structures that regulate the exchange of gases between a plant and its environment. Stomata are formed by two kidney-shaped guard cells surrounding a pore. The stomatal pore opening and closing allows CO2 to reach the mesophyll cells for photosynthesis, and keep the water inside the plant. Thus, stomata are not only important for plant productivity, but also were critical for the evolution of land plants. In Arabidopsis, stomata patterning follows the one-cell-spacing rule; there will be at least one pavement cell between two adjacent stomata. This kind of patterning ensures the optimal balance between CO2 uptake and H2O retention in the plant. It was proposed that, in Arabidopsis, stomata patterning is controlled by asymmetric cell divisions and local intercellular signaling. Here we will present a signaling cascade that plays a central role in regulating asymmetric cell division frequency and cell fate specification. Knocking out this signaling cascade leads to stomata cluster. Whereas constitutively activate this cascade ends up reduced frequency of asymmetric division and no stomata differentiation.

381 WVD2-Like Proteins Regulate Plant Growth Behavior and Anisotropic Cell Expansion

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Arabidopsis plants overexpressing *WVD2* (*WAVE-DAMPENED2*) exhibit dampened root waving and leftward root skewing when grown on tilted agar surfaces, compared with the wild-type seedlings. They also have shorter and stockier organs, and their petioles are twisted left-handedly. There are seven *WVD2-like* (*WDL*) genes in the *Arabidopsis* genome, which share high similarity to *WVD2* in a conserved region called the KLEEK domain (Yuen *et al*., 2003, Plant Physiology 131: 493-506). Using RT-PCR, we show that these *WDL* genes are expressed in all tissues examined, including cotyledons, hypocotyls, seedling roots, stems, flowers, and rosette and cauline leaves. With the exception of *WDL3*, which is preferentially expressed in stems and seedling roots, the remaining *WDL* genes are expressed at similar levels in different tissues. Overexpressing lines for most of the *WDL* genes show phenotypes similar to *WVD2*-overexpressing lines. T-DNA insertional knockout mutants in *WDL4* and *WDL5* exhibit stronger rightward skewing on tilted agar plates containing propyzamide or oryzalin (two microtubule-destabilizing compounds) relative to the wild-type *Columbia* (*Col*) seedlings. T-DNA insertional mutants in *WDL6* or *WDL7* show altered root growth sensitivity to these compounds but maintain wild-type root skewing. These results suggest that the *WDL* genes do not show complete redundancy in regulating root growth and development, and at least some of the *WDL* genes may encode proteins that regulate microtubule organization and/or dynamics. We are examining the global expression patterns and cellular localization of the WDL proteins using GFP and GUS as reporters.

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382 The Dof Transcription Factor, OBP3, Modulates Phytochrome and Cryptochrome Signaling by Altering Hormone Signaling in Arabidopsis

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Plants perceive subtle changes in light quality and quantity through a set of photoreceptors, including phytochromes (phy) and cryptochromes (cry). Upon light perception, these photoreceptors initiate signal transduction pathways leading to photomorphogenic changes in development. Many of these changes in development occur as a result of alterations in phytohormone levels or signaling. Using activation-tagging mutagenesis to identify novel light-signaling components, we have isolated a gain-of-function mutant, *sob1-D*, which suppresses the long-hypocotyl phenotype of the *phyB* mis-sense allele, *phyB-4*. The *sob1-D* mutant phenotype is caused by the over-expression of a Dof (DNA-binding with one finger) transcription factor, *OBP3*. A translational fusion between OBP3 and GFP is nuclear localized in onion cells. Tissue-specific accumulation of an OBP3::OBP3-GUS translational fusion is regulated by light in Arabidopsis. Hypocotyls of transgenic lines with reduced *OBP3* expression are less responsive to multiple intensities of white and red light. This aberrant phenotype in red light requires functional phyB, suggesting that OBP3 is a positive regulator of phyB-mediated inhibition of hypocotyl elongation. Furthermore, these partial-loss-of-function lines have larger cotyledons due at least in part to an increase in cell expansion. This light-dependent cotyledon phenotype is most dramatic in blue light, and requires functional cry1, indicating that OBP3 is a negative regulator of cry1-mediated cotyledon expansion. These results suggest a model where OBP3 is a component in both phyB and cry1 signaling pathways, acting as a positive and negative regulator, respectively. In addition to these phenotypes in the aerial portion of the plant, *OBP3-RNAi* roots are longer and have more lateral roots per root length when compared to the wild type. These cell expansion phenotypes in the hypocotyl, cotyledons, and roots, along with the lateral root initiation phenotype, suggest that OBP3 is a negative regulator of auxin signaling. Together these data suggest a more complex model where OBP3 is a general inhibitor of tissue expansion through an alteration in auxin signaling, with phyB and cry1 differentially modulating OBP3's role in this response.

383 A hormonally-specified growth checkpoint regulates axillary bud growth in Arabidopsis thaliana

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Plant adult body plan is largely determined as a consequence of the fate and function of shoot axillary meristems which are initiated on the axils of leaves. These axillary meristems can either remain dormant indefinitely or can actively grow outwards to become a vegetative shoot branch. In order to understand the mechanisms behind this transition from bud dormancy to activity we have used a global transcriptomics approach, using Affymetrix *Arabidopsis* gene chips, to identify transcripts that are differentially expressed in three experiments in which the transition to activity was imposed through different conditions. Analysis of these data revealed that genes are both up-regulated and down-regulated at the exit from dormancy and transition to activity. The genes that were down-regulated were down-regulated regardless of how dormancy was imposed however up-regulation of genes was controlled in a condition specific manner. The expression of these genes in the buds of bushy *Arabidopsis* mutants deficient in auxin signaling (*axr1-12*), or the synthesis or signalling of a novel branch-suppressing hormone (*max1-max4*), revealed that the transcriptome of their constitutively active buds resembles that of dormant wildtype buds. We therefore propose that bud activity is controlled at a growth checkpoint imposed relatively late in bud development, requiring both auxin and the MAX-dependent signal. We propose that the default for bud development is activity and that this is promoted by cytokinin and consistent with this we identified a member of the family of ARR response regulator proteins as being expressed at very high levels in constitutively active *max* and *axr1-12* buds. Double mutants created between the *max's* and a line containing a T-DNA insertion in this ARR (obtained from J. To and J. Kieber) display a reduction in branching. Other genes that have similiar expression patterns to this ARR have also being identified and will be tested to see if they too behave as candidate regulators.

384 Light-stimulated leaf growth in *Arabidopsis thaliana*

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Leaves of *Arabidopsis*, like other broad-leaved species, depend on light for expansion. Possible switches for the cascade of events leading to cellular expansion include the phototropins, phytochromes and cryptochromes. Light-induced leaf expansion is being studied in the photoreceptor mutants *phot1*, *phot2*, *phot1phot2*, *phya*, *phyb*, *cry1*, and *cry2* . Growth curves constructed for each intact rosette leaf of the photoreceptor mutants grown under white light show that growth is reduced in *phot1phot2* . We have developed a cell-based assay for light-stimulated leaf cell expansion in *Arabidopsis*. Leaf pieces, excised from normal light-grown plants, grow more rapidly in light than in dark when floated on a minimal medium of 10 mM KCl. This light-stimulated growth is accomplished by cell expansion without cell division. Further, the light-stimulated growth occurs even when photosynthesis is blocked by DCMU, showing that there are specific photomorphogenic signals that control the cell expansion process in leaves. Wavelength dependence of the growth response as determined using the leaf piece growth assay shows, for example, that *phot1* leaf pieces expand as well as wildtype in blue light, whereas tissue from the double mutant *phot1phot2* does not. The assay is also useful for determining polarity of the growth response. Length:width ratio of wildtype and mutant leaf pieces after incubation in red, blue or white light suggests that the photoreceptors not only stimulate cell expansion, but control the direction in which the excised piece expands.

385 Requirement of homeobox gene STIMPY for meristem growth and maintenance

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Most organs of flowering plants develop postembryonically from groups of pluripotent cells called meristems. The shoot apical meristem (SAM) is specified during embryogenesis and is the source of aerial organs. The SAM increases in size via cell division upon emergence, and reaches its mature state prior to floral transition. In Arabidopsis, the SAM is specified by two complementary pathways. *SHOOT MERISTEMLESS (STM)* defines the entire SAM region. *WUSCHEL (WUS)*, on the other hand, functions in a more restricted set of cells to promote stem cell fate, and is regulated by the *CLAVATA* genes in a negative feedback loop. However, little is known about how the growth of the SAM during vegetative development is regulated. Under ground, the root meristems are the origin of the root system. The primary root meristem forms during embryonic development, and most cell divisions in the root occur in the meristematic zone above the quiescent center. Till recently, there is little evidence that the shoot and root meristems share common regulatory mechanisms. We have characterized *STIMPY* (*STIP*; also called *WOX9*), a homeobox gene required for the growth of the vegetative SAM, and the maintenance of root growth. Loss of STIP function results in early seedling arrest due to the loss of cell division and premature differentiation of both the shoot apex and the primary root meristematic zone. Genetically, *STIP* interact with the *CLV/WUS* feedback loop in the SAM. In addition, *STIP* is required for lateral root initiation and the growth of other aerial organs. What sets *STIP* apart from known meristem mutants is that *stip* mutants can be fully rescued by stimulating the entry into the cell cycle using sucrose. Therefore *STIP* is likely to function by maintaining cell division and preventing premature differentiation in proliferating tissues. Taken together, *STIP* identifies a new genetic pathway integrating developmental signals with cell cycle control. We are currently investigate the mechanisms involved in *STIP* actions.

386 DNA-binding properties of a MYB protein needed for stomatal patterning

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Stomata are essential for plant productivity but the molecular function of genes involved in stomata development are only beginning to be determined. Mutations in the Arabidopsis FOUR LIPS locus produce clusters of stomata in direct contact. FLP is a negative regulator of division late in the stomatal cell lineage. Clusters of stomata in contact result when daughter cells produced by the symmetric division of a precursor cell termed a guard mother cell continue to divide like the GMC instead of terminally differentiating into stomata. FLP encodes an R2R3 MYB protein that contains two MYB repeats which in many other MYB proteins is responsible for DNA-binding. However, compared to typical R2R3-MYB proteins in Arabidopsis, FLP has some differences on the conserved regions, especially on MYB domain. Therefore, we investigated whether FLP binds to DNA. To identify putative FLP DNA-binding sequences, site-selection experiments coupled with electromobility shift assays (EMSA) were carried out. By screening a pool of double-stranded oligonucleotides using the recombinant FLP MYB domain, 26 different DNA sequences have been identified and further confirmed by EMSA. Sequence alignment using MotifSampler and mutation analyses of putative binding sites indicated that a core sequence is present in 23 out of 26 sequences, and this DNA sequence is recognized by the R2R3 MYB-domain of FLP. If confirmed, these results would show that FLP is a DNA-binding protein with a DNA-binding consensus distinct from all other plant or animal MYB-domain proteins that have been studied to date.

387 Characterization of *transport inhibitor response (tir2)* mutant in *Arabidopsis*

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Auxin is known to play an important role in plant development. However, many aspects of auxin biology are poorly understood. To identify genes that are required for auxin synthesis, transport, and signaling, we have previously screened for *Arabidopsis* mutants that are resistant to auxin transport inhibitors (Ruegger et al., 1997). The treatment of seedlings with an auxin transport inhibitor such as NPA results in auxin accumulation in the root tip and the inhibition of root elongation. Mutants that are resistant to NPA may have defects in synthesis, transport, or response. Roots of the *tir2* mutants display resistance to auxin transport inhibitors. In addition, *tir2* mutants have fewer lateral roots, very short root hairs, and a weak gravitropic response. To learn more about the function of the *TIR2* gene, we examined the lateral root number of *tir2* seedlings after treatment with auxin. If the *TIR2* gene is required for auxin signaling, *tir2* may be deficient in auxin-induction of lateral roots. However, if *TIR2* functions in auxin synthesis or transport, the mutant may produce a normal number of lateral roots in response to auxin. We found that *tir2* mutants formed fewer lateral roots than wild type seedlings even after auxin treatment. These data suggest that *TIR2* functions in auxin signaling. Furthermore, to understand the function of *TIR2* in more detail, we crossed *tir2* with the *tir7* , which is deficient in anthranilate synthase and has lower auxin levels in the root. The roots of both single mutants do not show any obvious defects in root meristem development. However, *tir2 tir7* double mutants displayed sever defects in root meristem organization. Some severely affected seedlings completely lacked quiescent center (QC) and columella cells. Furthermore, we were unable to detect expression of the auxin reporter DR5::GFP in these severely affected roots. These data suggest that the *TIR2* gene is required for auxin signaling during lateral root and root meristem development. Further analysis, including the cloning of the *TIR2* gene will reveal the functions of *TIR2* in root development.

Reference: Ruegger M et al., Plant Cell. 1997 ;9(5):745-57.

388 *MYR1* and *MYR2* are redundant genes that regulate flowering time, petiole elongation, and lateral shoot outgrowth in Arabidopsis

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Vascular tissues provide both the mechanical support to the plant body and the conducting cells for the transport of water, mineral solutes, hormones and other signaling molecules, amino acids and sugars. To identify genes that may regulate vascular tissue-specific functions, we performed a genome-wide comparative analysis of phloem-cambium and xylem transcripts. In this report we focus on *MYR1* and *MYR2*, two G2-like transcription factors with phloem-specific expression. *MYR1* and *MYR2* are expressed as splice variants affecting the predicted coiled-coil region and/or the MYB-related domain, yielding three and six isoforms for *MYR1* and *MYR2*, respectively. Using a GAL4-based yeast assay, we demonstrated that both N- and C-terminal regions of isoform MYR1-3 have transactivation activity. T-DNA insertion lines for *MYR1* and *MYR2* were identified and both single (*myr1* and *myr2*) and double (*myr1myr2*) mutants were analyzed. Under continuous light, *myr1* plants did not appear to differ from wild type (wt) plants. However, *myr2* plants flowered early, with an average of 16.6 rosette leaves compared to 18.9 rosette leaves for wt plants. This early flowering phenotype was slightly more pronounced in the *myr1myr2* plants where 15.3 rosette leaves were present at the time of flowering. *myr1myr2* plants exhibited additional differences in development compared to wt plants including elongated petioles, semi-erect leaf orientation and suppression of outgrowth of both secondary inflorescences and higher order branches on the primary inflorescence (i.e., increased apical dominance). Specifically, on eight-week-old plants, the average length of second-order branches on the primary inflorescence of *myr1myr2* plants was 14.9 cm compared to 23.7 cm for wt. Average second-order branch length for *myr1* and *myr2* plants was similar to that of wt plants at 23.6 cm and 26.0 cm, respectively. Third-order branch outgrowth was completely suppressed in *myr1myr2* plants, while wt, *myr1* and *myr2* plants produced an average of four third-order branches per plant ranging in length from 5.7 -7.9 cm. Outgrowth of one or two secondary inflorescences (average length = 14 cm) was observed for wt, *myr1* and *myr2* plants, while only one in three *myr1myr2* plants produced elongated secondary inflorescences with an average length of only 1 cm. These characteristics are reminiscent of shade avoidance responses, suggesting that MYR1 and MYR2 may be associated the photocontrol of Arabidopsis growth.

389 Conservation and divergence of flowering time gene expression in wild and domesticated sunflower

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Wild populations and domesticated cultivars of sunflower (*Helianthus*) vary greatly in the sensitivity of flowering time to daylength. Therefore, this genus is an excellent system for investigating how the genetic pathways controlling flowering time in response to photoperiod evolve. We have isolated homologs of several genes in this pathway and characterized their circadian expression patterns under different photoperiod conditions. Gene expression is conserved among three wild species, which include two facultative and one obligate short-day species, suggesting changes downstream or in parallel to this pathway have resulted in phenotypic differences. In contrast, domesticated cultivars have patterns of gene expression more consistent with their phenotypes. For example, day neutral cultivars show no circadian pattern in expression of the floral integrator gene FT while short day plants express FT in patterns similar to the short-day plant rice under short days but no expression under long days. Interestingly, expression of GI, a gene in the circadian clock, is conserved among cultivars, indicating changes downstream or in parallel to GI underlie the differences in FT expression.

390 TILLinG reveals selection at certain missense changes

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TILLinG (Targeting Induced Local Lesions in Genomes) is a general reverse genetic strategy that provides an allelic series of mutations in genes of interest. High-throughput TILLinG allows rapid, low-cost discovery of induced point mutations from a population of chemically mutagenized individuals and is especially well suited to plants where the reference population can be stored as seed. We have developed TILLinG as a service to the Arabidopsis community, as the Arabidopsis TILLinG Project (ATP, http://tilling.fhcrc.org:9366). In its first three years of operation ATP has detected, sequenced and delivered more than 5000 mutations in 400 genes requested by researchers. These data confirms 1) that EMS reliably induces G:C to A:T transitions in Arabidopsis 2) that the mutations available in our reference population are efficiently detected by high-throughput TILLinG, 3) that the full spectrum of point mutations including the more severe deleterious missense and knockout lesions are recovered and 4) that TILLinG permits the recovery of less severe mutations in essential genes. In published analysis of these induced mutations we have shown that the expected frequency of heterozygous silent, missense and truncation mutations are observed in these genes, but that there is selection against recovery of some homozygous, nonsense and splice junction changes (Genetics 164:731-740). In further analysis we show that there is selection against recovery of homozygous, missense changes at highly conserved residues in these gene products. Surprisingly, we also detect strong selection against recovery of homozygous arginine (codon:AGr) to lysine (codon:AAr) mutations, both basic amino acids and 7% of all missense changes recovered, while selection is not seen in homozygous glutamate (codon:GAr) to lysine (codon:AAr) mutations, a change from an acidic to a basic residue and 10% of all missense changes, nor in other classes of missense changes. Consistent with the hypothesis that AGr codons may be used to modulate the rate of translation, significantly stronger selection against the recovery of homozygous AGr to AAr mutations is measured when the analysis is limited to the codons at the start of the transcript.

391 Differential gene expression in the homoploid hybrid species *Helianthus deserticola* and its progenitors *H. annuus* and *H.petiolaris*

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A large number of studies dealing with gene expression in hybrid species have been conducted in recent years, usually in the context of allopolyploid taxa. Some of the most important factors in polyploid evolution are absent for homoploid hybrid species, although many factors that impact gene expression in allopolyploids are likely to play an important role in homoploid hybrid species as well. The annual sunflowers (genus *Helianthus*) represent an excellent study system for homoploid hybrid speciation. Three distinct homoploid hybrid species have resulted from hybridization between *Helianthus annuus and H. petiolaris*. The hybrid species (*Helianthus anomalus, H. deserticola, and H. paradoxus*) are ecologically and karyotypically divergent compared to each other and compared to the two parental species. Here, we report the first use of microarrays to analyze patterns of gene expression in a homoploid hybrid species and its progenitors. We describe the development and use of an EST-based microarray for *H. annuus* and closely related annual sunflowers. The array was used in a series of cross-species hybridizations to compare gene expression in *H. annuus, H. petiolaris, and H. deserticola*. Our goals were to examine patterns of gene expression in the homoploid hybrid species *H. deserticola* compared to the parental species *H. annuus and H. petiolaris*. Specifically we asked which genes showed transgressive levels of expression in the hybrid species compared to these two parental species.

392 Evidence of genetic conservation of diverse nectaries within the eudicots

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Nectaries are secretory organs widely present in flowering plants that function to lure floral pollinators. Due to diversity in nectary positions and structures, they are thought to have originated multiple times during angiosperm evolution, with their potential contribution to the diversification of flowering plants and pollinating animals being considerable. Using CRABS CLAW (CRC), a gene required for nectaries in Arabidopsis, the genetic bases of diverse nectary forms in eudicot angiosperm species were investigated. CRC expression is conserved in morphologically different nectaries from several core eudicot species and CRC is required for nectary development in both rosids and asterids, major phylogenetic branches of eudicots. However, in a basal eudicot species, no evidence of CRC expression in nectaries was found. Combining a mapping of nectary positions onto an angiosperm phylogeny and CRC expression analyses in eudicots, we propose that diverse nectaries in core eudicots share conserved CRC gene regulation, and that derived nectary positions in eudicots have altered regulation of CRC. Since the ancestral function of CRC lies in the regulation of carpel development, it was likely co-opted as a regulator of nectary development in basal eudicots, which may have led to the association of nectaries with reproductive organs in derived lineages.

393 Molecular Evolution of LEAFY transcription factor in land plants

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Understanding how molecular mechanisms can be linked to evolution of organism form is the founding theme of "evo-devo" studies. Transcription factors, which regulate and coordinate the expression of thousands of genes during development, represent candidate of choice to address the question of how molecular evolution of a developmental regulator can result in changes in the repertoire of gene it controls and ultimately translate into morphological evolution.

The plant-specific transcription factor LEAFY controls general aspects of the life cycle in a basal plant, the moss *Physcomitrella patens*. In contrast, LEAFY has more specialized functions in angiosperms, where it specifically induces floral fate during the reproductive phase. This raises the question of a concomitant change in the biochemical function of LEAFY during the evolution of land plants. We have identified that the DNA binding domain of LEAFY, although largely conserved, has diverged in activity. On the contrary, other, more rapidly evolving portions of the protein have few effects on LEAFY activity[1]. In a broader perspective of functional evolution of LFY among land plants, two testable hypotheses can be proposed. LFY might control similar networks of genes in non-flowering and flowering plants, with co-evolution of target sequences and LFY DNA binding specificity. Alternatively, there may have been a complete change of LFY function between basal taxa and flowering plants, in which an initial, albeit gradual change in biochemical activity was the prerequisite for recruitment and/or intercalation of new targets, such as AP1.

Our results establish a framework for the study of the functional/morphological evolution of plant form and flower invention and tentative unifying scenario will be discussed.

[1]: Maizel, A. et al. *Science* 308, 260-3 (2005)

394 Accumulation of male benefit genes and recombination arrest in Silene (white campion): on sexual dimorphism and Y chromosome evolution

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In mammals, X-Y chromosomes are very ancient (300Mya). Their study allowed to establish the "evolutionary strata" model (1,2), which elegantly accounts for the mechanism that has led to recombination arrest and X-Y differentiation. In brief, this model suggests that the series of chromosomal rearrangements which took place on the Y chromosome have acted as a basic and reiterated mechanism for recombination arrest along the X-Y chromosome pair. However, the model is most likely unable to explain the primary events of this evolutionary process. Certain dioecious plant species have evolved X-Y chromosome systems (white campion, papaya, sorrel, hops). They are evolutionarily much more recent than those of mammals (for ex., 30 Mya in white campion). We can therefore explore in such plants most, if not all, of the early events that have shaped a pair of autosomes into genuine X-Y chromosomes. We have shown (3) that a gradual process of recombination arrest is at work in white campion along large regions of the Y. We and others have generated an increasing number of X and Y linked genes and series of Y deletion mutants with informative sexual phenotypes: hermaphrodite, asexual, stamen developmental arrest, male sterile. For example, a few of the Y deletion mutants with defined male sterile phenotypes exhibit altered X-Y pairing at meiosis. Here we report on the use of a combination of deletion mapping with AFLP and gene specific markers, histological phenotyping of anther defects and X-Y pairing patterns during meiosis. The results allowed us to dissect the structural and functional organization of the Y chromosome and draw hypotheses on the initial recombination arrest event(s) and the way male benefit genes have accumulated on the Y.

1. Lahn BT, Page DC. 1999. Science 286: 964-7.
2. Skaletsky et al. 2003 Nature 423: 825-37
3. Nicolas et al, 2005 Public Library of Science Biology 3 : 47-56

395 Darwinian selection on a selfing locus in Arabidopsis thaliana

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The evolutionary transition from outcrossing to selfing is one of the most prevalent trends in flowering plants. In 1876, Charles Darwin proposed the reproductive assurance model to explain the prevalence of self-pollination in plants, suggesting that selfing can be evolutionarily advantageous when pollinators or mates are scarce in spite of inbreeding depression. To prevent selfing, *Arabidopsis lyrata* has a self-incompatibility system controlled by the female receptor gene *SRK* and the male ligand gene *SCR/SP11* in *S*-locus. *A. thaliana*, however, has pseudogenes of *SRK* and *SCR*, and the loss of functional alleles was responsible for the emergence of selfing.

We conducted evolutionary genomic analyses of *S*-locus to assess the evolutionary force and to estimate the timing of the transition. The *pseudoSCR1* gene in 21 *A. thaliana* accessions has low levels of nucleotide diversity compared with neighboring genes. This low value indicates that positive directional selection has driven the evolutionary fixation of the pseudogene allele of *SCR*. In contrast, *pseudoSRK* gene has high gene diversity with 3 divergent haplogroups. In addition to the major Col-type allele, haplogroup B (with frequency 0.003) and haplogroup C (with frequency 0.03) was found in worldwide collection, indicating that *SRK* was not responsible for the evolution of selfing.

Coalescent simulation of *pseudoSCR1* showed that this selection event most probably occurred very recently. The time frame is consistent with the expansion of the species range ~17,000 years ago owing to the last glacial retreats. This supports Darwin's reproductive assurance model, since rapid expansion would be accompanied by scarcities of mates and pollinators and thus selfing plants would have a selective advantage during long-distance dispersals.

Moreover, the evolution of selfing must have been followed by the rapid evolution in floral morphological traits to facilitate selfing, including small flower size and the pistil-stamen length suitable for autopollination. The hybrids between *A. thaliana* and *A. lyrata* had intermediate sizes of the organs, suggesting that those quantitative traits are not governed by simple recessive loci. Since those traits would have been deleterious if plants remain self-incompatible, they must have evolved after becoming self-compatible. These findings support the contention that adaptations such as those associated with key mating system innovations can occur rapidly.

396 Evolutionary diversification of the *LFY – TFL1* interaction in the rosette flowering species *Leavenworthia crassa*

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Inflorescence architecture in *Arabidopsis thaliana (Ath)* is conferred by the mutually antagonistic interaction of LFY and TFL1 function. LFY promotes floral identity in auxiliary meristems while TFL1 helps maintain indeterminacy in the inflorescence meristem (IM) and delays flowering. *Leavenworthia crassa (Lcr)*, a close relative of *A. thaliana*, does not produce an inflorescence, rather it generates solitary terminal flowers from auxiliary meristems throughout its adult phase while maintaining shoot identity in the apical meristem. *LcrLFY* expression in *A. thaliana lfy-* plants was able to rescue flowers indicating some conservation of LFY protein function between *L. crassa* and *A. thaliana*, but these transgenic plants also produce extra petals and terminal flowers. The latter phenotype suggests *LcrLFY* is not repressed by *AthTFL1* in the IM. Fusion of the *LcrLFY* 5' promoter to the GUS gene confirmed the *LcrLFY* promoter drives expression in the *A. thaliana* IM. To further examine the evolution of the *L. crassa* LFY locus, the *LcrLFY* 5' promoter was fused to the *AthLFY* coding sequence. This construct produced plants with terminal flowers and extra petals and was dominant to *AthLFY* indicating missexpression of the *AthLFY* protein via the *LcrLFY* promoter is sufficient to cause both phenotypes. To identify regions of the *LcrLFY* promoter necessary for expression in the *A. thaliana* IM, chimeric *LcrLFY AthLFY* promoters were constructed. The results revealed redundant *L. crassa* promoter fragments capable of driving expression in the IM. In addition, the effect of LcrLFY on *AthTFL1* expression levels in *A. thaliana* was measured by quantitative RT-PCR. *LcrLFY* transgenic plants showed a significant increase in *AthTFL1* mRNA levels beyond the control wild type plants suggesting LcrLFY may be able to activate *AthTFL1* transcription in the *A. thaliana* genomic background.

397 Conservation of YABBY protein functions between Arabidopsis and rice

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Members of the *YABBY* gene family generally participate in the promotion of abaxial cell fate in lateral organs in Arabidopsis. Some *YABBY* genes have additional functions such as the control of nectary and carpel development by *CRABS CLAW* (*CRC*). We previously revealed the functions of the *YABBY* gene, *DROOPING LEAF* (*DL*), in rice (*Oryza sativa*), which belongs to the grass species (Yamaguchi et al., 2004, The Plant Cell). Loss-of-functions of *DL* show homeotic conversion of carpels into stamens in flowers and midribless phenotype in leaves. *DL* is closely related to *CRC* of Arabidopsis and *DL* is specifically expressed in carpel primordia and in central region of leaf primordia. Ectopic expression of *DL* results in ectopic midrib formation in rice. Therefore, functions in carpel development are partially conserved between *DL* and *CRC*, however, *DL* and *CRC* appear to have distinct functions in midrib and nectary formation, respectively. Moreover, neither *dl* phenotype nor *DL* expression suggests the involvement of *DL* in abaxial promotion. Therefore, it is suggested that functions of *YABBY* genes are diversified among angiosperms.

To reveal if *YABBY* genes share conserved functions among angiosperms, we studied the conservation of molecular functions of YABBY proteins between rice and Arabidopsis. First, we tested if *DL* can rescue the phenotypic defects in *crc* mutant. As a result, *DL* expression from the *CRC* promoter almost rescued the defects in carpel and nectary development in *crc*. Next, we introduced *DL* under constitutive *35S* promoter into wild-type Arabidopsis. These transgenic plants showed needle-like or distorted leaves with abnormal adaxial-abaxial polarity as in *35S:FIL* or *35S:CRC* lines. In addition, these transgenic plants showed ectopic carpel development as in *35S:CRC*. It is noteworthy that ectopic *DL* expression leads to abnormal adaxial-abaxial polarity in Arabidopsis although *DL* is not involved in abaxial specification in rice. These results indicate that the protein functions of DL and CRC are conserved even though they seem to function differently in each original species. It is supposed that DL and CRC play similar roles at the molecular level in each plant, but the genetic networks involving *DL/CRC* have been modified during the evolution of angiosperms. Such modifications may have led to the diversification of plant morphology. Further molecular and genetic studies about *YABBY* gene functions in rice and Arabidopsis would reveal the fundamental function of *YABBY* genes in plant development and their roles in the evolution of plant architecture.

398 Two-locus F1 hybrid incompatibility of two wild *Arabidopsis thaliana* ecotypes

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Postzygotic hybrid incompatibility is a commonly observed type of reproductive isolation, which is a defining step in both animal and plant speciation. How hybrid incompatibility can arise in the first place presents a theoretical difficulty: by definition, mutations leading to infertile or unfit progeny are mal-adaptive. A potential solution to this problem is described by the Dobzhansky-Muller model, which posits that two (or more) genes evolve independently in individuals or populations that do not interbreed. Although not detrimental in their native genomic context, when combined through hybridization, the differently evolved gene pairs interact to cause lethality or sterility. We have identified a temperature sensitive incompatibility between two wild *Arabidopsis* ecotypes, Uk-1 and Uk-3, whose F1 and F2 segregation conforms to the expectations for a two-locus Dobzhansky-Muller type mechanism. The genetic incompatibility is dominant, but specific; crosses of either ecotype with a panel of 20 other ecotypes produce normal progeny. Uk-1/Uk-3 hybrid plants are normal at 23°C, but at 16°C (which is more representative of where Uk-1 and Uk-3 were collected), F1 plants show severe progressive growth arrest and only rarely produce flowers, which are infertile. Widespread cell death is observed in F1 leaves and correlates with severity of the phenotype. An additional seedling lethal phenotype is observed in the F2 and is associated with early-onset cell death in the meristem. We performed micro-array analysis on F1 and parent plants shifted from 23°C to 16°C to investigate the physiology underlying the F1 defects. The suite of genes differentially expressed in F1 progeny (but not in parents) at 16°C includes many genes with known or predicted roles in pathogen response. This suggests that an aberrant interaction of Uk-1 and Uk-3-derived genes involved in disease resistance signaling triggers a constitutive disease response, culminating in extensive cell death and growth defects. Preliminary mapping and genotype/phenotype associations suggest that the incompatibility is caused by interaction of one region on chromosome 3 from Uk-1, and one region on chromosome 5 from Uk-3. We are currently fine mapping the causative genes. Cloning of these genes will represent the first molecular identification of a gene pair involved in Dobzhansky-Muller type genetic isolation in plants, and provides a mechanistic model of how reproductive isolation can arise within a plant species.

399 Maternal Phenotypic Effects on Season of Germination Can Explain Life History Variation in Arabidopsis thaliana

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In natural populations of Arabidopsis thaliana season of germination determines life history. Spring annuals overwinter as seeds, and germinate and flower in spring. Winter annuals germinate in fall, overwinter as rosettes, and flower in spring. In many plants, germination depends on the maternal phenotype during seed production. A. thaliana produces seeds on branches from the main stem and branches from basal nodes. This study examines the effects of maternal branch type on germination in artificial seasonal environments. Seeds from main and basal branches of 41 accessions of A. thaliana were weighed and divided between a spring germination environment and a fall germination environment. Maternal branch type had significant effects on percent germination and seed weight, with the direction dependent on accession. There was a positive genetic correlation between seed weight and percent germination for seeds in the spring germination environment, and a negative one for seeds in the fall germination environment. These results suggest that production of seeds on different branch types can explain the presence of both spring and winter annuals in natural populations of A. thaliana.

400 Evolution under strong balancing selection : polymorphism at the self-incompatibility locus SRK in the genus Arabidopsis

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Self-incompatibility (SI) is the inability of a fertile hermaphroditic plant to produce viable offspring when self-pollinated. Theory predicts that balancing selection acting on the locus responsible for SI in plants (S-locus) should dramatically affect the evolution of genetic diversity at and around this locus. We analyzed S-locus sequence diversity in the genus Arabidopsis to derive empirical tests for three theoretical predictions.

1) At the molecular level, we tested an approach aimed at pinpointing nucleotide sites targeted by selection. Our approach is based on the observation that allelic lineages at the S-locus persist over extended time periods, eventually outdating the divergence time between species. Closely related species within the genus Arabidopsis may thus be expected to share a number of allelic lineages. Assuming that closely related pairs of alleles have trans-specifically retained identical specificity, the changed nucleotide positions are expected to reveal nucleotide sites that are not involved in specificity determination. In practice, we sequenced the pistil-expressed gene SRK in Arabidopsis halleri and investigated nucleotide polymorphism in the genus Arabidopsis by comparison with the published sequences in A. thaliana and A. lyrata.

2) At the population level, we tested whether the geographical distribution of alleles at the S-locus is more homogeneous than that of alleles at a set of unlinked microsatellite markers used as a putatively neutral reference.

3) At the level of the genus Arabidopsis, we tested whether the breakdown of self-incompatibility in the selfer A. thaliana relaxed selective constraint on the evolution of SRK sequences. We tested whether lineages leading to the A. thaliana alleles show accelerated evolution as compared to their functional counterparts in A. lyrata and A. halleri.

401 Genome inventory of sequence polymorphisms for *Arabidopsis thaliana*

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Variation in DNA sequence underlies the evolutionary process, and establishing the relationship between sequence polymorphisms and phenotypic effects is a fundamental challenge in modern biology. A critical first step towards this goal is the identification of genetic diversity in species and populations. Among multicellular organisms, a comprehensive inventory of sequence polymorphism currently exists only for humans. In *Arabidopsis thaliana*, analysis of sequence diversity has typically been limited to a small number of carefully selected loci that together comprise only a small fraction of the genome, and a comprehensive description of sequence polymorphism from a core set of ecotypes across the entire genome has been lacking (although see presentation by Magnus Nordborg at this meeting). We are using a high-density oligonucleotide array approach to generate array-based sequence for 20 *A. thaliana* ecotypes that were chosen to maximize both sequence diversity and utility for the *A. thaliana* research community. This approach allows ~ 50% of bases in the *A. thaliana* reference genome sequence (Columbia accession) to be called for each ecotype. The resulting dataset, which will consist of about 55-60 Mb of euchromatic sequence per ecotype, will allow the identification of much of the common single nucleotide polymorphism present in *A. thaliana*. In addition, analysis of pilot data suggests that deletion polymorphisms of greater than several hundred bp in size in *A. thaliana* can also be recovered using the array-based approach. We will describe the ecotypes for sequencing that have been chosen in consultation with Magnus Nordborg and co-workers. We will summarize initial sequence data for the 20 ecotypes for about 400 kb of test array data that has been selected in collaboration with Joe Ecker and co-workers. We will also present a preliminary analysis of the complete array-based sequence for two ecotypes. The complete sequence dataset for all 20 ecotypes will be generated by Fall of 2005, and we will discuss plans for sequence analysis as well as for the release of this large genomic sequence dataset to the *A. thaliana* research community.

402 The ecological genetics of germination timing in Arabidopsis thaliana

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Germination timing is a critical life history trait in Arabidopsis thaliana. Here we summarize several field and laboratory studies on the ecology and genetic basis of germination timing in A. thaliana. Field studies indicate that the timing of seed germination is geographically variable, depends on other life-history traits such as season of seed dispersal, and can explain up to 74% of the variance in fitness among genotypes in some conditions. Thus germination timing can impose a strong selective sieve that determines which genotypes can establish in a given location. Germination timing also influences the phenotypic expression of other life-history traits such as the timing and size at reproduction, and it can alter the strength and mode of natural selection on these traits. In this manner, germination timing can alter the evolution of post-germination life history traits. Germination timing is evolutionarily labile, with abundant genetic variation and weak pleiotropic constraints on evolving flexible germination responses to different environmental conditions. The evolutionary lability of germination timing may contribute to the ability of A. thaliana to expand its range across a wide geography. Recent work in A. thaliana investigating the genetic basis of germination responses to multiple seasonal environmental factors has identified a potential role of all five phytochromes in mediating germination responses to different environmental conditions. We found abundant functional redundancy but also functional specialization of different phytochromes. The functional specialization of phytochromes during germination may enable A. thaliana to distinguish different environmental conditions, which would be necessary for accurate germination cuing. The environment-dependent functional redundancy of phytochromes during germination may ensure that germination occurs when conditions are optimal for germination.

403 Variable patterns of transferred mitochondrial DNA in Arabidopsis thaliana ecotypes and related species

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The transfer of mitochondrial DNA (mtDNA) to specific sites on chromosomes one and two has previously been described in *Arabidopsis thaliana* ecotype Col-0. In this study, over 90 ecotypes of *Arabidopsis thaliana* and related species were screened using PCR to assess the distribution of these transfer events. Evidence for transfer of mtDNA to chromosome one was rare among ecotypes and related species. Data from markers for mtDNA transfer to chromosome two were grouped into four categories for different ecotypes. The pattern seen for Col-0 was rare among other ecotypes. In one case, differences in markers for mtDNA transfer to chromosome 2 were observed between individuals within the same ecotype. Microsatellite markers indicated a high degree of similarity between these individuals. PCR-based mapping studies of the regions flanking the large mtDNA insertion on chromosome two were performed for representative ecotypes from the four categories of mtDNA transfer and select species. Additional markers as far as 64 kb from one end of the mtDNA integration site in ecotype Col-0 were found to be absent in most of the other accessions that were screened. Results from these studies suggest large scale rearrangements of chromosome 2 DNA at and around the site of mtDNA transfer and provide new molecular markers for discrimination between ecotypes.

404 Genetic Basis of sister species divergence in Clarkia (Onagraceae)

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Clarkia (Onagraceae) is a genus of annual wildflowers found primarily in California. The genus is an important evolutionary model for plant speciation. The cross Clarkia breweri x C. concinna produces a fertile hybrid and is being used to generate a map for Clarkia using intron targeted markers. The cross has unique aspects regarding speciation, adaptation to different pollinators and morphological divergence. Two important aspects of speciation are the petal shape and aroma of the flowers. The primary difference in petal shape appears to be controlled by a single locus closely linked to invertase and linalool synthase. Linalool synthase seems to be the main locus responsible for scent. Results of primer design show that primers designed from Arabidopsis thaliana, Pisum sativum, Medicago truncatula, Lycopersicon esculentum, and others will amplify Clarkia (Onagraceae) DNA. The primers created will allow for many other relationships to be studied within the Clarkia genus. One relationship under scrutiny is between the Clarkia species C. lingulata and C. biloba. The proposed progenitor C. biloba contains eight chromosomes while C. lingulata contains one more chromosome thought to be a combination of two chromosomes of C. biloba. This study has shown that though this last chromosome is excess material it is not clear if it is made from copies of the single genes on the progenitor's chromosomes or repetitive DNA.

405 Ecotype-specific evolution of centromeric satellites in Arabidopsis thaliana

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Arabidopsis centromeres contain large array of satellite repeats of 178-bp length, which is thought to provide the centromere function. It is believed that the satellite repeats in the array are homogenized by occasional unequal crossing-over, but it is not known how they are homogenized among chromosomes. It is also not known how the copy number of the satellite repeats is controlled. Here we report evolution of diverged satellite sequence in one chromosome in one Arabidopsis ecotype. The presence of the diverged satellites in that centromere is associated with increase of satellite repeat number and deletion of significant part of the adjacent heterochromatic region. We discuss possible implication of these observations.

406 Global survey of gene expression diversity in *Arabidopsis thaliana*

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Natural phenotypic variation is often quantitative and occurs in both simple and complex physiological traits. At their core, these traits are the manifestation of genome-wide DNA sequence variation as it is filtered through variation at the gene expression, proteomic and metabolomic levels before being displayed as a heuristic phenotype. Even though polymorphic gene expression controls numerous traits, such as flowering time and plant/pest interactions, little is known about the genomic distribution of variation that underlies differential expression. We are asking a series of questions to better understand gene expression variation within Arabidopsis. Which genes are differentially expressed? How does genetic variation within the species relate to expression level polymorphisms? How does gene expression variation relate to the underlying nucleotide polymorphism level?

We have used Affymetrix ATH1 microarrays to survey the transcriptome of seven *Arabidopsis thaliana* accessions in the presence and absence of exogenously applied salicylic acid (SA). We used a factorial design with three biological replicates per treatment (+/- SA) per three time points (4, 28, 52 hr post treatment) per accession, and a total of 126 microarrays. These accessions encompass ~80% of the low-to-moderate frequency nucleotide polymorphisms in Arabidopsis. Using a split-plot ANOVA, we detected on average 2250 genes that were significantly differentially expressed between any two Arabidopsis accessions under the conditions of this experiment. Most expression differences were of low frequency within this collection of accessions and, as such, the lists of differentially expressed genes were significantly different between each accession pair. Thus, a large fraction of the Arabidopsis genome showed differential expression even under this limited set of experimental conditions. This suggests that experiments including more developmental and environmental conditions would show that the majority of the genome is differentially expressed. We are also testing if there is a correlation between sequence diversity and expression diversity. Finally, we are using the SA treatment to estimate the impact of genotype x treatment interaction on expression level polymorphisms.

407 Epistatic and Seasonal Selection at the Major Flowering Time Gene FRIGIDA

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How natural selection acts on combinations of genes underlying complex traits has long been a central question in evolutionary biology. However, only recently has it become possible to address this question directly, as the genes that contribute to natural variation in complex developmental traits have been identified. We tested whether natural variation at two interacting flowering time genes, *FRIGIDA* (*FRI*) and *Flowering Locus C* (*FLC*), is associated with fitness among European *A. thaliana* accessions. We report that in field experiments, the direction of selection on natural alleles of *FRI* reversed depending on allelic variation at *FLC* and the season of germination. In particular, functional *FRI* alleles were associated with high winter survivorship in the fall generation only when in the FLC^{A} haplogroup background, and functional *FRI* alleles were associated with low seed production in the spring generation only when in the FLC^{B} haplogroup background. Additional results indicate that these fitness differences can be explained by time to bolting in the fall but not in the spring generation. Our analyses employed controls for population stratification, and show that controlling for population structure enabled the detection of associations that would otherwise have been missed. These results show epistasis for fitness among naturally polymorphic loci underlying a complex trait, and suggest that epistasis, in combination with environmental heterogeneity, is likely to be important for the maintenance of genetic variation. These results also provide an evolutionary explanation for the over-representation of the functional *FRI* FLC^{A} genotype and the under-representation of functional *FRI* FLC^{B} genotype across Europe. In addition, these results suggest that two factors were necessary for the rapid evolution of *FRI* deletion-null alleles: climates permitting a successful spring generation and the presence of FLC^{B} alleles.

408 How to measure a cost of adaptation?

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Associating fitness costs to adaptive genes is a logical and in many cases valid assumption that is based on both theory and observations. First, genetic changes conferring adaptation to the new 'environment' may involve large modifications of the previous phenotype and may therefore induce a fitness penalty in the previous environment. Second, polymorphisms at adaptive genes seem to persist in populations due to the counter-selection of the adaptive genes in the absence of the corresponding selective pressure. The methods generally used to determine the fitness cost of adaptive genes can be sorted into two distinct groups. The first group is based on a direct comparison of a measured set of traits that are viewed as the major components of fitness, among homozygous adapted and non-adapted individuals. This direct approach may provide information about the specific trait modification responsible for the cost. The second group involves quantifying the changes in the adaptive allele frequencies in populations with reduced selection pressure over several generations, or in populations located along a transect between selected and unselected areas in an artificial cline approach.

The evolution of herbicide resistance can be viewed as an extreme case of adaptation to a strong selective pressure. We used several herbicide resistant mutant lines in the model species *Arabidopsis thaliana* to estimate fitness cost with different direct and indirect methods. Because several small modifications between resistant and susceptible individuals can turn into a significant interaction when integrated over the whole life cycle[1,2], we found that methods measuring change in resistance allele frequencies were more powerful to detect costs and probably more reliable for giving the 'real' cost value[3].

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409 Hitchhiking effects associated to selection on the self-incompatibility locus (S-Locus) in *Arabidopsis halleri*

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We investigated the hitchhiking effect on genomic regions associated to the S-locus in the self-incompatible *Arabidopsis halleri* L. (Brassicaceae). As the S-locus is apparently subject to very strong negative frequency-dependent selection, theory predicts that linked loci would present higher levels of synonymous nucleotide diversity within populations and reduced among-populations genetic differentiation with respect to unlinked loci. Because of recombination, this effect should decrease with increasing distance from the selected locus and should not be recognisable at distances above few hundreds of Kb. In the present study, we assessed levels of sequence polymorphism (π, θ_w), among-populations genetic differentiation (F_{ST}), and deviations from neutral allele frequencies distribution (Tajima's D) at synonymous sites from three genes located around the S-Locus. Thirty individuals from 6 European populations were genotyped for two genes located downstream of the S-Locus, namely At4g21390 (following *A. thaliana*'s genome annotation) and At4g21430, and for one located upstream, namely At4g21350, at distances from the S-Locus respectively of about 7.3, 20.7 and 26.6 Kb, based on *A. thaliana*'s physical map. The results will be compared to a published survey of sequence polymorphism within and among populations at eight genes unlinked to the S-locus in *A. halleri*, and to an analysis of polymorphism at the gene At4g21350 in the self-compatible *A. thaliana* at the species level.

410 Independent ancient polyploidy events in the sister families Brassicaceae and Capparaceae

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Recent studies have elucidated the ancient polyploid history of the *Arabidopsis thaliana* (Brassicaceae) genome. The studies concur that there was at least one polyploidy event, identifiable by numerous duplicated gene pairs in syntenic blocks, which occurred some 20 to 60 mya and near the divergence of the Brassicaceae from its sister family, the Capparaceae. Using a comparative genomics approach, we have asked the question of whether this polyploidy event was unique to members of the Brassicaceae or if it was shared with the Capparaceae. We have isolated and sequenced three genomic regions from diploid *Cleome spinosa* (Capparaceae) that are homologous to a duplicated region found on At3 and At5. The three genomic regions in *Cleome* are each unique and are mosaics of At3 and At5 specific genes, with no clear orthology/ paralogy gene pair relationships. Genes found in duplicate in *A. thaliana* also tend to be replicated in *Cleome*. Surprisingly, molecular evolutionary analyses found that the triplication in *Cleome* occurred more recently, and independently, of the duplication event in *A. thaliana*, but that the rate of gene loss is higher in *Cleome*. The occurrence of independent ancient polyploidy events in closely related lineages has profound implications for homology-based research approaches, and provides an interesting opportunity to examine duplicate gene evolution.

411 Evolution of Centromere-Binding Proteins and their Interactions with Centromere DNA in the Brassicaceae

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During cell division, proper chromosome segregation involves assembling a kinetochore protein complex at the centromere region of every chromosome. A fundamental yet unresolved question is how inner kinetochore proteins initially choose the site for kinetochore assembly and subsequently maintain its identity as a centromere throughout the cell cycle. The challenge in understanding these phenomena is that inner kinetochore proteins, namely centromere-binding proteins (CENP) A, B, and C, share high sequence similarity between plants, yeast, and animals, yet the centromere DNA to which they associate lacks a conserved sequence and is instead composed of repetitive DNA including rapidly evolving satellite DNA (Henikoff *et al.*, 2001). These observations have led to the hypothesis that CENPs evolve adaptively across species to maintain protein-DNA interactions at the centromere for chromosome inheritance. The goal of this study is to identify variable and conserved structural features within the three constitutive centromere-binding proteins that are required for establishing and maintaining a species-specific functional plant centromere *in vivo*. To accomplish this, we will exchange CENP-A, CENP-B, and CENP-C within the Brassicaceae family to define the degree of natural variation able to sustain CENP function at the centromere. Some *CENP-A, CENP-B,* and *CENP-C* genes have been described (Cooper and Henikoff, 2004; Talbert *et al.*, 2004) and we will clone the remaining set from *A. thaliana, A. arenosa, C. rubella, O. pumila,* and *S. irio*. The limit of CENP complementation will be directly tested by expressing foreign fluorescently-tagged CENPs into wild type and mutant *Arabidopsis* lines and monitoring whether chromosome inheritance is maintained. Further studies will link functionally important changes to alterations in 3-D structure.

412 Using synthetic *RPP1* gene cluster to model *R* gene evolution in *Arabidopsis*

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Recombination between paralogous genes within a gene cluster causes gene rearrangements and the formation of novel chimeric genes with potential new functions. Plants disease resistance genes (*R* genes) are frequently organized as gene clusters that encode proteins required for recognition of invading pathogens. In this study, we constructed a reconfigurable synthetic *RPP1* gene (for resistance to Paranospora parasitica) cluster (synth*RPP1*) composed of three linked *R* gene homologs. The synth*RPP1* gene cluster was transformed into *Arabidopsis* CW84, which is susceptible to a wide variety of downy mildew isolates. Transgenic plant lines that contain a single copy synth*RPP1* were selected. The initial synth*RPP1* inserts were alternatively reconfigured *in vivo* to produce two alternative *RPP1* alleles. Lines containing the reconfigured synth*RPP1* alleles will be crossed to allow meiotic homologous recombination to form chimeric *RPP1* genes. The chimeric *RPP1* genes will be identified by a gain-of-Luciferase phenotype (luc^+). Luc^+ plants will be isolated and characterized for chimeric *RPP1* gene structure and function. Studies will include determining the frequency of several distinct types of meiotic recombination, mapping of recombination resolution sites, and determining whether chimeric *RPP1* genes confer altered or novel downy mildew recognition profiles.

413 Altered Inheritance and Genetic Mapping of an *Arabidopsis thaliana* RNase Activity Mutant

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Changes in RNase activity have been associated with many developmental, metabolic, and environmental response processes in plants; however, little is known about the functions of RNases in these processes. Mutant RLD plants with extra RNase activity in the 35-38 kD region of inflorescence stem extracts were identified previously. When the mutant plants were crossed to wild-type RLD plants the mutant phenotype was inherited in a manner consistent with a single locus recessive mutation. However, when mutant plants were crossed to wild-type Columbia plants to generate mapping populations, the mutant phenotype was inherited in a dominant manner. Various explanations for the apparent change in penetrance of the mutant phenotype between the RLD and Columbia ecotypes are being tested. The mutation responsible for the extra RNase activity has been tentatively mapped to the region from 52-58 cM on chromosome three.

414 On the Role of Introns: Reverse-complement and Exact Sequence Matches of Intron n-mers to Coding Sequences of Neighbouring Genes

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We asked the question: has the genome evolved to eliminate the possibility of introns causing self-RNAi. Using bioinformatic methods, we performed an in silico analysis of this possibility by looking at 22-mer matches between introns and the coding sequences of all genes in the Arabidopsis genome. Intriguingly, there are 88 pairs of genes that are neighbours on the genome for which a 22-mer or longer of the intron from one gene matches in reverse complement to the coding sequence of the neighbouring gene, whereby these gene neighbours are not orthologs. We have termed these rPINs for reverse-complement proximal intron n-mer matches. Interestingly, there are 787 genes in which a 22-mer or longer of the intron sequence from one of these genes matches the coding sequence of the neighbouring gene or the coding sequence of the same gene exactly. We have termed these ePINs for exact proximal intron n-mer matches. These data suggest that self-RNAi has been selected against, although there might still be a role to play in the case of the 88 pairs of rPIN genes. Also, why are exact match ePINs roughly ten times more abundant than rPIN matches and what role do ePINs play? In addition, 22-mer fragments of intron sequences of genes near to the centromere match to coding sequences of genes close to the centromere across all 5 Arabidopsis chromosomes. These phenomena can be observed in Arabidopsis thaliana, C. elegans (with restrictions), and Drosophila melanogaster. We present the results of our analyses, and discuss possible implications with respect to gene regulation and gene repair.

415 Antiquity of microRNAs and their targets in land plants

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Plant microRNAs (miRNAs) function to mediate specific post-transcriptional regulation of target messenger RNAs, many of which are implicated as master regulators of development. Several miRNA-target regulatory circuits have been shown to be necessary for distinct developmental processes in *Arabidopsis*. Many *Arabidopsis* miRNAs are conserved between eudicots and monocots, and in at least one case, an *Arabidopsis* miRNA is conserved in basal plants. Development and implementation of a miRNA-specific microarray resulted in miRNA expression profiles for major *Arabidopsis* organs during wild-type development; intersection of these data with existing messenger RNA profiles revealed a significant negative correlation between miRNA and target accumulation. We also used the miRNA-specific microarray as a phylogenetic profiling tool to probe RNA samples derived from specimens representative of major clades of land plants, and found that out of the 23 *Arabidopsis* miRNA families tested, expression of 11 was detected in a gymnosperm, eight in a fern, three in a lycopod, and two in a moss. We developed an empirical strategy for detecting miRNA target messenger RNAs from unsequenced transcriptomes and directly demonstrated that the targets of several miRNAs in non-flowering plants as deeply branching as ferns and mosses are homologous to the targets in *Arabidopsis*. Therefore, several individual miRNA regulatory circuits have ancient origins and have remained intact throughout the evolution and diversification of plants. Because it is easy to envision that small changes in the temporal, spatial, or environmental regulation of these modules over time could have had large phenotypic effects on plant morphology, we suggest that these highly conserved miRNA-target interactions have been substrates for the natural selection of plant forms.

416 Intra- and Interspecific Hybridizations between Genetically Modified (GM) and Non-genetically Modified (Non-GM) Brassica

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Reciprocal crosses were made between the genetically modified (GM) canola cv. *Brassica napus* RaideRR) resistant to Roundup® (glyphosate) herbicide and the non-GM *B. napus* cvs. Sponsor, Defender, Westar and GRIN 41; reciprocal crosses also were made between RaideRR and *B. rapa* (GRIN 42 and 43). When the GM parent was used as the pollen donor, higher numbers of viable seeds (e.g. 2,929 for GM pollen and 672 for non-GM pollen) and earlier seed germination (e.g., 100% germination at 24h for the GM pollen as compared to 60h for the non-GM pollen) were observed in the F_1 and BC_1 progeny. The greater compatibility of *B. napus* x *B. napus* crosses as compared to interspecific *B. napus* x *B. rapa* crosses was reflected in the higher seed yields of the intraspecific crosses. No significant differences were observed in dates of emergence of the flower bud and flowering of the F_1 progeny between the GM and non-GM canola. However, F_1 progeny between *B. napus* and *B. rapa* exhibited earlier formation of flower buds and flowering date, 34-42 days after seeding (DAS) and 39-41 DAS respectively, as compared to intraspecific crosses of *B. napus* (48-59 DAS and 52-63 DAS). This was attributed to the earlier emergence of *B. rapa* flower buds (26-46 DAS) and earlier flowering dates (29-49 DAS). Analysis of leaves by the Traitchek™ lateral flow test confirmed the presence of *CP4EPSPS* which confers resistance to glyphosate in all F_1, F_2, and BC_1 progeny. Mapping populations have now been produced for quantitative trait loci (QTL) analysis of traits potentially related to ecological fitness such as seed production, viability and germination. To address these research issues, 54 microsatellite primers from the *Brassica* database have been selected for evaluation to look for polymorphisms between the parental GM and non-GM canola, F_1 and BC_1 hybrid progeny.

417 Using Cabbage Leaf Curl Virus to Dissect Silencing and Defense Pathways in Arabidopsis thaliana

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Using Viral-Induced Gene Silencing (VIGS) constructs of the Geminivirus, Cabbage Leaf Curl Virus (CaLCuV) we knocked down expression of the Hen1 transcript, a novel plant gene involved in the miRNA and siRNA pathways. Knockdowns of the Hen1 gene in tandem with the endogenous ChlI, magnesium cheletase gene, in *Arabidopsis* showed attenuated viral symptoms, and either no silencing of the ChlI, or incomplete silencing of ChlI. The incomplete ChlI silencing is very similar to the silencing phenotype found in Rdr6 knockdowns using the CaLCuV, however Rdr6 knockdowns showed increased viral symptoms, whereas Hen1 knockdowns showed attenuated symptoms. These findings suggest that Hen1 is needed for silencing of an endogenous gene in *Arabidopsis*. The attenuation of symptoms in Hen1 knockdowns could be due a direct or indirect interaction of Hen1 with the virus. Hen1 could also play a role in viral symptoms due to altered miRNA function in infected plants.

418 Identification and Characterization of Insulators Protecting Gene Expression in the *Arabidposis* Centromere

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Sequences with the ability to insulate regions from positional effect and heterochromatin spread have been identified in several systems, including the sub-telomeric region in yeast and the B-globin locus in mammals. Despite a generally repressive heterochromatic environment, the genetically defined Arabidopsis centromeres contain expressed genes. Frequently one or more of these genes are grouped together in regions < 10kb surrounded by repetitive DNA containing transposons, retroelements and other centromeric repeats. By mapping the chromatin state surrounding these islands we will identify boundary sequences that form the barrier between the transcriptionally active unit and heterochromatic milieu. The functionality of these sequences will be tested by a transformation based assay. Constructs containing a reporter gene flanked by candidate boundary sequences will be used for transformation and the activity of the reporter gene will be monitored for insertions in both euchromatic and heterochromatic region. Regulatory elements required for barrier activity will be identified genetically by mutagenizing lines expressing the barrier-reporter construct in heterochromatin and screening for loss of reporter activity.

419 Molecular Mechanism of EMF-mediated Floral Repression

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EMBRYONIC FLOWER(EMF) genes are required to maintain vegetative development via the repression of flower homeotic genes. *EMF1* encodes a putative transcriptional regulator, while *EMF2* encodes a Polycomb group (PcG) protein. Two PcG protein complexes exist in *Drosophila*: PRC1 and PRC2. PRC2 is recruited to the target sites via PcG responsive elements and subsequently PRC1 is recruited to maintain the repressive chromatin complex. The core members of the PRC2 complex comprise four proteins first identified in *Drosophila* as the Esc, P55, E(z) and Su(z)12 proteins. There is now strong evidence that structurally and functionally equivalent complexes occur in *Arabidopsis*. EMF2 is a homolog of Su(z)12 and interacts with CURLY LEAF (CLF), homolog of E(z), which interacts with FIE, homolog of Esc; and FIE interacts with MSI1, homolog of P55. These proteins apparently form a protein complex and function to repress floral homeotic genes, such as AG and AP3. Mutations in each of these genes cause early flowering and exhibit mild homeotic transformations in flowers. The PRC1 complex is not found in plants. Therefore plants might use distinct mechanisms to achieve gene silencing effects. Little is known of the role of EMF1 in floral repression and its relationship to the EMF2 complex. EMF1 could potentially serve to initiate the repression of the floral homeotic genes while EMF2 complex maintains its repression. Alternatively EMF1 could be part of the PcG protein complex, in lieu of the PRC1 in maintaining inactive state of the flower homeotic genes. To investigate the relationship between EMF1 and the PcG protein complex, we carried out in vitro and in vivo protein interaction studies. While EMF1 does not interact with EMF2 and FIE, it interacts with MSI1. To characterize the mechanism of floral repression, we are investigating the DNA elements of the AG gene required for EMF1- and EMF2-mediated repression. Ectopic AG transcriptional activities occurred in either *emf1* or *emf2* mutants only if both AG promoter and second intron are present, indicating that the two EMF proteins function via a common mechanism. Immunological and protein tagging approaches are used to study temporal and spatial expression of EMF proteins. We are also constructing chimeric genes to regulate the expression of EMF activities in a temporal and spatial manner in order to study when and where EMF activities are required for floral repression.

420 Methylation in coding region causes strong gene silencing of the *phyA* locus in *Arabidopsis*

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A novel type of gene silencing in *phyA* locus of *Arabidopsis* was discovered, wherein the native *PHYA* gene is strongly silenced (based on phenotype) at high frequency (77%) by a homologous construct designed to carry out homologous recombination mediated *PHYA* knock-out. The silencing occurs only in those lines that contain multi-copy integration loci. The silencing of *phyA* gene is characterized by seedlings with long hypocotyls and unexpanded cotyledons when grown in Far-red (FR) light for 3 days. When such seedlings are brought to white light they survive, whereas WT plants die. From one of the transgenic lines, 68lf3, two progeny plants, $phyA^{GS-7}$ and $phyA^{GS-17}$, were recovered that maintained silencing in the absence of the transgene locus indicating that silencing occurred at the transcriptional level. Southern analysis of these two lines indicated no structural changes in the *phyA* gene. Low levels of reversion were observed initially but the phenotype stabilized in subsequent generations. Northern analysis of 5 day dark grown seedlings revealed reduction in the levels of *PHYA* mRNA in these two lines as compared to WT plants. To check if these two lines represented epi-mutation, methylation profile of $phyA^{GS}$ lines was compared with WT plants. Southern analysis with two methylation sensitive enzymes *Hpa*II and *Hha*I revealed 4 unique sites in the *PHYA* coding region, which were methylated specifically in $phyA^{GS-7}$ and $phyA^{GS-17}$. McrPCR assay showed that methylation is confined to the coding region and is not present in the promoter or 5'-UTR of the $phyA^{GS-7}$ and $phyA^{GS-17}$ alleles. Methylation was correlated with the mutant phenotype and the silenced *phyA* alleles were not found to be paramutagenic. Silenced phenotype has been found to be quite resistant to 5-azacytidine. Results of genetic crosses with *DNA methylation1* (*ddm1*) locus and *met1*, which encode putative chromatin remodeling factor and methyltransferase, respectively, will be presented.

421 Disruption of DNA methylation induces genome-specific changes in gene expression in natural Arabidopsis allotetraploids

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Combination of evolutionarily-diverged genomes in allopolyploids generates dynamic changes in gene expression and genome organization. The expression patterns of orthologous genes are rapidly and/or stochastically established in synthetic allotetraploids, and the silenced genes are maintained by DNA methylation and chromatin modifications. However, it is unclear how DNA methylation affects genome-wide expression of homoeologous genes originating from A. thaliana and A. arenosa ancestral genomes in natural A. suecica. Here we report comparative analysis of up- or down-regulated genes in the met1-RNAi A. suecica lines with non-additively expressed (different from the mid-parent value) genes in the synthetic allotetraploids. Decrease in DNA methylation in A. suecica induces A. arenosa-specific demethylation in centromeres and altered expression of >200 genes encoding transposons and predicted proteins that are located near centromeres and heterochromatic regions, whereas the non-additively expressed genes in the synthetic allotetraploids are distributed randomly along the chromosomes and encode various proteins in metabolism, energy, cellular biogenesis, cell defense and aging, and hormonal regulation. The reactivated genes in met1-RNAi A. suecica lines are predominately derived from the A. thaliana genome, indicating its hypersensitivity to changes in DNA methylation. The data suggest that allotetraploid genomes coincidently display chromosomal specific changes and genome-dependent regulation of homoeologous genes in response to DNA methylation perturbations.

422 Role of small interfering RNAs in A. thaliana rRNA gene expression patterns

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Small interfering RNAs of the 24nt class have been shown to direct chromatin modifications with impact on the transcriptional state of the targeted gene. In the A. thaliana rRNA gene promoter region, 24nt siRNAs are restricted to a discrete region flanking the transcription start site and their accumulation is dependent on AGO4, DRM2, DCL3 and NRPD2a genes, all known players of the RNA dependent DNA methylation pathway. In the A. suecica allotetraploid, an A. thaliana and A. arenosa hybrid, A. thaliana rRNA genes are typically silenced and subjected to the phenomenon of nucleolar dominance. Intriguingly, we found 24nts NOR siRNAs flanking the transcription start site in A. suecica to be derived from A. arenosa, the dominant rRNA gene subset in the hybrid, but not from A. thaliana NOR sequences, apparently contradicting the notion that siRNAs are required for the maintenance of a silenced state. We are currently investigating the rRNA small RNA population in A. suecica plants in which hypomethylation and hyperacetylation are chemically induced and known to disrupt nucleolar dominance. Together with RNAi knockdown lines of the major players in the RNAi pathway, these studies should provide important insights into the phenomenon in the allotetraploid species.

423 DNA translesion polymerases and plant genomic integrity

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DNA damage induced by UV-B light, reactive oxygen species, or other agents threaten plant genomic integrity. DNA lesions present during S-phase can block replication. Persistent collapsed replication forks can give rise to lethal double-strand breaks. Specialized translesion polymerases (TLPs) with inherently low fidelity synthesize DNA past template lesions, with relatively 'error-free' or 'error-prone' results. TLPs are necessary for orderly S-phase progression in DNA-damaged cells and may determine the frequency and spectrum of mutagenesis. Whether bypass is mutagenic or not depends on the particular TLP or combination of TLPs involved, and the particular template lesion being bypassed. There are four TLPs conserved across eukaryotic kingdoms. Pol κ, Pol η and Rev1 are members of the Y-family of DNA polymerases and share highly conserved domains. The fourth conserved eukaryotic TLP is Pol ζ, a member of the B-family of DNA polymerases, which includes the major replicative polymerases. Unlike the high fidelity replicative polymerases, Pol ζ is able to elongate distorted primer-template termini that arise at DNA lesions. Within the conserved domains are invariant amino acids that apparently bestow on these specialized polymerases abilities to synthesize DNA past lesions. There are also variant amino acids within the conserved domains, which might affect substrate specificity. We suggest that TLPs from organisms have evolved to optimize efficiencies and fidelities in bypass of DNA lesions that are frequently induced by environmental and endogenous mutagens that the organism encounters. To address this hypothesis, we are studying the biological roles of TLPs in the model plant Arabidopsis and biochemically comparing TLP orthologs from microbes, mammals and Arabidopsis. We are establishing plants deficient for each of the four TLPs by T-DNA insertion, and constructing various double mutants. We are also isolating sets of orthologous TLPs by parallel purification procedures and establishing assays for biochemical comparisons. (Supported by NSF grant MCB-0345061 to John B. Hays)

424 Control of Nucleolar Dominance by Histone Deacetylase HDA6

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HDA6, an Rpd3-like histone deacetylase in A. thaliana, regulates a variety of epigenetic phenomena including: transgene silencing, RNA dependent DNA methylation, repetitive DNA silencing, and heterochromatic condensation of the rDNA. We have recently shown that HDA6 also controls nucleolar dominance. Nucleolar dominance describes the phenomena in genetic hybrids in which the rRNA genes from one parental species are transcriptionally silent while the rRNA genes from the second parental species remain transcriptionally active. We show that when HDA6 expression is knocked down in Arabidopsis suecica, the transcriptionally silent rRNA genes become reactivated. In these knock-down lines, histone H3 and H4 associated with the previously silenced rRNA genes becomes hyperacetylated and the promoter of the inactive genes becomes hypomethylated. To determine how HDA6 affects nucleolar domininance, we purified epitope tagged HDA6 from A. thaliana using pEarleyGate plant expression vectors. In support of HDA6 acting on acetylated histone H3 and H4, we have recently shown that purified HDA6 from A. thaliana deacetylates histone H3 and H4. Histone deacetylase inhibitors sodium butyrate and trichostatin A also reactivate the silent class of rRNA genes in genetic hybrids. HDA6 activity is inhibited by histone deacetylase inhibitors sodium butyrate and trichostatin A further linking HDA6 to the control of nucleolar dominance.

425 Multi-species analysis of centromere regions in the Brassicaceae family

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Centromeres — large hetrochromatic DNA domains that mediate chromosome segregation—are conserved in function among eukaryotes, although centromere sequences diverge rapidly. Centromeres exhibit evolutionary dynamics that are distinct from the euchromatic chromosome arms, including extensive duplications, invasion by mobile elements, rearrangements, and movement or emergence at new chromosomal positions. We are examining evolution of plant centromere regions, in members of the Brassicaceae family that are close relatives of *Arabidopsis thaliana*, including *Arabidopsis arenosa*, *Capsella rubella*, and *Olimarabidopsis pumila*. *A. thaliana* centromere 3 sequence was used to identify homologous regions from each of the related species for comparative sequence analysis. Analysis of approximately 100-kb contiguous sequenced regions from the three related species revealed conserved gene content and order (collinearity) among the three species and *A. thaliana*. Surprisingly, however, gene density was much higher in the three relatives than in the homologous *A. thaliana* centromere 3 region, which contains highly expanded intergenic regions and is relatively gene-poor. Six predicted genes in the three relatives occupied genomic regions less than 1/10th that of the homologous centromere 3 region in *A. thaliana*. This pattern was consistent when gene densities from centromere 5 were compared, possibly suggesting that *A. thaliana* centromere regions have undergone a rapid expansion, while the genome as a whole has undergone extreme size reduction. This expansion of intergenic regions appears to be predominantly due to transposon insertion, rather than duplications or rearrangements, which are prominent in human and mouse centromere regions. FISH carried out on pachytene chromosomes confirmed that each of the sequenced BACs localizes to the pericentromeric heterochromatin of the three close relatives, in proximity to the centromere-specific satellites. These findings differ from those obtained from comparative sequence analyses of euchromatic portions of these genomes, where genes are maintained at similar density and order, and intergenic regions are of similar sizes. Our results indicate that genomic regions flanking the centromere satellite cores exhibit sequence changes in marked contrast to those detected in the euchromatin; such changes among closely related species highlight the dynamic nature of centromere regions.

426 Genomic and Molecular Evolution of Centromere Satellites in Brassicaceae Species

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Higher eukaryotic centromeres contain thousands of satellite repeats organized into tandem arrays. As species diverge, new satellite variants are homogenized within and between chromosomes, yet the processes by which particular sequences are dispersed are poorly understood. We isolated and analyzed centromere satellites in plants separated from Arabidopsis thaliana by 5 to 20 million years, uncovering more rapid satellite divergence compared to primate alpha-satellite repeats. We also found that satellites derived from the same genomic locus were more similar to each other than satellites derived from disparate genomic regions, indicating that new sequence alterations were homogenized more efficiently at a local, rather than global, level. Nonetheless, the presence of higher order satellite arrays, similar to those identified in human centromeres, indicated limits to local homogenization and suggested that sequence polymorphisms may play important functional roles. In two species, we defined more extensive polymorphisms, identifying physically separated and highly distinct satellite types. Taken together, these data show that there is a balance between plant satellite homogenization and the persistence of satellite variants. This balance could ultimately generate sufficient sequence divergence to cause mating incompatibilities between plant species, while maintaining adequate conservation within a species for centromere activity.

427 Meiotic chromosome maintenance in *Arabidopsis*

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The correct segregation of chromosomes during mitosis and meiosis is critical to ensure genetic integrity of progeny cells. This process has important consequences for reproduction as well as genome evolution. To better understand the factors required for proper segregation of chromosomes in *Arabidopsis*, we are developing a system that will allow determination of the frequency of chromosome loss events during pollen meiosis. Individual chromosomes marked with an easily scored visual marker will be followed through meiosis in the *quartet* mutant, where tetrad analysis is possible. We will examine the use of addition lines carrying an extra copy of one of the five chromosomes for this study. Future prospects of this approach, including the investigation of the effects of aneugenic compounds on pollen meiosis and genetic approaches to identify factors required for high fidelity chromosome transmission through meiosis will be discussed.

428 Distinct DNase I hypersensitive sites are found in transcriptionally-competent gene promoters in *Arabidopsis*

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In eukaryotic nucleus, the DNA is organized into higher-order chromatin structure to a variable level of condensation. In mammalian cells, generally, inactive genes are known to be located in condensed, inaccessible chromatin, whereas active genes are found in more open, accessible chromatin. On the other hand, in plants, as in other eukaryotes, constitutive heterochromatin segments (centromeres and nucleolus organizing regions) are organized as cytologically defined, highly-condensed chromocenters. However, in the remaining chromosomal regions where most genes reside, detailed knowledge regarding chromatin condensation is lacking. In this study, we evaluated the degree of chromatin condensation by measuring DNase I sensitivity at 500 bp resolution across an 80 kb *Arabidopsis* genomic region. This region contains 30 protein-coding genes ranging over 500-fold in their expression level in leaves. This is the first study of chromatin sensitivity at high resolution over multiple genes in plants. As a result, general DNase I sensitivity was similar irrespective of individual gene expression state throughout this 80 kb region, suggesting that chromatin condensation was not correlated with gene expression level. However, to our surprise, distinct DNase I hypersensitive sites (DNase I HSs) were found at the 5' and 3' ends of most genes, with 28 of 30 genes possessing such sites. Further analysis of several well-characterized genes showed that, upon transcriptional activation, the 5'-DNase I HS was extended resulting in the opening of promoter proximal *cis*-acting elements and TATA box. In addition, DNase I HSs were present in transcriptionally-competent (expressed or inducible) gene promoters and absent from incompetent (unexpressed nor inducible) gene promoters. These results suggest that the DNase I HSs play important roles in gene expression, and that local chromatin structure regulates gene expression potential by controlling the accessibility of transcription regulators to *cis*-acting elements in *Arabidopsis*.

429 Genome Rearrangement at Non-Standard Configuration of Ac/Ds Transposon Ends in Arabidopsis

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Transposable elements (TEs) are DNA sequences that can be mobilized from one site in the genome to another. The transposition reaction requires the enzyme transposase which catalyses double-strand breaks at the terminal-inverted-repeat sequences at either end of the transposable element, and insertion of the TE at another site in the genome. Transposition reaction also can occur if the terminal regions are in direct configuration or inverse. While normal transposition could result in gene disruption by inserting TE into a functional element, aberrant transposition reactions are known to result in various genome rearrangements including large deletions, duplications, and inversions. We have studied genome rearrangements involving maize Ac/Ds transposon ends inserted into Arabidopsis. A number of rearrangements have been identified and results will be reported here.

430 Natrual variation of a subtelomeric sequence in Arabidopsis thaliana

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The subtelomeric regions of chromosomes are evolutionary dynamic regions subject to frequent DNA rearrangements. Unlike many plant species, where repetitive sequences are dispersed in the subtelomeric regions, no significant repetitive sequences are noted in the model plant *Arabidopsis thaliana*, suggesting that a different genome evolutionary processes operate on the subtelomeric regions in this plant with a relatively small, simple genome. To gain insights into the dynamics of subtelomeric regions and its implication in telomere maintenance in *Arabidopsis thaliana*, we investigated sequence variation of the subtelomeric region of chromosome 1 north among 36 natural accessions. Using genomic sequence of the accession Columbia as a reference and a PCR approach, we observed that the general organization of this subtelomeric region (spanning approximately 3.5 kb from the telomere to the first annotated ORF) is conserved among the 36 accessions. Sequences of a ~2-kb region proximal to the telomere repetitive sequence revealed various DNA rearrangements, which coincide with the haplotype relationships of the 36 accessions surveyed. These DNA rearrangements include: (1) various large sequence insertions and deletions up to 429 base-pairs; (2) a 1.2-kb sequence inversion that accompanies deletions of flanking sequences of 387 base-pairs and 429 base-pairs (found in four accessions); (3) insertion of a copia-like transposon LTR in replacement of a 343-baspair sequence; (4) a translocation of 104-bp mitochondrial sequence in the telomere repetitive sequence present in one-third of the accessions. We have also found that two *Arabidopsis suecica* accessions, LC1 and 9502 (obtained from Comai and Pikkard laboratories), share conserved subtelomeric sequence of *A. thaliana* chromosome 1 north, supporting previous phylogenetic studies indicating that *A. thaliana* is one of the parents of the amphidoploid *A. suecica*. Further, the subtelomeric sequences of both *A. suecica* accessions have the sequence inversion observed in the four *A. thaliana* accessions characterized herein, suggesting the parental origins of LC1 and 9502 are closely related to these four accessions. The various DNA rearrangements observed in this study support the view that subtelomeric regions are dynamic, and provide a record of the diverse DNA rearrangements that occur within the deceivingly simple structure of Arabidopsis subtelomeres.

431 Genetic and epigenetic consequences of a paracentric inversion in Arabidopsis thaliana

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A heterochromatic knob (hk4S) is located in the short arm of chromosome 4 in the Arabidopsis accessions Columbia (Col) and Wassileskija (Ws) (Fransz et al. Plant J. 1998, 13: 867-87). Previous investigation (Fransz et al. Cell 2000, 100: 367-376) revealed a reversed order of two BAC clones in the proximal half of chromosome arm 4S in the knobless accessions Landsberg and C24 compared to Col and Ws. We therefore examined the region around the knob hk4S to determine the nature of the chromosomal rearrangement. By pachytene-FISH with DNA probes from 10 BACs we established a paracentric inversion of 1.25 Mb. This inversion has moved a part of the pericentric heterochromatin to an interstitial position, generating the heterochromatic knob hk4S in the common ancestor of Col and Ws. These accessions are therefore evolutionary closely related. Detailed examination by FISH to DNA fibers and interphase nuclei narrowed down the position of both the distal and proximal breakpoints of the inversion event. Based on the breakpoint positions we analysed the recombination frequency in the inversed region using classical and AFLP markers. We found no recombination between knob (Col) and knobless (Ler) accessions. Moreover, 27 AFLP markers in this chromosome segment revealed no polymorphism between the Col and Ws genomes compared to regions beyond the borders of the inversion. These data suggest that the DNA sequence in the paracentric inversion region is highly conserved between the Col and Ws. Since the heterochromatic knob is flanked by euchromatic segments we analysed the condensation state of the knob in interphase nuclei. FISH analysis of the proximal arm 4S in interphase nuclei from knob and knobless accessions revealed that flanking euchromatin regions affect the condensation degree of the heterochromatic island. Genetic and epigenetic consequences of the inversion event are discussed.

432 Plants with altered small RNA pathways respond to abscisic acid differently

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Recent reports suggest that hormone signaling including abscisic acid (ABA), which controls seed development and vegetative responses to drought and cold stress, are regulated by microRNAs (miRNAs)(Lu and Fedoroff, 2000; Han et al., 2004), but molecular mechanisms are unknown. The viral helper component proteinase (P1/HC-Pro) affects the accumulation and function of miRNAs and small interfering RNAs (siRNAs)(Dunoyer et al., 2004). A tobacco B3-domain transcription factor NtRAV, which has homology to the ABA signaling factors VIVIPAROUS-1 (VP1)/ABA-INSENSITIVE-3 (ABI3), physically interacts with HC-Pro and negatively regulates post-transcriptional transgene silencing (PTGS)(Bowman et al., 2004). We have shown that overexpression of AtRAV2 in maize mesophyll protoplasts is sufficient for transactivation of ABA-inducible gene expression by synergizing/interacting with the ABA effectors VP1, ABI5, ABF3 and ABI1-1. Detached leaves of *Nicotiana tabacum* overexpressing P1/HC-Pro or NtRAV lost significantly less fresh weight over time compared to the wild type, and P1/HC-Pro seeds were hyperdormant, suggesting ABA hypersensitivity similar to phenotypes observed in the *hyponastic leaves-1 (hyl1)* Arabidopsis mutant. Consistent with these results, a knockout mutant of AtRAV2 and the *enhancer of hua-1 (hen1-1)* miRNA methyltrasferase mutant (Yu et al., 2005) both transpired more than wild type, and *hen1-1* was hypersensitive to cold-stress-induced ion leakage. Because the miRNA metabolism mutant *hyl1* does not affect PTGS and results in ABA hypersensitivity (Han et al., 2004), while the PTGS and miRNA effectors HC-Pro, RAV, and HEN1-1 have various positive or negative effects on ABA signaling, our data suggest that ABA signaling may be affected by both the miRNA and the siRNA pathways.

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433 Dynamics of neo-centromere formation in Arabidopsis

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Centromeres are the recombination deficient regions of eukaryotic chromosomes that nucleate kinetochores, mediate sister chromatid cohesion and proper chromosome segregation. Unlike the gene-rich euchromatic chromosome arms, centromeres contain highly repetitive satellite sequences, transposons, retroelements and a few transcribed genes that perform a variety of functions. These varied properties lead to significant challenges in characterizing the requirements for centromere function. In the last few years, our lab has characterized the evolution and modification of centromeres, both in *Arabidopsis* and related species. We have developed methods to rapidly convert BAC clones containing centromere DNA to forms that are readily introduced into *Arabidopsis* via *Agrobacterium* transformation. We have generated a series of *Arabidopsis* T-DNA lines that contain various centromeric fragments, including satellites. We are developing systematic assays to assess whether such fragments are integrated into chromosomes or autonomous. The *Arabidopsis quartet* mutant is useful for this analysis, making it easy to monitor meiotic segregation of the introduced constructs. These assays will enable us to discern the genetic and epigenetic requirements for the newly introduced DNA to acquire centromere functions. For those T-DNA lines that have acquired novel centromere activity (either in autonomous constructs or dicentric chromosomes), we are assessing the stability and inheritance of the novel centromere DNA through generations of *Arabidopsis*.

434 RdRP-dependent RNA Silencing of Transgenes with Intrinsic Direct Repeats or Leaky Transcription Terminators

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In plants, RNA silencing can be induced efficiently by inverted repeats, presumably through double stranded RNA generated from the complementary sequences of the transcripts. RNA silencing can also be induced by sense transgenes in some transformants that are often associated with certain specific events such as high levels of expression, increased copy number and/or specific DNA arrangement of transgenes. Recently, it has been shown that transgenes with intrinsic direct repeats (IDR) can induce RNA silencing at very high frequencies (80-100%). Here we show that IDR-induced RNA silencing is dependent on the putative RNA-dependent RNA polymerase, SDE1/SGS2. Furthermore, insertion of a transcription terminator sequence at the end of the first repeat substantially decreased the frequency of RNA silencing, suggesting that read-through transcription of the direct repeats is important for the high efficiencies of RNA silencing. To further analyze the roles of read-through transcription of transgenes in RNA silencing, we have tested various *35S:GUS* constructs that differ in their terminator sequences in both wild type and *sde1* mutant plants. The *35S:GUS* construct with a single 35S or nos terminator was associated with high frequencies of transgene silencing (55-65%). The *35S:GUS* construct with 35S and nos double terminators had a relatively low frequency of gene silencing (20-25%) while the construct with no terminator sequence at its 3' end led to silencing of the GUS transgene in all tested transgenic lines. Thus, transcription read-through may play a critical role in high frequencies of transgenic silencing in transgenic plants.

435 Characterization of *Arabidopsis* Topoisomerase IIA binding and cleavage sequences

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Topoisomerase IIA (TopoIIA) is an essential enzyme involved in controlling DNA topology. Although topoisomerase II from bacteria, viruses, and animals has been extensively characterized, no characterization of the DNA recognition, binding and cleavage properties of the plant TopoIIA has been reported (Singh et al. 2004). Although TopoIIA-mediated DNA recognition has been shown to be species-specific (Fortune and Osheroff 2000), consensus sequences derived for *Drosophila* and vertebrate enzymes are used in different analyses including the analysis of the flanking sequences of the transgene loci (Sawasaki et al. 1998; Shimizu et al. 2001; Makarevitch et al. 2003). The detailed characterization of the specificity of the plant TopoIIA recognition would be useful in different studies of the DNA metabolism in plants. We developed a protocol for purifying nuclear TopoIIA from *Arabidopsis* thaliana seedlings that yielded large quantities of active, stable topoisomerase II and characterized its kinetics and cleavage properties. AtTopoIIA showed reproducible cleavage patterns in experiments *in vitro*. AtTopoIIA exhibited "poisoning" by etoposide resulting in substantially increased intensity of cleavage. We precisely mapped locations of 103 AtTopoIIA cleavage sites in *Arabidopsis* genomic clones. To confirm the validity of these sites detected *in vitro*, we tested cleavage of 9 sites *in vivo*. In all cases, we detected cleavage at the predicted locations in *Arabidopsis* seedlings. The consensus cleavage site derived from analysis of the 103 mapped sites possessed several interesting properties including palindromic symmetrical structure, alternating purine/pyrimidine patterns, and cleavage on the border between a stem and a loop in the stem-loop secondary structures. Prediction algorithms based on animal TopoIIA consensuses predicted 5 times more TopoIIA sites in the *Arabidopsis* genomic clones that we analyzed compared to the actual number of sites detected. These algoritms also predicted only about 34% of the detected sites. Although the consensus for AtTopoIIA recognition was quite different from animal sequences, the consensus by itself was very difficult to implement into TopoIIA cleavage prediction algorithms. However, the secondary structure motifs and alternating purine/pyrimidine stretches determined for AtTopoIIA are similar to those described for animal enzymes, suggesting that the combination of the consensus sequences and secondary DNA structure motifs described from different sources might prove useful for future in silico predictions of TopoIIA cleavage sites.

436 Establishment of the winter-annual habit in Arabidopsis requires *EARLY FLOWERING IN SHORT DAYS*, a putative histone H3 lysine 4 methyl transferase

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Many naturally occurring accessions of Arabidopsis are late flowering unless vernalized and thus behave as winter annuals. In the absence of vernalization (a prolonged period of cold exposure) high levels of the floral inhibitor *FLOWERING LOCUS C (FLC)* act to block flowering. Following vernalization, *FLC* expression is epigenetically downregulated and late flowering is eliminated. Recently it has been shown that the repression of *FLC* by vernalization is mediated by repressive modifications in chromatin structure (H3K9 and H3K27 methylation). In a screen for positive regulators of *FLC* we identified several alleles of a previously described mutant, *EARLY FLOWERING IN SHORT DAYS (EFS)* . We have found that *efs* mutations completely suppress *FLC* expression, thus *EFS* acts as a positive regulator of *FLC*. Interestingly, *efs* mutants flower earlier than *flc*-null mutants indicating that the expression of other floral inhibitors may also be affected by *EFS*. We cloned *EFS* using a T-DNA-tagged allele and found that it encodes SET-domain-containing protein. Many SET-domain proteins are known to act as histone methyl transferases. The SET domain of EFS is similar to that of ASH1 from Drosophila, which is involved in gene activation by methylation of lysine 4 of histone H3. Consistent with a similar role for *EFS*, we have found that H3K4 methylation is decreased in the *FLC* gene in the *efs*-mutant background. Thus *FLC* is likely a direct target of *EFS*. We have also found that the *FLC* related genes, *FLM/MAF1* and *MAF2*, are also downregulated in the *efs* mutant. Thus EFS activity is required for proper expression of this clade of genes. Because *FLM/MAF1* and *MAF2* also function as repressors of flowering, the coordinate downregulation of *FLC, FLM/MAF1*, and *MAF2* may account for the strong early-flowering phenotype of the *efs* mutant.

437 Lipases in Plant Defense and Death

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Plant defense responses are constitutively on in the Arabidopsis thaliana ssi2 mutant plant. In addition, the ssi2 mutant allele confers a spontaneous cell death phenotype. The SSI2 gene encodes a stearoyl-ACP desaturase, which converts stearic acid (18:0) to oleic acid (18:1), suggesting a role for lipids in plant defense and death. Microarray analysis identified several genes, which encode putative lipases that are expressed at elevated levels in the ssi2 mutant plant, in comparison to the wild type plant. In order to study the involvement of these putative lipases in plant defense and death, we have identified T-DNA insertions within these genes and are evaluating their effect on ssi2-conferred phenotypes.

438 DNA methylation and DNA methyl-binding domain genes in rRNA transcriptional regulation

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Our lab investigates transcriptional regulation and chromatin structure in the Arabidopsis using nucleolar dominance as a model to understand the processes that regulate transcription. Nucleolar dominance occurs in hybrids of many species in which the rRNA genes from one parent are silenced, while the rRNA genes of the second parent are preferentially transcribed. We have used this endogenous silencing process to identify mutants in which the silenced rRNA genes are transcriptionally activated. Previous work in our lab demonstrated that by inhibiting histone deacetylation and DNA methylation, previously silent rRNA genes were transcriptionally reactivated. Futhermore, perturbation of histone deacetylation induces changes in the DNA methylation state, and vice-versa . The role of histone deacetylation was further confirmed by analyzing knockdowns of the Arabidopsis histone deacetylases HDT1 and HDA6, which relieve silencing.

Data indicating the epigenetic regulation of rRNA gene transcription led us to investigate other known chromatin modifying genes for changes in rRNA transcription. We found RNAi mediated knockdowns of both Methyl-CpG binding (MBD) proteins and the Drm2 DNA methyltransferase that affect rRNA transcription. We demonstrate that the change in rRNA transcription in the Drm2-RNAi mutant lines is accompanied by a shift in the histone methylation state of the underdominant loci, resulting in an increase in methylated Histone H3 lysine 4 and a decrease in Histone H3 lysine 9 methylation. Furthermore, Drm2 is shown to bind to the rRNA gene promoter and Drm2 mutants demonstrate a specific loss of DNA methylation at one site in the rRNA promoter. Two MBD proteins, MBD6 and MBD10, may interact with the methylated rRNA promoter to regulate transcription. RNAi knockdowns of both genes show reactivation of the silenced rRNA genes. On going research focuses on identifying possible genetic and biochemical inter-relationship between Drm2 and the MBDs, as well as the possible interactions between the MBDs, HDACs and Drm2. These new data, coupled with previous data from our lab, flush out the interdependent mechanisms involved in rRNA gene regulation the role of chromatin in regulating gene transcription in Arabidopsis.

439 A link between miRNA and siRNA pathways mediated by ARGONAUTE1 and DICER-LIKE1

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The Dicer and Argonaute families of proteins are essential for carrying out RNA interference (RNAi) in a diverse set of organisms. *DICER-LIKE1* and *ARGONAUTE1* of *Arabidopsis* are required for microRNA (miRNA) activity, and mutations in either gene severely perturb normal development. To identify genes misregulated in these mutants, we examined global gene expression in the ago1 and *dcl1* backgrounds by microarray analysis. 8–25% of genes displayed significant changes in expression in *ago1* and *dcl1*, with 1–6% of genes showing activation. Higher mRNA levels accumulated for 11 genes at multiple developmental stages in both mutants. Five of these genes, representing three families, were targets of previously characterized miRNAs, but several known microRNA target genes did not change significantly in expression in *ago1* or *dcl*1. Similar levels of mRNA cleavage products were observed in wild-type *ago1* and *dcl1* for miRNA target genes that were upregulated in *ago1* and *dcl1* as well as those that did not change in expression, indicating that an alternate or redundant mode of RNAi might contribute to their regulation. Some antisense RNAs corresponding to spliced mRNA accumulated at higher levels in *ago1* and *dcl1*, and 21–22-nt. small interfering RNA (siRNA) were present in wild-type plants, but not in *ago1, dcl1*, the RNA-dependent RNA polymerase mutants *rdr2* and *sde1/sgs2*, or the Dicer-like mutant *dcl3*. We are further investigating the relationship between AGO1, DCL1, miRNA metabolism and the production of these secondary siRNAs by examining a series of miRNA-resistant transgenic lines.

440 Analysis of a suppressor mutant of the FKBP-like twisted dwarf1 (twd1) mutation in Arabidopsis

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The *AtFKBP42* gene of *Arabidopsis* encodes a membrane-bound immunophilin-like protein which interacts with plasma membrane bound ABC transporters AtPGP1 and AtPGP19. This interaction leads to regulation of polar auxin transport facilitated by the ABC transporters. Null mutants of the *AtFKBP42* gene are characterized by dwarfism due to impaired cell elongation and disoriented cell growth, which coined the name *TWISTED DWARF1 (TWD1)* for this gene. A suppressor mutant screen resulted in the isolation of *twd1-sup* which is a suppressor mutation of the T-DNA induced *twd1-2* knock-out allele. The phenotype of the suppressor line is intermediate between *twd1-2* knock-outs and wild-type (Ws-2). Genetic analysis showed dominance of the suppressor mutation over *twd1-2*. Segregation ratio of phenotypes from crosses of wild-type and *twd1-sup* provides strong evidence for an intragenic suppressor. However, no sequence alterations of the *TWD1* locus could be found in knock-out and suppressor lines. The NPTII selection marker of the inserted T-DNA is silenced in twd1-sup plants but not in *twd1-2*. This reduction of NPTII expression can be overcome by treatments with the methylation inhibitor 5-azacytidine (5-azaC). In addition, prolonged treatments of suppressor plants with 5-azaC caused reversion of the intermediate suppressor phenotype to the phenotype of knock-out plants. The same phenomenon has been observed in crosses with methylation mutants such as *ddm1*, *met1* and *hog1*. These facts indicate a role of epigenetic effects, probably caused by differential methylation, to be the cause of the suppressor phenotype.

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441 Genetic and Epigenetic Study of de novo Centromere Formation in Arabidopsis thaliana

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Centromeres play a critical role in ensuring proper segregation of chromosome by specifying site for kinetochore assembly and spindle attachment during cell division. Our lab is interested in studying the genetic and epigenetic determinants at the centromere that specify its function in *Arabidopsis thaliana*. Using tetrad analysis in *A. thaliana*, Copenhaver *et al*. (1999) were able to map all five centromeres. The mapped region is enriched in repetitive DNA, with a 180bp repeat being the predominant satellite (Nagaki K *et al*. 2003, Hall SE *et al*. 2003). In addition to the presence of repetitive elements, centromeric DNA also shows strand-biased methylation patterns (Luo and Preuss, 2003). The primary question this study aims to answer is: what genetic elements in the centromeric region of *A. thaliana* are sufficient to confer centromeric activity. To this end, DNA fragments from different regions of the *A. thaliana* genome will be integrated into the chromosome by *Agrobacterium*-mediated transformation. These DNA fragments will include BAC clones carrying centromeric DNA repeats, gene rich euchromatic sequences, heterochromatic DNA from the NOR, and the chromosome 4 knob. Following transformation of these DNA fragments into the chromosome, *de novo* formation of centromeres at these inserted sequences will be investigated by using various genetic, cell and molecular biological methods. The candidates that show *de novo* centromere formation will be further analyzed for epigenetic changes brought about by centromere formation. These candidates can then be used to find the minimal sequence required for centromere function.

442 Low-light exposure induces disruption of heterochromatic chromocenters in Arabidopsis

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Arabidopsis nuclei display a flexible response to specific biotic and abiotic stresses. This may well reflect the plasticity of the plant to respond to changing environmental conditions. An alteration in quality or quantity of the perceived light can result in a stress situation. As a consequence the plant may display a physiological 'shade avoidance' response characterized by hyponastic growth and elongation of the petiole of rosette leaves. Here we show that 90% decrease of light led to a dramatic reduction of visible DAPI-positive heterochromatin in mesophyll nuclei of the accession Columbia (Col). The heterochromatin fraction reached its minimum after 96 hours, showing ~75 % less heterochromatin. FISH analysis revealed complete de-condensation of pericentromeric repeats and also de-condensation of the centromeric 180 bp repeat. Only the 45S rDNA region maintained a certain level of condensation. The decrease of heterochromatin is a reversible process, since raising the light intensity to original levels restored normal values of heterochromatin content. The extent of chromatin reduction appeared to depend on the ecotype and the light regime. We are currently investigating the nuclear response to low light in a number of photoreceptor mutants in order to dissect the signalling pathway leading to heterochromatin de-condensation in relation to shade avoidance responses.

443 Large-scale chromatin decondensation accompanies cell de-differentiation during protoplast culture

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We focus on understanding the structure, formation and function of heterochromatin in the interphase nucleus of Arabidopsis thaliana. Heterochromatin in this model system is confined to 6 to 10 discrete nuclear domains, the so-called chromocenters, each of which can be microscopically identified. Chromocenters contain one or more centromeric regions and display most characteristics of silenced chromatin, such as a high level of chromatin condensation, late replicating activity, presence of repetitive elements, di-methylation at lysine 9 of histone H3 and 5-methylcytosine-rich DNA. In contrast, the surrounding euchromatin mainly consists of gene-dense regions containing acetylated histones and histone H3 di-methylated at lysine 4. Previous work has demonstrated that the typical organization of chromocenters becomes partly disrupted in several gene-silencing mutants such as ddm1, met1, sil1 and hog1 (Soppe et al, 2002; Fransz et al, 2003). Due to the relocation of pericentromeric sequences away from heterochromatin, the mutant chromocenters decrease in size. Interestingly, we have recently observed comparable nuclear phenotypes in wild-type leaf mesophyll tissue under specific biotic and abiotic conditions. Here we show that nuclei display reduced heterochromatin content upon protoplast formation. Moreover, the strong reduction of heterochromatin domains involves not only the delocalization of pericentromeric sequences but also of the centromeric 180bp tandem repeat. As a consequence, the chromocenters are completely disrupted. Surprisingly however, we did not detect changes in the methylation level of DNA or lysine 9 of histone H3 in heterochromatin. We are currently investigating the process of de-condensation events resulting in the disappearance of chromocenters. Since the process is reversible, this system provides the opportunity to study formation of heterochromatin.

444 The role of microRNAs in non-additive gene regulation in Arabidopsis allopolyploids

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The combination of Arabidopsis thaliana and A. arenosa genomes that diverged ~5.8 Mya induces regulatory incompatibilities and altered interactions in the synthetic allopolyploids, leading to non-additive gene regulation. Here we test a hypothesis concerning RNA-mediate gene regulation in allopolyploids. MicroRNAs (miRNAs) are involved in post-transcriptional regulation of target genes, many of which play important roles in plant and animal development. Transcriptome analysis indicates over 200 genes that are expressed non-additively in the synthetic allotetraploids match the targets of 22 miRNA families identified in Arabidopsis. Up- or down-regulation of the miRNAs is correlated inversely with the expression of the target genes in the allotetraploids. Although miRNAs are very conserved between the progenitor species, intergenic regions encoding miRNA precursors diverge between A. thaliana and A. arenosa. A subset of miRNA precursors in the allotetraploids is preferentially processed from the A. thaliana parent, and the genes surrounding the MIR loci display the same parental expression patterns, indicating that the chromosomal regions in the vicinity of miRNA precursors are altered during polyploidy formation. Furthermore, miR163 is highly expressed in A. thaliana diploids but down-regulated in A. thaliana autotetraploids and repressed in A. arenosa and A. suecica. The data suggest that interspecific hybridization induces incompatibilities of miRNA processing machineries, leading to differential accumulation of miRNAs that down-regulate the target genes regardless of their origin, which provides a mechanism for rapid phenotypic and developmental changes in the synthetic allopolyploids.

445 Multiple hydrophobic clusters in Arabidopsis CBF1 redundantly contribute to transcriptional activation

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Arabidopsis genome has up to 5% genes (~ 1500) encoding transcriptional factors (TFs). Many TFs are unique in plants including the members of C-repeat Binding Factors (CBF), a subfamily of the AP2/EREBP family. Relative little work has been done to biochemically characterize the structure and function of a plant TF. Here we present our understanding of CBF1's trans-activation by the strategy of systemic alanine scan mutagenesis. By domain swap experiments, we show that the N-terminal 115 amino acids were sufficient to direct CBF1 to its cis-acting elements and C-terminal 98 amino acids were functional in trans-activation. By extensive mutational analysis including truncation and alanine substitution through the C-terminal activation domain, we show that CBF1 contains four hydrophobic clusters (HC) redundantly contributing the transcriptional activation. The overexpression of these mutants in plants confirmed this feature and also indicated that the disruption of a single HC did not seriously abolish the activity unless combined with the disruption of a second. The HCs consist of several hydrophobic residues that are delimited from one another by short stretches of Asp, Glu, Pro and other residues favoring the formation of loops. This structural pattern is conserved across plant taxa as revealed through alignment of Arabidopsis CBF1 with homologous sequences from a diverse array of plant species. Overexpression in plants of the CBF1 C-terminal activation domain fused with yeast GAL4 DNA binding domain also resulted in severe stunting of growth, a phenotype which was alleviated if the activation domain was rendered ineffective. Taken together these results suggest that high level overexpression of an active, activation domain compromises plant growth.

446 Identification of multiple mutations in the microRNA target site of *TCP4* and of a mutation in the miR319a microRNA by suppressor mutagenesis

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MicroRNAs (miRNAs) are small regulatory RNAs of 19 to 22 nucleotides in length, which regulate gene expression at the posttranscriptional level. In contrast to the mechanism of translational inhibition proposed for animal miRNAs, plant miRNAs mostly target transcripts for cleavage, requiring extensive base paring between the miRNA and target transcript. Previous studies have revealed the importance of miRNA-Jaw (miR319a) in plant development. Ectopic miR319a expression in *jaw-D* plants causes pleiotropic developmental defects, including epinastic cotyledons, crinkly leaves, and a modest delay in flowering time. MiRNA-Jaw negatively regulates transcript levels of five *TCP* transcription factors that are thought to be involved in the regulation of cell proliferation. We carried out a suppressor screen to analyze the miR319a/*TCP* interaction *in vivo*, and to identify other factors necessary for the phenotypic effects of ectopic miR319a expression. 16 potential suppressor mutants were identified, called suppressor of *jaw-D*, (*soj*). These affect the miR319a pathway at different levels. Here we focus on four point mutations that impair the miR319a guided cleavage of the *TCP4* transcript. Three of these mutations are located within the miR319a target site of *TCP4*, whereas one was found in the mature miR319a. We show that all mutations partially impair the efficiency of miR319a guided cleavage of *TCP4*. Although the mutant *TCP4* alleles have no dramatic effects in the endogenous genomic context, their overexpression in transgenic plants leads to severe defects in early plant development. In summary, suppressor mutagenesis of miRNA overexpressers is an efficient tool for studying miRNA-target interaction *in vivo*.

447 The Arabidopsis SKP1-LIKE1 (ASK1) protein is most abundant in leptotene and likely regulates homologous recombination in male meiosis

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The ASK1 protein in Arabidopsis is the Skp1 component of many Skp1-Cullin-F-box protein ubiquitin ligases that regulate the degradation of diverse proteins. It is known that *ask1-1*, a null mutant of *ASK1*, is severely affected in male meiosis and is male sterile. The defects in *ask1-1* male meiosis are multifaceted and occur in nearly the entire course of the meiotic cell cycle: starting at the leptotene stage with the failure of the nucleolus to migrate to the nuclear periphery, followed by asynapsis and non-separation of homologous chromosomes at the later stages of meiosis I. In addition, we found in *ask1-1* abnormally large and stable proteinaceous structures on male meiotic chromosomes using transmission electron microscopy, and stable association of a subset – perhaps 5 pairs – of the 20 chromosomes in meiosis II using fluorescence microscopy. To shed light on whether these defects occur independently or a later defect secondarily results from an earlier defect, we investigated, during male meiosis, the dynamics of an ASK1-green fluorescence protein (GFP) fusion protein expressed under the *ASK1* promoter. We found that the amount of the ASK1-GFP was most abundant in leptotene, moderate in zygotene and pachytene, and low in other stages of the meiotic cell cycle. These observations are consistent with the first observable defect occurring in leptotene of *ask1-1*, and suggest that the non-separation of chromosomes likely results from a primary defect in early prophase I. The timing of the ASK1 expression in the wild type and the meiotic defects in *ask1-1* also suggest that ASK1 is likely involved in regulation of homologous recombination. To test this possibility, we crossed a marker of homologous recombination into the *ask1-1* background. The marker consists of two partial but overlapping glucuronidase (GUS) coding regions that upon recombination restore the function of the *GUS* gene. The frequency of blue microspores detected by the histochemical assay for GUS should positively correlate with the recombination frequency. Our preliminary study suggests that homologous recombination events were increased in *ask1-1* male meiosis. Therefore, ASK1 likely functions in early male meiosis to regulate the stability of certain recombination-related proteins.

448 The biogenesis of endogenous trans-acting siRNAs in Arabidopsis is initiated by miRNA-directed cleavage

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Trans-acting small interfering RNAs (ta-siRNAs) are endogenous siRNAs that direct the cleavage of non-homologous transcripts by sequence-specific base pairing. Previously we described 3 closely-related non-coding genes (*TAS1a*, *TAS1b* and *TAS1c*) that produce ta-siRNAs, and showed that *DCL1*, *SGS3*, and *RDR6* are required for the production of ta-siRNAs from these genes. Here we report a new source of ta-siRNAs, *TAS2*, and describe the function of *DCL1*, *SGS3*, and *RDR6* in the biogenesis of ta-siRNAs from these loci. We show that the biogenesis of siRNAs from *TAS1* and *TAS2* is triggered by the cleavage of their primary transcripts by *miR173*. This event produces a 3' cleavage fragment that is transformed into siRNAs by the sequential activity of SGS3, RDR6 and an unknown DCL. Following the transformation of the 3' cleavage fragment into a dsRNA, DCL produces siRNAs from this fragment, starting at the miRNA cleavage site. Because siRNA biogenesis begins at a defined site, this process generates a reproducible set of siRNAs. Our results indicate that miRNAs can initiate an RNAi cascade, and thus have significant effects on expression of genes that are not direct targets of the miRNA.

449 Arabidopsis mutants with altered responses to HDAC inhibitors, including HC-toxin

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HC-toxin is the primary determinant of the leaf spot and ear mold disease in maize caused by race 1 of the fungus Cochliobolus carbonum. Being a cyclic tetrapeptide, HC-toxin is a potent inhibitor of histone deacetylases (HDACs) not only from maize but most other organisms, including Arabidopsis. Despite this and despite the fact that it lacks HC-toxin reductase activity, which is responsible for resistance to C. carbonum in maize, Arabidopsis is able to resist infection by C. carbonum. Is this because Arabidopsis has an alternative mechanism to contend with HC-toxin? We opted to address this question by generating and identifying EMS-induced Arabidopsis mutants that exhibit altered responses to HC-toxin as well as another HDAC inhibitor, CBHA (m-carboxycinnamic bis-hydroxamic acid). A genetic screen was devised that resulted in the identification of a number of mutants exhibiting either enhanced sensitivity or tolerance to these HDAC inhibitors. The ways these mutations are being characterized will be described and reasons for why and how these mutants confer their respective sensitive or tolerant phenotypes will be explored. It is anticipated that these mutants will not only allow us to understand how HDAC inhibitors interfere with plant defenses, but also facilitate insights into the realm of chromatin remodeling in plants.

450 MAPKs and stress hormone signaling during ozone exposure

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Changing environmental conditions, atmospheric pollutants and resistance reactions to pathogens cause production of reactive oxygen species (ROS) in plants. ROS in turn trigger the activation of signaling cascades, such as the mitogen-activated protein kinase (MAPK) cascade and accumulation of plant hormones, jasmonic acid, salicylic acid and ethylene. We have used ozone (O_3) to generate ROS in the apoplast of wild type Col-0 and hormonal signaling mutants of *Arabidopsis thaliana* and show that this treatment caused a transient activation of 43 kDa and 45 kDa MAPKs. These were identified as AtMPK3 and AtMPK6. We also demonstrate that initial AtMPK3 and AtMPK6 activation in response to O_3 was not dependent on ethylene signaling, but that ethylene is likely to have secondary effects on AtMPK3 and AtMPK6 function, whereas functional salicylic acid signaling was needed for full level AtMPK3 activation by O_3. In addition, we show that *AtMPK3*, but not *AtMPK6*, responded to O_3 transcriptionally and translationally during O_3 exposure. Finally, we show *in planta* that activated AtMPK3 and AtMPK6 are translocated to the nucleus during the early stages of O_3 treatment. The use of O_3 to induce apoplastic ROS formation offers a non-invasive *in planta* system amenable to reverse genetics that can be used for the study of stress-responsive MAPK signaling in plants.

451 Stress Responses to Polycyclic Aromatic Hydrocarbons Include Generation of Reactive Oxygen Species, Cell Death and Changes in Gene Expression

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Polycyclic aromatic hydrocarbons (PAHs) are of global environmental concern because they cause many health problems including cancer and inflammation of tissue in humans. Plants are important in removing PAHs from the atmosphere; yet, information on the physiology, cell and molecular biology, and biochemistry of PAH stress responses in plants is lacking. We will present studies on the PAH stress response in Arabidopsis thaliana exposed to the three-ring aromatic compound, phenanthrene. Morphological symptoms of PAH stress include growth reduction of root and shoot, deformed trichomes, reduced root hairs, chlorosis, late flowering and appearance of white spots, which later developed into necrotic lesions. At the tissue and cellular levels, plants experience oxidative stress. This was indicated by localized H2O2 production and cell death, which were detected using 3, 3-diaminobenzidine and trypan blue staining, respectively. Gas chromatography-mass spectrometry and fluorescence spectrometry analyses showed that phenanthrene is internalised by the plant. Gene expression of the cell wall loosening protein expansin was repressed, whereas gene expression of the pathogenesis related protein PR1 was induced in response to PAH exposure. Whole genome analyses after PAH exposure suggest strong similarities to ozone stress and pathogen defense pathways. Additionally, we will discuss potential cross-talk with hormone pathways.

452 Differential regulation of salt tolerance genes between *Arabidopsis thaliana* and the halophytic *Arabidopsis* Relative Model System, *Thellungiella halophila*

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Thellungiela halophila is a halophytic *Arabidopsis* Relative Model System exhibiting 90%-95% identity with *Arabidopsis* at the cDNA level. It can tolerate very high (500mM) levels of NaCl salinity whereas *Arabidopsis*, a glycophyte, shows severe symptoms of stress at 100mM NaCl. Glycophytes and halophytes employ common mechanisms to cope with salt stress and it is hypothesized that differences in salt tolerance arise due to changes in the regulation of a basic set of salt tolerance genes. We compared the expression of key salt tolerance genes between *Arabidopsis* and *Thellungiella*. The plasma membrane Na^+/H^+ antiporter SOS1 controls loading and retrieval of Na^+ into and from the xylem and *sos1* mutants are salt-sensitive. We observed that basal shoot *SOS1* mRNA levels differed little between the two species under unstressed conditions but were more strongly induced by salt in *Thellungiella*. Root *SOS1* mRNA levels showed little species-specific difference in their response to salt but were constitutively higher in unstressed *Thellungiella*. Consistent with these findings, *Thellungiella* shoots accumulated Na^+ to a lower extent than *Arabidopis* under saline conditions.

Proline is a compatible osmolyte that accumulates in many plants under salt stress. *Thellungiella* accumulates low levels of proline under unstressed conditions while under salt stress *Thellungiella* accumulates more proline than *Arabidopsis*. Basal and salt-mediated expression of proline biosynthetic *P5CS* genes were similar in the two species. Strikingly, mRNA encoding the proline-degrading enzyme, PDH, was undetectable in *Thellungiella* shoots suggesting that repression of proline degradation may allow *Thellungiella* to accumulate more proline than *Arabidopsis*. We confirmed that *Thellungiella* had a reduced capacity for proline degradation by growing *Arabidopsis* and *Thellungiella* seedlings on media containing excess proline. Other studies have shown that a reduced capacity for proline degradation leads to hyper-accumulation of proline and subsequent inhibition of growth when a high level of proline is included in the growing media. We observed a 63% reduction in *Thellungiella* root elongation as well as impaired shoot growth in proline-containing media whereas *Arabidopsis* was unaffected.

Taken together, our results suggest that the species-specific differences in the regulation of genes involved in key salt stress mechanisms in *Arabidopsis* and *Thellungiella*, contribute to the ability of *Thellungiella* to withstand extreme levels of salinity.

453 Functional Characterization of the Nicotianamine Synthase (*NAS*) Gene Family in *Arabidopsis thaliana*

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Iron is an essential nutrient but is toxic in excess. In addition, iron is often not readily available for uptake from the soil, so plants have evolved tightly regulated iron uptake strategies to ensure that the plant maintains appropriate levels of iron within cells. Strategy I plants utilize a combination of a ferric chelate reductase enzyme and a ferrous iron transporter to effectively take up iron, whereas Strategy II plants rely on the biosynthesis and secretion of phytosiderophores that chelate ferric iron in the rhizosphere and shuttle it back into the cells. Interestingly, many Strategy I species, including *Arabidopsis*, synthesize the phytosiderophore precursor nicotianamine (NA), even though they do not make the phytosiderophores. Previous studies have indicated that NA may play a role in long distance transport of metals in both strategy I and strategy II plants. NA is made from three molecules of S-adenosyl methionine, through the action of the enzyme nicotianamine synthase (NAS). In this study, we examine the expression and function of the four *Arabidopsis NAS* genes try to elucidate the precise role(s) of NA in this strategy I species. Our results demonstrate that the *NAS* genes are expressed in a tissue specific manner and are differentially regulated by iron, copper, zinc and nickel, indicating possible roles for NA in metal homeostasis. We have focused our initial efforts on *NAS1* because it is expressed in roots and its expression is induced by iron deficiency. We created transgenic lines with altered *NAS1* gene expression. Elemental analysis of the transgenic lines suggests that NA is necessary for the accumulation of copper. Transgenic lines with reduced *NAS1* expression accumulate reduced levels of copper whereas 35S-NAS1 lines accumulate increased levels of copper. Our data also demonstrate that *NAS1* plays roles in iron, zinc and nickel metabolism.

454 Transcriptional regulation of *Arabidopsis thaliana* P450 genes in response to phenobarbital

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Cytochrome P450 genes constitute one of the largest *Arabidopsis* gene families (272 genes and pseudogenes) with their coding sequences spanning 0.6% of the genome. Proteins encoded by this highly diverse P450 superfamily participate in cellular functions ranging from regulation of plant development to xenobiotic detoxification. Extensive divergence of catalytic site as well as noncatalytic site residues has resulted in a high degree of primary sequence variation. One approach for assigning functions to these many genes is to define modulations of gene expression in response to chemical treatments with the expectation that these changes represent the signature of the cellular response to the compound studied.

In our analysis, we have examined the effects of phenobarbital as a prototype for environmental pollutants and a large group of xenobiotics known to induce drug-metabolizing enzymes in mammals. Microarrays containing gene-specific elements for 265 *Arabidopsis* P450s and 362 biochemical pathway and physiological function markers were hybridized with probes directed against RNAs obtained from 10 mM phenobarbital (PB)-treated 7-day-old seedlings at two time points (6 and 24 hrs). Six P450 genes *(CYP81F4, CYP81H1, CYP85A2, CYP90A1, CYP90B1, CYP710A2)* and several other genes (iron-superoxide dismutase, zeanthin epoxidase, protein kinase, water-channel protein, etc.) were selectively and significantly induced in response to PB at 6 hrs. A collection of these and four other P450 genes were also induced at 24 hrs. Four P450 genes involved in glucosinolate synthesis *(CYP79B2, CYP79B3, CYP83A1, CYP83B1)* and jasmonic acid synthesis *(CYP74A1)* and other genes (Cu/Zn superoxide dismutase, etc.) were significantly repressed under the same conditions. RT-PCR methodologies have confirmed the expression patterns of these transcripts in response to PB as well as other abiotic stresses. Computational analyses of the -2000 kb intergenic regions of the induced and repressed gene sets have identified several conserved and over-represented DNA sequence motifs. Collations of these response patterns with respect to plant biochemical pathways coupled with molecular modeling and expression screening for potential substrates are providing the basis for defining functions for these enzymes.

455 Screening of a 35S-cDNA Arabidopsis lines library for ABA-insensitive dominant mutants shows negative regulation by a *PP2C* in ABA signaling

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To identify new loci in abscisic acid (ABA) signaling, we screened a library of 35S-cDNA Arabidopsis expressing lines (LeClere and Bartel, Plant Mol. Biol. 2001, 46:695-703) for ABA-insensitive dominant mutants in seed germination assays. One of the identified mutants, 393.1, germinated on 2.5 μM ABA, a concentration that completely inhibits wild-type seed germination. Backcrosses and F2 analyses indicated that the mutant exhibit a dominant phenotype and that the ABA-insensitivity was linked to the T-DNA. The cDNA of this mutant identified by PCR was the full-length cDNA of a protein phosphatase 2C similar to ABI1 and ABI2, and Northern blot analysis demonstrated that the *PP2C* is indeed over-expressed in 393.1. A more detailed characterization of the mutant indicates that over-expression of *PP2C* not only causes ABA-insensitivity in seed germination and post-germination growth, but also during ABA-induced stomatal closure responses. A homozygous T-DNA insertion line in this *PP2C* was recovered from the Signal Collection at The Salk Institute. This *pp2c* mutant exhibits a clearly opposite phenotype to the over-expressor 393.1, namely a strong ABA-hypersensitivity in all of the physiological responses tested. Moreover, Northern blot analyses clearly indicate that the *PP2C* transcripts are rapidly up-regulated by both ABA and drought-stress. Thus, all of these data unequivocally demonstrate the negative regulatory function of this *PP2C* in ABA signaling.

Aurelien BOISSON-DERNIER and Josef KUHN contributed equally to this work.

456 PIK1 (PHYTOCHROME INTERACTOR WITH KELCH REPEATS 1) is a positive acting component of light signaling

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Arabidopsis seedlings undergo profound developmental changes when transitioning from dark-grown to light-grown conditions. This transition is mediated in part by members of the phytochrome (phy) gene family. To understand the early steps of phy signaling, we performed a yeast two-hybrid screen to isolate phy interacting proteins. Here we present the characterization of one of these proteins. PIK1 interacts both in yeast and in vitro with phyA and phyB, independent of phy conformation. PIK1 localizes to the nucleus and the ER. A T-DNA knockout, pik1-1, shows a long hypocotyl phenotype in continuous red light, suggesting that PIK1 acts as a positive component in red light signaling. Interestingly, while phyB levels remain unchanged in pik1-1, phyA degradation is delayed in the mutant. Preliminary data shows that PIK1 interacts with degradation machinery. A role for protein degradation in light signaling has been well established, with the ubiquitin E3 ligase activity of COP1 targeting both phyA and downstream components such as LAF1 and HY5 for degradation. Isolation and characterization of PIK1 will further our understanding of the role of regulated protein turnover in phy signaling and give us further insight into phyA degradation.

457 Genome-wide identification of transcription factors involved in the initial phase of salt stress in rice

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Rice is an important food crop and model plant for cereal genomes, and various abiotic stresses limit rice production. To survive against these stresses, plants response and adapt with complex mechanisms, including development, morphological and biochemical strategies, altering the expression of complex networks. The rice genome has been sequenced and detailed analyses reveal that a significant percentage of the encoded proteins correspond to transcription factors (TF), which are often organized into regulatory cascades. The knowledge about stress signal transduction could be also vital for continued development of rational breeding and transgenic strategies to improve stress tolerance in crops. Based on this background, we are interested to investigate the differential expression of rice transcription factors in response to salt stress. Different indica varieties were obtained from the Institute of Biotechnology (Hanoi, Vietnam) and were ranked based on the index of relative tolerance. A resistant and a sensitive variety were selected for further experiments. Based on a Pfam search, around 2600 rice transcription factors were identified in our group which are subsequently organised in the rice transcription factor database (http://ricetfdb.bio.uni-potsdam.de). Due to usually low expression level of transcription factors we decided to employ RT-RT-PCR to screen for differentially expressed genes in the root tissue upon salt stress. For this purpose primers for all rice TF genes were designed and synthesised. Transcription level of 71% of all target genes could be measured in these two varieties in salt- or non-stress conditions. A total of 313 transcription factors were identified as salt regulated. These TF are belonging 35 families included bZIP, AP2 and zinc finger, which have been showed to be related to salt response. Upon the identification of differentially expressed genes, the mechanism of regulation should be further studied using bioinformatics as well as molecular-biological and physiological methods.

458 The role of temperature in redundant auxin biosynthesis pathway utilization

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Auxins represent a class of diffusible, growth-promoting compounds that play critical roles in a wide variety of plant physiological processes. Indole-3-acetic acid (IAA) is the primary auxin found in most plants and its levels are under tight regulatory control. We have been working to identify genes involved in auxin regulation by pairing genetic and analytical methods. Genetic methods include generating mutants defective in crucial steps in the IAA synthesis pathway. The analytical methods include stable isotope labeling coupled with gas chromatograph mass spectrometry (GC-MS) to monitor Trypotophan (Trp)-dependent and Trp-independent IAA synthesis. We are specifically interested in the role of temperature in the utilization of these two pathways.

Three mutants of use in this study are the cyp79B2 and cyp79B3 single knockouts and the cyp79B2cyp79B3 double knockout. CYP79B2 and CYP79B3 encode cytochrome P450s that metabolize Trp to indole-3-acetaldoxime (IAOx). IAOx is a branch point compound between IAA and indole glucosinolate synthesis pathways in Arabidopsis. We show results from experiments designed to test the hypothesis that the CYP79B2 and CYP79B3 Trp-dependent IAA synthesis pathway is regulated by temperature.

459 RFI2, a RING-domain Zinc-finger protein, integrates phytochrome and circadian signaling with photoperiodic flowering control

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Light is arguably the most important resource for plants, and an array of photosensory pigments enable plants to develop optimally in a broad range of ambient light conditions. The red and far-red light-absorbing photosensory pigments or phytochromes regulate seedling de-etiolation responses, photoperiodic flowering, and circadian rhythm. We have identified a long hypocotyl mutant under red and far-red light, rfi2-1 for red and far-red light insensitive 2-1. rfi2-1 was also impaired in phytochrome-mediated end-of-day far-red light response, cotyledon expansion, far-red light block of greening, and light-induced expression of CAB3 and CHS. RFI2 was identified through the segregation of two T-DNA insertions into different recombinant lines, genetic rescue, and phenotypic characterization of rfi2-2. RFI2 encodes a nuclear protein with a C3H2C3-type zinc finger or RING-domain known to mediate protein-protein interactions. In addition to its defective de-etiolation responses, rfi2-1 flowered early, similar to phyB-9 but in contrast to phyA-211, under both long-day and short-day photoperiods. The early flowering phenotype is accountable by an enhanced expression of CO, a gene that promotes floral transition, in rfi2-1. Further genetic analysis indicated an epistasis of co-2 to rfi2-1. RFI2 also showed a long-day photoperiod-enhanced expression and a free-running circadian rhythm similar to CO. However, the free-running rhythms of TOC1 and CCA1 expression were not affected in rfi2-1, suggesting a function of RFI2 downstream of the clock and under the control of the clock. RFI2 therefore reveals a previously unidentified step that integrates phytochrome and circadian signaling to control photoperidic flowering.

460 Circadian regulation of auxin signaling in *Arabidopsis*

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Plants use an internal clock to efficiently coordinate endogenous processes with environmental rhythms. To better understand the physiological relevance of circadian regulation and the mechanism behind it, we investigated the circadian transcriptome of *Arabidopsis* using Affymetrix ATH1 arrays. Over 10% of the genes detectably expressed in seedlings are clock-regulated at the steady-state mRNA level. Pathways and processes with an over-representation of circadian-regulated genes include primary and secondary metabolism, specific types of transcription factors, aspects of post-translational modification, and hormone biosynthesis and signaling. We also identified and confirmed the functionality of promoter motifs that confer circadian rhythmicity in different phases than the previously identified evening element. To further investigate the relationship between the clock and plant growth and development, we focused on interactions between the clock and the plant hormone auxin. The expression of an unexpectedly large proportion of auxin-signaling components and auxin-responsive genes was found to be circadian, with genes that promote auxin signaling (ARFs) out of phase with antagonists (Aux/IAAs) and auxin-inactivating enzymes (Group II GH3s). We then demonstrated that the synthetic auxin-responsive promoter DR5 confers dawn-phased circadian expression on a luciferase reporter gene. *DR5::LUC* expression is induced following exogenous treatment of plants with auxins. Furthermore, the acute induction of *DR5::LUC* by exogenous IAA is gated by the clock, demonstrating that plant responsiveness to exogenous auxin is under circadian regulation. This induction is greatest when the circadian-regulated abundance of transcripts encoding two ARFs are at peak levels and those encoding six AUX/IAAs and three IAA-amido conjugating enzymes (Group II GH3s) are at their lowest levels, suggesting that rhythmic auxin signaling and/or rhythmic inactivation of auxins may contribute to circadian regulation of auxin responses. Given the ability of auxin to promote cell elongation, this circadian regulation may have a role in phenomena such as circadian control of hypocotyl elongation. These findings illustrate the power of a genomics approach to studying circadian mechanisms.

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461 Arabidopsis *ONSET OF LEAF DEATH* genes regulate leaf senescence by modifying Age-Related Changes

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The onset of leaf senescence is controlled by leaf age and ethylene can promote leaf senescence within a specific age window. Thus, ethylene-induced senescence depends on age-related-changes (ARCs) of individual leaves. We exploited the interaction between leaf age and ethylene and isolated mutants with altered leaf senescence that were named as *onset of leaf death* (*old*) mutants. The relationship between ethylene and age in the induction of leaf senescence was tested in eight *old* mutants and compared to the wild type. Plants with a constant final age of 24 days were exposed to ethylene for 3 to 16 days. Increasing ethylene treatments of 3 to 12 days caused an increase in the number of yellow leaves in the wild type. However, an ethylene exposure time of 16 days resulted in a decrease in the amount of yellowing. Thus, ethylene can both positively and negatively influence ARCs, depending on the length of the treatment. The *old* mutants showed and altered response to the ethylene treatments. *old1* and *old11* were hypersensitive to ethylene in the triple response assay and an ethylene exposure time of 12 days already resulted in a decrease in the amount of yellow leaves. The other *old* mutants did not show a decrease in yellow leaves with an ethylene treatment of 16 days. The results revealed that the effect of ethylene on ARCs can be modified by at least 8 genes. The nature of the *OLD* genes in relation to the senescence phenotype will be discussed.

462 Identification and characterization of transposon activation tagged mutants for Abiotic stress tolerance

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Plants are sessile and thus have to withstand environmental challenges such as soil salinity, drought and cold temperatures which affect plant growth and decrease crop productivity. An understanding into the mechanisms providing Abiotic stress tolerance would help produce plants that are tolerant to such stresses and ensure crop stability. We have generated an Arabidopsis activation tagged populations using the En-I transposon system with about 13,000 lines. Our aim was to use this population to screen for various Abiotic stress resistance e.g. drought, salinity and cold. We first selected lines which were compromised for fitness (e.g. low seed set, poor growth) that would comprise plants that had overexpression of stress tolerance pathways. A selected sub-population of about 279 lines were screened for drought resistance, where the plants were put under water deficit beyond wilting point and allowed to recover after rehydration. The drought screening revealed 9 mutants that were found to be significantly resistant. These mutants will be studied further by identifying the activated genes and overexpressing them in Arabidopsis with the constitutive 35s promotor and check for recapitulation of the stress tolerance phenotype. Subsequently, these mutants will be tested for salt and cold resistance and their mechanism for resistance will be investigated. From a screen of a set of mutants identified on basis of a morphological phenotype 5 of them were found to be drought resistant. One of these 5 mutants called *shine (shn)* was characterized further. The gene responsible for the *shine* phenotype belongs to the AP2/EREBP transcription factor family. The *shn* mutant displays bright green shiny leaves due to a six-fold increase of cuticular wax, composed primarily of alkanes. The *shn* mutant and 35S: SHN Arabidopsis overexpression lines show significant drought tolerance compared to wild type. Chlorophyll leaching assays and fresh weight loss experiment indicated that overexpression of the *SHN* gene increased the cuticle permeability. Most significantly there is a 30% reduction in stomatal index, presumably reducing water loss due to transpiration. There are 3 closely homologous AP2 family members with characteristic domains that define the *SHN* clade, as they functionally confer similar phenotypes when overexpressed. Expression analysis using promoter–GUS fusions of the 3 *SHN* clade genes reveal expression in distinct tissues having a role in plant protective layers. Expressing these genes in different cultivated crops would be useful to make them drought tolerant.

463 UV induced Programmed cell death

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Exposure to UVC radiation can bring about programmed cell death in Arabidopsis. Doses up to 50kJ have been shown to cause PCD through experiments with caspase-inhibitors and the induction of a DNA ladder, one of the well known hallmarks of PCD in multicellular systems.

Irreversible bleaching occurs after UVC has been administered; this occurs over a time course of approximately 96h, and is light dependent. The mechanism of light-dependent cell death is likely to be linked to signalling pathways involving photoreceptors or to the chloroplast, or indeed, both. Other workers have shown that phytochrome signalling is likely to play a role in hormone-mediated cell death. We show that chlorophyll degradation, increase in fluorescence emission by PSII and hydrogen peroxide production are all detectable during UVC-induced PCD thus the role of the chloroplast is likely to also be important. Work with wild type and photoreceptor mutant cell suspension cultures is underway to establish the nature of early events in light-dependent PCD.

Up regulation of specific genes has been reported during PCD. In contrast other experiments have indicated that PCD can be translation independent, thus requiring no new gene expression to occur. Our current work tries to establish what different forms of PCD occur dependent or independent of translation.

464 FRD3 is Required for Efficient Root to Shoot Iron Translocation

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Iron, while being an essential micronutrient, becomes toxic if present at high levels. Plants therefore possess carefully regulated mechanisms to acquire iron from the soil. The Strategy I response, found in all plants except the grasses, involves the release of protons into the rhizosphere, the induction of Fe(III) chelate reductase and increased Fe(II) transport activities. The *frd3* mutant of *Arabidopsis thaliana* exhibits constitutive expression of these iron uptake responses and is chlorotic. We have shown these phenotypes are the result of mislocalization of iron in both the root and the shoot of Arabidopsis plants. Reciprocal grafting experiments with *frd3* and wild type Arabidopsis plants have demonstrated that the phenotype of a grafted plant is determined by the genotype of the root, not by the genotype of the shoot. This indicates that FRD3 function is root-specific and points to a role for FRD3 in delivering iron to the shoot in a usable form. Direct measurement of iron levels in shoot protoplasts has shown that intracellular iron levels in *frd3* are only about half the levels in wild type. Histochemical staining for iron revealed that *frd3* mutants accumulate high levels of ferric iron in their root vascular cylinder, the same place the FRD3 protein is located. Additionally, *frd3* xylem exudate contains approximately half as much iron as does xylem exudate from wild type plants. Taken together, these results clearly indicate a role for FRD3 in root to shoot iron translocation. The FRD3 protein belongs to the multidrug and toxin efflux (MATE) family; other MATE proteins are known to export low-molecular weight organic molecules. Therefore, we hypothesize that FRD3 loads into the xylem an iron chelator or other factor necessary for efficient iron uptake out of the xylem or apoplastic space and into leaf cells. Currently we are testing this hypothesis by examining the levels of known iron chelators in xylem exudate from wild type and *frd3* mutants. We are also expressing the FRD3 protein in both yeast and Xenopus oocytes to examine transport function.

465 Cellular Model for Oxidative Stress Tolerance Activated by Glycine Betaine

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Glycine betaine (GB) is a naturally occurring osmoprotectant that can improve plant tolerance to several different kinds of stress, including stresses caused by chilling, frost, salt and high-light intensities. GB can be applied directly to plants or be produced by transgenics expressing GB biosynthetic genes. Based on the low concentrations of GB that can cause improvements in stress tolerance, we hypothesized that at least part of GB's effect could be ascribed to the activation of the expression of stress tolerance genes. By a strategy based on high-throughput gene expression analysis with microarrays, we have now identified 24 genes activated by GB in leaves of Arabidopsis. Several of these GB-activated genes reinforce intracellular processes protecting cells from oxidative damage while others appear to be involved in setting up a scavenging system for reactive oxygen species (ROS) in walls of leaf cells. Genes for establishing the extracellular ROS scavenging system include two transcription factors, membrane trafficking proteins such as RabA4c and RabB1b, the cell wall peroxidases ATP3a and ATP15a, extracellular ascorbate oxidase and the plasma membrane NADPH oxidases FRO6 and FRO7. On the basis of evidence from gene expression studies, knockout mutants and transgenic plants expressing GUS driven by the RabA4c promoter, we propose that the extracellular ROS scavenging system represents an important oxidative stress protection mechanism in plants that complements other mechanisms already described. It is also interesting to note that oxidative stress may represent a common feature of stresses caused by chilling, frost, salt and high-light intensities. Our goal is to use GB as a model for obtaining a better understanding of how plants survive conditions of stress.

466 The Role of the Heme-Oxygenase Gene Family in Photomorphogenesis

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The oxidative cleavage of heme by heme oxygenase (HO) to form biliverdin IXa is the committed step in the biosynthesis of the phytochrome chromophore. There are four HO genes (HO1-4) encoded within the *Arabidopsis* genome. The *ho1(hy1)* and *ho2* mutants have previously been described. *ho1* mutants have long hypocotyls, decreased chlorophyll accumulation, and are deficient in (3E)-phytochromobilin, the chromophore of plant phytochromes. The *ho2* mutant has a similar but less dramatic phenotype indicating that it plays a minor role in photomorphogenesis. Here, we attempt to define the function of HO3 and HO4 in *Arabidopsis*. Through a phenotypic analysis of T-DNA insertion mutants in *ho3* and *ho4* in combination with other HO family member mutants, we show that both HO3 and HO4 function in photomorphogenesis. The *ho1* phenotype is enhanced with the addition of *ho3* or *ho4*. The phenotype of the *ho1/ho3/ho4* triple mutant is enhanced further. Our focus is to further describe the specific role of each HO, and to establish whether HOs have non-photomorphogenic roles. Ultimately, we plan to create a quadruple HO mutant that will be completely deficient of HO activity resulting in a plant completely deficient of functional phytochromes.

467 Nitrogen biosensing in *Arabidopsis thaliana*

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Nitrogen deficiency in plants initiates an array of responses from physical changes to alterations in the expression of entire sets of genes. Although detailed studies have been conducted on the morphological and physiological changes during nitrogen starvation, little is known about how the plant perceives the status of nitrogen in the plant in various tissues through the life cycle. We have developed nitrogen biosensing lines in *Arabidopsis thaliana* that harbor nitrogen starvation-inducible luciferase (LUC), GFP and GUS reporters. Expression of these reporters is mediated by Gal4 which is under the control of a promoter that responds to nitrogen (*AtAmt1.1*, ammonium transporter). The effects of light (amount and intensity) and various nitrogen sources have been characterized in these lines.

468 Overlapping and Distinct Roles of PRR7 and PRR9 in the Arabidopsis Circadian Clock

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Autoregulatory feedback loops represent the main molecular mechanism of the circadian oscillators studied so far. In *Arabidopsis*, the first identified feedback loop was based on the transcription factors *CCA1* and *LHY* and the pseudo-response regulator *TOC1*. *PRR7* and *PRR9* are, as *TOC1*, members of the circadian regulated pseudo-response regulator gene family, but in contrast to *TOC1* their peak of RNA expression lays in the morning. We have studied the role of these morning expressed PRRs by analyzing the circadian rhythms of the single and double mutants of *PRR7* and *PRR9*. Detailed analysis of the free running period of *prr7-3* and *prr9-1* under different light qualities and quantities suggests that these genes play distinct roles in the regulation of the light signals to the central oscillator. The double mutant *prr7-3 prr9-1* displays a very strong circadian phenotype, having a free running period of up to 35h, and significantly compromised rhythms under constant darkness, which indicates that *PRR7* and *PRR9* also play some overlapping roles in the regulation of the circadian rhythms. In addition, we could show that CCA1 and LHY act positively on *PRR9* and *PRR7* expression, and that this effect is likely to be direct. Therefore, we propose that as demonstrated recently in animals, the *Arabidopsis* circadian oscillator is composed of several interlocking positive and negative feedback loops.

469 The Beta Subunit of the Heterotrimeric G-Protein is Required for a Plastidic Response to a Pulse of Green Light

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Light quantity, quality and duration guide the progression of developmental transitions in plants. During the transition from darkness to light a programmatic shift in gene expression prepares the developing seedling for autotrophic growth. Transcriptome profiles corresponding to photomorphogenic development are well understood and feature blue or red light induction of transcripts associated with chloroplast function. However, treatment with a short, single pulse of green light leads to rapid down-regulation of a suite plastid transcripts within 1 h. The response persists in all known photomorphogenic mutant backgrounds and is specific to green light. The plant heterotrimeric G-protein has been previously implicated in light responses. A reverse-genetic approach was employed to test the hypothesis that the green light response is G-protein mediated. A candidate transcript from the microarray, *psaA*, was used to assay the response. In the G-alpha subunit mutants rapid down-regulation of *psaA* transcripts is identical to that of wild-type. However, in the G-beta subunit mutants down-regulation of *psaA* transcript levels is not observed, and the transcript actually accumulates above dark levels. These findings indicate that the G-beta subunit is indispensable for the normal response to this minor treatment with green light, and present the exciting possibility that the plant G-protein may be delivering environmental information to the chloroplast.

470 RFK1, an F-Box Kelch Domain Protein required for Blue-Light Destabilization of *Lhcb* Transcripts

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In pea and Arabidopsis the steady-state level of the blue-light-induced *Lhcb* transcript is regulated by two antagonistic systems. The first system increases transcript abundance in response to a low-fluence pulse of blue light by increasing the rate of *Lhcb* transcription. This system is balanced by the blue high-fluence system that destabilizes the transcript in response to a single pulse of high-fluence blue light. Sequences in the 5'-UTR are sufficient to confer blue high-fluence induced destabilization. This report describes the identification and characterization of *Rfk1* (*RNA-associated, F-box, Kelch domain 1*), a gene encoding a novel F-box / kelch domain protein that associates with the 5'-UTR of the pea *Lhcb*1*4 transcript. The activity was identified in a yeast-based screen for Arabidopsis proteins that interact with the pea *Lhcb* 5' UTR. T-DNA insertion *rfk1* mutants lack blue-high-fluence induced *Lhcb* transcript destabilization. The mutants also exhibit blue-light specific defects in hypocotyl elongation, indicating that RFK1-mediated transcript destabilization likely affects additional facets of photomorphogenic development. These findings indicate that RFK1 may be targeting proteins that influence *Lhcb* transcript stability and likely other transcripts required for normal photomorphogenic development.

471 A Novel Plant-Specific Protein Family Involved in Abscisic Acid Response

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Abscisic acid (ABA) is a plant hormone that regulates many aspects of plant development including seed maturation, premature germination, and stress tolerance. A key ABA-response locus, *ABA-insensitve 5 (ABI5)*, was identified in our lab by screening for mutations that result in ABA insensitivity at germination. ABI5, which accumulates in germinating seeds in response to ABA, high salinity and drought, is a transcription factor with a basic leucine zipper (bZIP) DNA binding motif. To identify proteins that interact with ABI5 and potentially regulate its activity, we performed a yeast two-hybrid screen of an Arabidopsis cDNA library. Four members of a small highly conserved plant-specific protein family were identified as ABI5 interacting proteins (A5IPs). This family includes four members in *Arabidopsis thaliana* and at least one member each in *Oryza sativa* (rice) and *Helianthus annuus* (sunflower), but lacks homology to protein domains of known function. To date, we have physiological and genetic data to suggest that these proteins are involved in at least two ABI5-dependent processes: gene expression during seed maturation and stress-induced inhibition of germination. Analysis of Arabidopsis loss- and gain-of-function lines suggests that distinct members of this family have similar or antagonistic roles in ABA signaling. Expression of the A5IPs is differentially regulated by various stresses, including ABA, high salinity, and osmotic stress. We have also analyzed yeast two-hybrid interactions between the A5IPs and an ABI5-related subfamily of bZIPs. Members of this bZIP subfamily appear to be involved in various stress responses at distinct developmental stages. Several of the A5IPs interact very strongly with a subset of these bZIPs, which may represent additional targets of the A5IPs *in planta*. Additional yeast two-hybrid data indicates that one of the A5IPs can form homodimers and heterodimerize with other A5IPs. This A5IP appears to be localized to the nucleus. We are in the process of performing a yeast two-hybrid screen with this family member to identify additional protein interactors that will provide clues to its biochemical function.

472 Dissection of ZTL functional domains in the regulation of circadian clock and plant development

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The plant circadian clock controls a wide variety of metabolic, physiological and developmental processes such as flowering time, hypocotyl elongation, stomata opening, photosynthesis activity and gene expression. ZTL is a clock-associated protein in which loss-of-function mutations cause a longer circadian period in all light conditions and shorter hypocotyls in red light. ZTL belongs to a small family of F-box proteins that are in unique combination with two other domains: a LOV (light, oxygen and voltage sensing) domain at the N-terminus and six repeats of a kelch domain C-terminal to the F-box. ZTL forms into an SCF complex (SCFZTL) and functions in the degradation of a key player of the circadian central oscillator, TOC1. However, our knowledge of the coordination of the three distinct domains for ZTL function is still very limited. For a better understanding of the involvement of the three domains of ZTL in these processes, we characterized the phenotypes of transgenic plants that constitutively express high levels of either the LOV or LOV-F domains of ZTL (LOV OX; LOV-F OX). Constitutive expression of LOV or LOV-F cause similar phenotypes: slightly longer period, longer hypocotyls and late flowering in long days. We find that the longer period phenotype is due to the reduction of the endogenous ZTL protein level while the RNA level remains unaffected, suggesting the a protein-protein interaction involving the LOV domains may facilitate ZTL protein degradation. We also show that the LOV and LOV-F domains may be involved in light-dependent inhibition of hypocotyl growth in parallel with Cry1 in blue light, and in coordination with phyB in red light. The late flowering phenotype of LOV and LOV-F overexpressers is due to the reduction of CO gene expression in light period, which in turn results in low level expression of flowering promoting factor FT. Another ZTL family member, FKF1, regulates CO gene expression. Since the message level of FKF1 in LOV and LOV-F OX transgenic plants is unchanged, a potential interaction between LOV domains of ZTL and FKF1 is postulated. This is also supported by recent reports that LOV domain can form dimers and mediate protein-protein interactions among ZTL family members.

473 Arabidopsis thaliana diacylglycerol kinase 2 (AtDGK2) exhibits cold-, wound- and mannitol inducible gene expression

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Evidence indicates that phosphatidic acid (PA) is an important lipid mediator that regulates a number of cellular functions, including growth, development and stress responses in plants. PA may be synthesized by two signaling pathways, one involving phospholipase C (PLC) and diacylglycerol kinase (DGK), and the other involving only phospholipase D (PLD). As diacylglycerol kinases (DGKs) generate PA from diacylglycerol (DAG), we investigated whether DGKs are involved in mediating stress responses in which PA may participate in plants. In Arabidopsis thaliana, seven candidate DGK genes (named AtDGK1 to AtDGK7) encode putative DGK isoforms (Gómez-Merino et al., 2003). We have cloned the AtDGK2 cDNA and demonstrated that it encodes a functional DAG kinase that associates with membranes when expressed as a recombinant protein in bacteria. We observed that AtDGK2 transcript accumulates rapidly, within 30 minutes, after exposure to cold (4°C) and enhanced expression remained for up to 24 h (Gómez-Merino et al., 2004). Here, we further investigate the expression of the AtDGK2 gene in response to environmental stress. We found that AtDGK2 gene expression is rapidly induced by wounding remaining at an elevated level for up to 24 h. Analyses of promAtDGK2::GUS reporter lines confirmed wound-inducible gene expression. Furthermore, expression of the AtDGK2 gene is induced by mannitol treatment, its transcript level increases progressively within 30 min and 4 h after treatment. We conclude that AtDGK2 is induced by multiple abiotic stresses. We hypothesize that its protein product may play a role in mediating plant responses to several environmental cues. We are currently characterizing loss-of and gain-of-function mutants to further elucidate the role of AtDGK2 in plant biology.

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474 Phytohormones regulate various aspects of the circadian systems

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Both phytohormones and the circadian clock control the life cycle of plants, and coordinate endogenous events with the external environment. These processes have been believed as distinct pathways. We found that phytohormones and the circadian system are actually tightly coupled. To investigate phytohormone influence on circadian rhythms, the classical plant hormones were exogenously added in separate experiments and rhythms were assayed *via* the promoter:*luciferase* system. The data were analyzed mathematically for various clock parameters. Interestingly, many hormones control various aspects of the circadian system, including period, phase, amplitude, and accuracy. In particular, we found that cytokinins delayed circadian phase, that auxins regulated circadian amplitude and clock accuracy, that brassinosteriods shortened the circadian periodicity and increased amplitude, and that abscisic acid lengthened circadian periodicity and reduced amplitude. To confirm the hormone pharmacology, we analyzed circadian rhythms in a variety of hormone synthesis and signaling mutants. Many of these mutations affected the circadian systems in ways predicted from the pharmacology. We further went on to characterize the genetic mechanism by which one hormone response leads to an input to the clock. The response regulator ARR4 and the photoreceptor phytochrome B were identified as components that integrate the circadian cytokinin signal. We then tested whether the clock controls hormone biology. The circadian expression profiles of hormone-related genes, encoding elements of hormone synthesis, perception, and trafficking, were found to be under clock control, suggesting that hormone signal flux oscillates. We are also investigating whether the steady-state levels of endogenous phytohormones are clock regulated. In preliminary data, several phytohormone levels were found to be rhythmic. Our interpretation is that multiple hormonal inputs affect the circadian system, and that hormone responses are themselves likely clock regulated. Plants therefore have a unique and flexible clock system(s), fine-tuned by multiple hormones that functions as an integrated, feedback system. This is of likely benefit to enhance a plant's perception of the day under changing environmental conditions.

475 Integrated metabolite and transcript profiling of nine *Arabidopsis* accessions to define processes important for acclimated and non-acclimated freezing tolerance

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Many temperate plant species such as *Arabidopsis* increase their freezing tolerance when exposed to low, nonfreezing temperatures. This process of cold acclimation is a multigenic and quantitative trait associated with complex physiological and biochemical changes. Changes of gene expression during this process are well-documented. Recently, gene expression profiling has revealed hundreds of changes in response to low temperature. However, these studies have focused mainly on single genotypes making it difficult to separate treatment dependent changes from those functionally related to the process of cold acclimation. Additionally, these studies have not investigated how such changes of gene expression correlate with changes at the metabolite level. We used parallel metabolite and expression profiling for nine *Arabidopsis* accessions before, and after, two weeks of cold acclimation (4°C). Quantitative measurements of leaf electrolyte leakage revealed significant differences in both non-acclimated and acclimated freezing tolerance. Using three independent experiments, expression profiling was performed using Affymetrix ATH1 arrays and metabolite profiling using GC/TOF-MS. A thorough statistical analysis of both datasets was performed prior to functional testing and the integration of transcript and metabolite data into metabolic pathways. These data provide a comprehensive survey of the massive transcript and metabolite changes during cold acclimation. There is considerable overlap between the responses shown by different accessions, although we also identified differences in the specificity and magnitude of these changes. Moreover, we identified changes that correlate significantly with acclimated freezing tolerance across the different accessions. Finally, we identified changes that were associated with the significantly higher non-acclimated freezing tolerance of one accession in comparison to the other accessions. Whilst there are many changes specific to each of these analyses there is also significant overlap which may indicate basic principles of cold acclimation in *Arabidopsis*. Of broader relevance, the use of natural variation combined with metabolite and transcript profiling offers a novel and significant advance in the identification of processes functionally involved in the interaction of plants with their environment.

476 Coupling of the circadian clock and the 26S proteasome to modulate light signaling pathways in .*Arabidopsis*

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Light is an important environmental cue to plants and much of their physiology is influenced by light. The rhythms of the circadian clock have a profound influence on light signaling, such that the strength of the response to a light stimulus varies depending on the time of day it is given. This diurnal difference in responsiveness is a hallmark of circadian gating. The ubiquitin/26S proteasome system is responsible for the turnover of specific target proteins, and its activity is a key regulatory step in plant signaling processes like hormone and light responses. Identification of the condemned protein is accomplished by an E3 ubiquitin ligase that often utilizes an F-box protein as an adapter to recognize and bind the target protein. Previous work has shown that the F-box protein Attenuated Far-Red Response (AFR) plays a positive role in phytochrome A (phyA)-mediated light signaling in .*Arabidopsis*. Investigation into the target of the AFR E3 ligase has uncovered a novel signaling cascade that relies on the type of proteins initially associated with signaling in response to the hormone auxin. As such, this pathway may represent an integration point for hormone and light information. Yeast two-hybrid and co-immunoprecipitation suggest that AFR physically interacts with a transcriptional repressor belonging to the AUX/IAA family. Interestingly, this AUX/IAA protein, IAA9, also interacts with the C-terminal portion of phyA, and previous reports suggest that it is phosphorylated by this photoreceptor *in vitro*. Steady-state levels of epitope-tagged IAA9 *in vivo* are significantly elevated in plant lines lacking AFR function. Furthermore, genetic analysis demonstrates that IAA9 acts downstream of AFR as a repressor of light signaling. IAA9 belongs to a large class of transcriptional repressors that typically inhibit transcription by forming heterodimers with a transcription factor of the AUXIN RESPONSE FACTOR (ARF) family. Yeast two-hybrid experiments have identified the ARF protein that specifically interacts with this AUX/IAA protein. Current work is aimed at identifying the target genes that lie downstream on this transcription factor, which will serve to integrate it action into the phyA signaling network. Finally, seedlings lacking ARF function do not display the characteristic increase in light responsiveness that is observed following onset of dawn. This finding raises the possibility that circadian expression of AFR plays an important role in circadian gating of far-red light signaling.

477 Investigating the Function of ARG1 and ARL2 in the Root Gravitropic Response of Arabidopsis

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The *Arabidopsis* protein ARG1 and its paralog ARL2 are required for early signaling in root and hypocotyl gravitropism. They contain N-terminal DnaJ domains and predicted C-terminal coiled-coils. ARG1 is a peripheral membrane protein that associates with membranes of the secretory and vesicle trafficking pathways, and is required for the rapid alkalinization of gravity-sensing columella cells' cytoplasm upon gravistimulation (1). Experiments with the auxin-responsive DR5-GUS and GFP reporters demonstrate that ARG1 and ARL2 also mediate auxin flux through the root tip and lateral redistribution of auxin toward the lower flank of gravistimulated roots. Lateral auxin redistribution is required for the root gravitropic response and is thought to be mediated by a relocalization of the auxin efflux facilitator PIN3 to the new bottom side of columella cells upon gravity stimulation (2). We have used polyclonal antibodies raised against PIN3 to demonstrate that PIN3 fails to relocalize in columella cells of the arg1-2 and arl2-3 mutants, even 40 minutes after initiation of the gravity stimulus. These results demonstrate that ARG1 and ARL2 are required for the gravity-responsive relocalization of PIN3, further defining a role for these proteins in the early phases of root gravitropic signaling, during or prior to PIN3 relocalization.

(1) Boonsirichai et al., (2003) Plant Cell 15:2612
(2) Friml et al. (2002) Nature 415:806

478 *LUX ARRHYTHMO* Encodes a Novel Myb Transcription Factor Essential for Circadian Rhythms

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In higher plants the circadian clock orchestrates fundamental processes such as light signaling and the transition to flowering. We isolated mutants of the circadian clock from an Arabidopsis mutagenized reporter line by screening for seedlings with long hypocotyl phenotypes and subsequently assaying for abnormal clock-regulated *CAB2::LUC* expression. This screen identified five mutant alleles of a novel clock gene, *LUX ARRHYTHMO* (*LUX*), that significantly affect amplitude and robustness of rhythms in both constant white light and dark conditions. In addition, the transition from vegetative to floral development is accelerated and hypocotyl elongation is accentuated in these mutants under light:dark cycles. We genetically mapped the mutations by bulk segregant analysis with high-density oligonucleotide array genotyping to a small single Myb domain transcription factor related to other clock components and response regulators in Arabidopsis. The negative arm of the Arabidopsis circadian clock, *CIRCADIAN CLOCK ASSOCIATED* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), is repressed in the *lux* mutants, while *TIMING OF CAB2 EXPRESSION* (*TOC1*) is activated. We demonstrate that CCA1 and LHY bind to the evening element motif in the *LUX* promoter, which strongly suggests that these proteins repress LUX expression, as they do TOC1. The data is also consistent with LUX being necessary for activation of *CCA1* and *LHY* expression.

479 HY5 interacting B-box proteins regulate plant growth and development

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Light signaling results in dramatic changes of the transcriptional program during seedling de-etiolation. This is causing inhibition of hypocotyl elongation, promotion of cotyledon expansion and synthesis of a number of pigments, including chlorophyll and anthocyanin. The bZIP transcription factor HY5, which act downstream of several photoreceptors, is an important positive regulator of these processes. In the absense of light, the activity of HY5 is negatively regulated by COP/DET/FUS mediated degradation of the HY5 protein. We have found that HY5 interacts with STH and three related B-box containing proteins and that the interactions require the DNA binding bZIP domain in HY5 and the B-box domains in the STH like proteins. Phenotypic analysis of T-DNA insertion mutants in the four STH like genes revealed pigmentation, hypocotyl and root phenotypes suggesting a positive role in HY5 regulated processes. Furthermore, STH enhances the ability of HY5 to activate reporters for HY5-regulated genes (e.g. chalcone isomerase) in a transient protoplast assay. Thus, we present genetic, biochemical and cell biological data suggesting that these B-box proteins positively regulate HY5 activity in the light.

480 Dissecting ABA and Stress-related Signaling Networks Using ABA Analogs

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The phytohormone (+)-abscisic acid (ABA) plays an essential role in regulating responses to abiotic stresses such as drought. ABA analogs are structurally similar to natural ABA and many exhibit ABA-like effects. HyperABAs such as (+)-8'-acetylene ABA (PBI425) are analogs that are more persistent in vivo and exhibit stronger biological effects than ABA itself. Other ABA analogs such as (-)-ABA and (-)-2', 3'-dihydroacetylenic abscisic alcohol (PBI51) exhibit weaker and selective or anomalous ABA-like biological effects. To dissect ABA and stress signaling network, genome-wide profiling of gene expression in response to (+)-ABA, (-)-ABA, PBI51 and PBI425, drought, rehydration were compared using Arabidopsis oligonucleotide microarrays (26K). The results revealed 1) High similarities existed in gene regulation among ABA, ABA analogs and drought treatments and the overall regulatory effects were consistent with their biological activities; 2) More ABA responsive genes including many novel genes not reported before were identified using hyperactive PBI425; 3) The differences in expression patterns between (+)-ABA and analogs revealed that the ABA signaling network could be dissected into signaling modules (regulons) enriched in specific gene functions. These differences might reflect hormone-receptor interactions, differential transport/metabolism or cell/tissue specificity.

481 Arabidopsis photomorphogenic regulatory network discovery *in silico* and *in vivo*

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We have developed new computational tools to facilitate *cis*-regulatory DNA motif analysis from Arabidopsis transcriptional profiling and genomic sequence data. The tools use the enumerative method for motif discovery and are being implemented for public access via the web. They allow both the discovery of new regulatory motifs and the analysis of promoters according to whether known regulatory motifs are overrepresented and conserved in their position within a promoter sequence.

We have applied these tools to furthering knowledge of the transcriptional regulatory network of light signaling in Arabidopsis, which is extensively but incompletely characterized. Interest in this regulatory network has previously been focused on the immediate / early events that transduce signals from the photoreceptors. The downstream events in this transcriptional cascade are currently less well understood. Using the tools we have developed for regulatory motif discovery, four motifs have been identified in phytochrome A-inducible promoters. We have shown that all four sequence motifs are capable of conferring light-inducibility on a reporter gene in vivo. These elements are over-represented in the promoters of genes that respond later in the de-etiolation process, and may represent new transcriptional regulatory pathways under the control of phytochrome A.

By using the output of the motif discovery software to define a set of features which in combination define light-regulated promoters, we have been able to develop a support vector machine model describing the combinations of motifs that can trigger induction of gene expression by phytochrome A. Using this model we can predict the behavior of a large number of Arabidpsis light-induced genes *in silico*, based solely on the sequence of their promoters. The annotation and prediction of gene expression from promoter sequence in Arabidopsis will be discussed.

482 Exploring a new detergent-inducible promoter active in higher plants and its potential biotechnological application

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Abstract

For the biotechnological use of higher plants, it is necessary to ensure the formation of the desired recombinant product in the right organ at the right time, and in the right cellular location, in large quantities and without exerting toxic effects in the cell. For many purposes, it may be desirable to exert exogenous control over the production of recombinant proteins using chemically controllable promoters. At the Department of Plant Physiology of the Ruhr-University Bochum, the promoters of 12-oxophytodienoic acid reductases (OPR1 and OPR2) have been isolated in order to investigate jasmonic acid biosynthesis. Although OPR1 is not the isoform that is important for in vivo jasmonate production, the OPR1-gene has a very sensitive promoter which can be activated by mechanical and chemical inducers (Biesgen and Weiler, 1999; Sanders et al 2000; Schaller et al 2000; Stintzi et al 2000; He and Gan, 2001). One of those chemical inducers is the non-ionic detergent Tween 20. A number of deletions of the OPR1-promoter have been designed and tested for their properties and range of applications, using reporter gene analysis (β-glucuronidase) to assay for promoter strengths and characteristics by Northern Blot and GUS fluorometric assays. Microarray analyzes were performed in order to search for new Tween 20-inducible promoters and to elucidate how the *Arabidopsis thaliana* genome is affected after Tween 20 application. Several genes were tested using RT-PCR and computer analysis, only one was chosen for further promoter analysis. The aim of the project is to advance our understanding of detergent application-oriented promoter-design in the biotechnological use of higher plants.

Key words

Inducible promoter, detergent, Tween 20, OPR, promoter-GUS-fusion.

483 FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*

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Plants monitor changes in day length and process this information to modulate growth and developmental events such as flowering. In the model plant *Arabidopsis thaliana*, key mechanisms for the regulation of photoperiodic flowering are the temporal control of CONSTANS (CO) expression and activity. The FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) protein has been shown to be crucial in regulating diurnal *CO* transcription, although the molecular mechanism is unknown. We demonstrated that FKF1 exerts control over the stability of a Dof (DNA with one finger) transcription factor, CYCLING DOF FACTOR 1 (CDF1), that appears to suppress *CO* transcription. FKF1 physically interacts with CDF1 in yeast and *in vitro*, and CDF1 protein is more stable in *fkf1* mutants. Plants having elevated levels of *CDF1* flower late and have reduced expression of *CO*. In addition, CDF1 binds to specific elements of the *CO* promoter *in vitro*. As our data also indicates that the regulation of *CO* expression involves components whose functions overlap with CDF1, we propose that FKF1 precisely controls *CO* expression in part by degrading the repressor CDF1 in the late afternoon. This mechanism contributes to generate the daytime *CO* expression pattern that is critical for day length discrimination.

484 An *Arabidopsis HOS6* Locus Confers Both ABA Hypersensitivity And Tolerance To Dehydration Stress

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To investigate essential components that control stress signalling and acclimation in plants, we initiated a large scale stress response screen using *Arabidopsis* plants carrying a transgene consisting of the firefly luciferase coding sequence *(LUC)* driven by the stress-responsive *RD29A* promoter. We report here, the isolation and characterization of a mutant, *hos6-1* (for high expression of osmotically responsive genes), in which expression of *RD29A::LUC* was hyperactivated by, ABA, salinity stres and low temperature. Our results show siginificant differences in several stress responses between *hos6-1* mutant and wildtype plants. Germination rate and root growth of *hos6-1* was hypersensitive to ABA. The diurnal water loss from intact plants also showed increased sensitivity to ABA treatment. Moreover *hos6-1* plants dipslayed a significant increase in tolerance to soil dehydation. After witholding of water for 20 days, the shoot fresh weight of *hos6-1* plants was significantly higher than the wild type whereas there was no difference in dry weight. Furthermore when the wilted plants were rewatered, 70% of the *hos6-1* plants recovered and only 5% of wild type plants were able to survive. The basta resistance of *hos6-1* cosegregates with ABA hypersensitive phenotypes. HOS6 appears to play an important role in ABA signalling and phenotype response to deydration stress and we are focusing on determining the identity of HOS6 protein.

485 An Arabidopsis Cyclin –Like Protein, CLP, is involved in plant development

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Cyclins are known as play critical roles in regulation of the cell cycle. The Arabidopsis genome contains 50 putative cyclin proteins, which can be classified into 10 different groups. Only very few functional studies of plant cyclins have been reported to date. Here we show that *CLP*, one of the cyclin family of proteins, plays important roles in plant development. We screened Arabidopsis T-DNA insertion mutant pools in the C24 background, which carry the firefly luciferase reporter gene driven by *RD29A* promoter (*RD29A::LUC*). One mutant showed extremely high constitutive bioluminescence level, without any treatment. TAIL-PCR analysis of this mutant has revealed a T-DNA insertion in the first exon of the Arabidopsis gene encoding a cyclin family protein. This protein has one cyclin_N domain and one cyclin-like domain, but does not have the D-box domain, which is involved in cyclin proteolysis by the ubiquitin-dependent proteasome pathway and is conserved in both cyclin A and cyclin B protein groups. The *CLP* mutation strongly effects leaf morphology and size, the mutant plants also exhibit wider rosette leaves and have more trichomes on the leaves and stems compared to C24 wild type plants. *CLP* plants also have abnormal petal and stamen numbers in the terminal flowers. We suggest that CLP plays important roles in maintenance of plant organ morphology.

486 Hormonal interactions in plant abiotic stress responses

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We have isolated a series of rcd-mutants (radical-induced cell death) that display visible HR-like lesions coupled with increased reactive oxygen species accumulation when exposed to ozone. The rcd1 mutant is insensitive to abscisic acid, ethylene and methyl jasmonate, has constitutively more open stomata than the wild type and the expression and regulation of several ABA-regulated genes (e.g., RAB18, RD29A) is compromised. In the triple response rcd1 displays only slight ethylene insensitivity, but induction of ethylene dependent genes is compromised similarly as in ein2. Induction of jasmonate marker genes in rcd1 is significantly lower than in the wild type, and again, equivalent to ein2, but higher than in the jasmonate-insensitive jar1. The mutation in rcd1 disrupts an intron splice site in a gene that encodes a protein belonging to a small protein family. Members of the RCD1 protein family contain a WWE-domain for protein-protein-interactions, two canonical nuclear localization sequences and poly-ADP-ribosylase (PARP) core domain, which suggests that RCD1 belongs to the (ADP-ribosyl)transferase-subfamily of WWE-containing proteins. Yeast two-hybrid analysis identified several RCD1-interacting proteins, most of which are transcription factors or nuclear-localized proteins related to salt and osmotic stress (e.g., DREB2A). ABA-debendent cold acclimation capability and freezing tolerance of rcd1 is not compromised. Thus, RCD1 seems to be involved in processes that affect interplay between hormonal signaling cascades and identifies a branch in ABA-pathway that affects ABA-dependent gene expression and stomatal regulation, but not cold acclimation or freezing tolerance.

487 Sugar-mediated Regulation of Phosphate Starvation Induced Response in Arabidopsis

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Phosphate (Pi) is the second most important plant nutrient that is responsible for dynamic changes in carbon fluxes. The interaction between Pi and carbon metabolism coordinates several crucial processes that control plant growth and development. In addition plant Pi levels regulate the expression of genes involved in sugar metabolism/synthesis. In this study we have shown the effect of carbon supply on the regulation of expression of Phosphate Starvation Induced (PSI) genes. High light condition or supplementation of sucrose in the medium was found to be essential for the expression of PSI genes under both Pi limiting as well as sufficient conditions. This influence on the gene expression was not confined to sucrose but also observed with other metabolizable sugars studied. Interestingly non-metabolizable sugars palatinose, turanose and 3-O-methylglucose did not induce the expression of PSI genes. Further studies using *gin2* mutant revealed a reduction in the level of expression of PSI genes during phosphate starvation. However no significant changes were observed in the level of free Pi between wild type and *gin2*. The data provides further insight into the effect of various sugars on the regulation of PSI responses and potential role of hexokinase in sugar signaling during PSI responses.

488 Identification of amino acid residues critical for post-translational regulation of the IRTI metal transporter by iron in Arabidopsis

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Although iron is an essential nutrient, it is toxic if accumulated at high levels. Hence, iron uptake and sequestration are controlled by precise regulatory mechanisms. Strategy I plants, including the dicots and non-grass monocots, induce expression of a set of genes in response to iron deprivation. The Arabidopsis *FRO2* (Ferric Reductase Oxidase) gene encodes the inducible ferric chelate reductase responsible for reduction of iron at the root surface. IRT1 (Iron Regulated Transporter) is the major high affinity iron transporter responsible for iron uptake from the soil in Arabidopsis; IRT1 belongs to the ZIP family of metal transporters. Previously, we showed that metals coordinately regulate the transcript abundances of *FRO2* and *IRT1* and that expression of *FRO2* and *IRT1* is subject to post-transcriptional regulation by iron. IRT1 protein accumulates only in iron-deficient roots of *35S-IRT1* plants, while root ferric chelate reductase activity is elevated only in iron-deficient roots of *35S-FRO2* plants. IRT1 contains an intracellular loop that may be critical for post-translational regulation by metals; of particular interest is a histidine motif (HGHGHGH) that is found in many ZIP family members. In addition, we targeted two intracellular loop lysine residues that could serve as attachment sites for ubiquitin. We constructed a set of mutants: IRT1H154Q, IRT1H156Q, IRT1H158Q, IRT1H160Q, IRT14HQ (quadruple his mutant), IRT1K146R, IRT1K171R and a double mutant (IRT1K146R,K171R). All mutants were able to rescue the iron- and zinc-limited growth defects of the *fet3fet4* and *zrt1zrt2* yeast strains, respectively. This result demonstrates that mutation of the his or lys residues does not eliminate the ability of IRT1 to transport iron or zinc. Each of the IRT1 variants and an IRT1intact construct were expressed in plants from the 35S promoter. Mutation of the histidine motif did not alter IRT1 protein stability. However, western analysis revealed that *35S-IRT1K146R,K171R* plants accumulate IRT1 protein when grown on iron-sufficient and iron-deficient medium while *35S-IRT1*, *35S-IRT1K146R* and *35S-IRT1K171R* plants accumulate IRT1 protein only when grown on iron-deficient medium. *35S-IRT1K146R,K171R* plants also accumulate higher levels of metals than *35S-IRTI*, *35S-IRT1K146R*, *35S-IRT1K171R* and WT. These findings suggest a role for ubiquitination in metal-induced removal of IRT1 from the plasma membrane.

489 Functional Profiling of Genes Involved In Early Phytochrome Signaling

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We are using reverse genetics to evaluate functional involvement in phytochrome (phy) signaling of genes identified by two different approaches. First, we have systematically isolated T-DNA insertional mutants in several bHLH (basic Helix-Loop-Helix) family members closely related to PIF3 (Phytochrome Interacting Factor 3). Our ongoing mutant analyses of PIF family members have indicated that they have distinct functions in different facets of seedling photomorphogenesis. Both *pif1*- and *pif3*-mutants have defects in greening. The *pif4*- and *pif5*-mutant seedlings display a hypersensitive morphological phenotype and overexpression of PIF4 and PIF5 leads to a *phyB*-null like phenotype. As a second approach, we have used our previously reported oligonucleotide microarray analysis to identify phy-dependent rapid-response (within 1h) genes. We have systematically analyzed nearly all of the T-DNA insertional mutants available for three functional categories of these early response genes: transcription factor genes, putative signaling component genes and genes of unknown function. We have evaluated 34 loci and found that 41% of these genes displayed marginal to robust light-dependent phenotypes when mutated, indicating that they were necessary to a greater or lesser extent for optimal seedling deetiolation. Of these, 8 new loci (23% of all genes tested), in addition to 6 genes already known to contribute to optimal seedling deetiolation were identified. Some of these genes show functional specificity to treatment with either continuous red or far-red light, while others function in deetiolation response to both continuous red and far-red light. By comparison, a global study of gene-deletion mutants in yeast showed that only 1-7% of genes significantly affected in expression in response to various growth conditions were also functionally necessary for optimal growth under those conditions. In *Arabidopsis*, although a number of studies have used microarray analysis to identify genes responding to external treatments stimulating specific biological pathways, no systematic analyses of the fraction of these genes functionally necessary for optimal response to the signal have been reported. In addition, in order to investigate the mechanism by which phy signals to PIF4 and PIF5 in the cell, we are examining the involvement of phytochrome in regulating the levels of these two proteins.

490 Genetic and physiologic interactions between ZTL and ELF3

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In plants, the circadian clock regulates many aspects of development, including hypocotyl length elongation and photoperiodic induction of flowering. ZTL is a clock- related F-box protein and mutations in ZTL cause a fluence-rate dependent long period phenotype. ELF3 is a novel protein of unknown biochemical function, and elf3 mutations cause light-dependent circadian dysfunction, elongated hypocotyls, and early flowering. Hence, both genes have roles in control of circadian period and photomorphogenesis. To investigate the genetic and physiological interactions between ZTL and ELF3, we crossed elf3-1 and ELF3 overexpressors with ztl mutants (ztl-1, ztl-3) and ZTL overexpressors. We analyzed the flowering phenotype in long and short days, light responsiveness of hypocotyl elongation, and circadian cycling in different light qualities and constant darkness. We found that the early flowering elf3-1 mutations have an additive interaction with ztl mutations, or with ZTL overexpression to regulate the circadian clock and hypocotyls elongation in Arabidopsis. However, control of flowering by these two genes is more complex and the relationship between these genes and CO, FT and FKF1 will be presented.

491 Transcriptome analysis of class B-HSF double knockout mutant in Arabidopsis

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In *Arabidopsis thaliana* there are 21 different HSF genes. Based on amino acid homology, plant HSFs are assigned to two major classes A and B. A striking peculiarity of class B-HSF is the lack of AHA motifs in the carboxyterminal domain, which are crucial for activator function of class A-HSF. The heat-inducible expression of class B-factors, HsfB1 and HsfB2b, suggests that their function is required for delayed effects in the HS response. In order to functionally characterize HsfB1 and HsfB2b, we have isolated T-DNA double knock out mutants. The effect of ***hsfB1/B2b*** mutation on heat shock gene expression was investigated by RT-PCR and Microarray profiling. Using Affymetrix ATH1 genome microarray analysis we found that only 31 genes or 16 genes were differentially expressed in the mutant compared to wild type after heat stress or respectively at normal temperature. Only 3 genes were affected at both temperatures. There is no overlap of ***hsfB1/B2b*** dependent genes with target genes affected in class A-HSF double knockout mutant (***hsfA1a/1b***).

492 A rice hydroxyproline-rich glycoprotein family gene shows high similarity to yeast decapping enzyme gene, dcp1

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The regulation of mRNA turnover is powerful way to control gene expression. An important control step in the mRNA turnover is removal of the m7G-cap structure at the 5' end. The yeast decapping enzyme, dcp1, is known to catalyze the cleavage of the m7G-cap structure (Beelman et al., 1996). By using yeast two hybrid system, we have isolated a hydroxyproline-rich glycoprotein family gene showing high similarity to yeast dcp1, which seem to be involved in rice MAP kinase signal transduction pathway. Expression profiling by Northern blot analysis revealed that this gene is a stress-related gene, supporting its involvement in MAPK signaling cascade pathway. Arabidopsis also has putative dcp1 that has 57.22% homology with this gene. To know more about the function of the gene in plants, physiological responses of mutant line to various environmental stresses, such as water deficit, salinity, low or high temperature, are being analyzed using Arabidopsis T-DNA tagging line.

493 Genetic analyses of *shot1* and *2*, suppressor of *hot1-4*, involved in thermotolerance of Arabidopsis

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*At*Hsp101, a member of the Hsp100/ClpB family, is a hexameric ATPase believed to function in disaggregation of denatured proteins. We have first described that *At*Hsp101 is a major component controlling acquired thermotolerance in higher plants (PNAS 97: 4392). We have also defined a new functional domain based on the phenotype of a semi-dominant mutation, *hot1-4*, in the coiled-coil domain and further isolated intragenic suppressor mutations of *hot1-4* (Plant Cell 17:559). In this study, we analyzed two extragenic suppressor mutants of *hot1-4* to uncover essential substrates or partner proteins of *At*Hsp101, or components of other mechanism required for thermotolerance. ***shot1***(recessive suppressor of *hot1-4*) eliminated the 38 °C sensitivity, but did not restore thermotolerance function to *hot1-4*. In the *hot1-4* background, the *shot1* mutant shows a short hypocotyl phenotype in the dark and near wild type levels of *At*Hsp101 accumulation. The *shot1* mutant displays no thermotolerance defective phenotype in the absence of the *hot1-4* mutation. This suggests that *shot1* affects interaction of *At*Hsp101 with functional partner proteins during an early step in the development of thermotolerance. Current mapping data indicates that *shot1* is located at the bottom of Chromosome 3 between the CIW4 and nga6 markers. In contrast to *shot1*, the ***shot2*** mutant showed a reversion of the thermotolerance defect of *hot1-4* in both pre-adaptation and acquired thermotolerance. *shot2* is a dominant extragenic suppressor of *hot1-4* and is a homozygous lethal mutant, suggesting a direct functional interaction with *At*Hsp101. Further study of these two extragenic suppressors should contribute significantly to our understanding of how Hsp100/ClpB cooperates with substrates and/or cofactors *in vivo*.

494 The circadian clock provides memory of prior light exposure during hypocotyl growth

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Most organisms use a circadian oscillator to predict daily rhythmic environmental changes, but little is known about how the circadian system interacts with acute environmental signals (light, heat, and water) to control growth. We monitored *Arabidopsis* seedling growth for several days after germination under short day conditions or in constant dark. To observe seedlings during both day and night, we used infra-red (IR) illumination and captured time-lapse images with IR-sensitive CCD cameras. We found that rhythmic elongation depends on light, since no circadian rhythm of hypocotyl elongation was observed in entrained plants grown in constant dark. Under diurnal cycles we found that hypocotyls grew at maximum rate around dawn and stopped growing during the night. This is different from the previously reported growth maximum at dusk observed under continuous light (Dowson-Day (1999) Plant J. 17, 63-71). Furthermore, we found that the growth patterns of arrhythmic circadian clock mutants (*elf3-1* and *CCA1-OX*) were distinct from WT, with growth only regulated by the presence of light (growth inhibition while illuminated and rapid growth in the dark), indicating that light control of hypocotyl elongation has circadian clock independent pathway(s). In wild-type plants the inhibitory effects of light on hypocotyl elongation are sustained during the dark period and this effect depends on the circadian clock. These results show that for growth control, the clock does not function to "gate" response to light but rather to allow memory of prior light exposure. Surprisingly, clock regulated growth persists in *elf4*, which contrasts with the previously reported arrhythmic *CCR2* expression in *elf4*. The amplitude of rhythmic hypocotyl elongation is reduced in *elf4*, indicating that *ELF4* is involved in regulating this sustained response. *gigantea-2* (*gi-2*) shows low amplitude of rhythmic hypocotyl elongation under both short day and 4hr L: 4hr D conditions without this sustained response.

In order to examine how the circadian clock modulates light signaling, we are first focusing on a key regulator of light signaling, HY5, a bHLH transcription factor. HY5 protein abundance is being compared between WT and *CCA1-OX* seedlings to determine if the clock impinges on light signaling upstream of this point. Also to investigate phytochrome and cryptochrome involvement in this response, time-lapse analysis of hypocotyl elongation under monochromatic red and blue light will be presented.

495 Arabidopsis Metallothioneins: Roles of Seed-Specific Type 4 MTs and Regulation of MT2a Expression in Response to Copper and Oxidative Stress

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Metallothioneins (MTs) comprise a conserved family of proteins characterized by low molecular weight, high cysteine content and strong affinity toward heavy metals. MTs are found in virtually all eukaryotes and in some bacteria. In yeasts, mammals and bacteria, MTs play vital roles in heavy metal detoxification, storage and distribution of essential metals, and protection against oxidative stress. Although several classes of MTs have been identified in flowering plants, their physiological roles remain enigmatic. Here we describe functional characterization of the *MT4a* and *MT4b* genes, which encode seed-specific Type 4 MTs from *Arabidopsis thaliana*. Both *MT4a* and *MT4b* transcripts accumulate during late embryogenesis and decline rapidly following seed imbibition. MT4a and MT4b were equivalent in their capacity to restore copper tolerance to the yeast *Δcup1* mutant. In contrast, MT4a was far more efficient than MT4b in suppressing the zinc hypersensitive phenotype of the yeast *Δzrt1/Δcot1* mutant. These results, in spite of their sequence similarity, suggest that the two MTs may have different metal binding properties with MT4a having a specific role in zinc homeostasis. The Arabidopsis knockout mutants that are deficient in MT4a and MT4b were obtained from the GABI-KAT and SINS databases, respectively. To test whether MT4a and MT4b are involved in deposition of essential metals in the developing embryos, seeds derived from the mutants and wild type will be analyzed for their metal contents. Alternatively, to examine a role of these MTs in metal tolerance, germination ratios will be compared between the mutant and wild-type seeds grown in media containing excess concentrations of metals. The current results of these characterizations will be presented.

Expression of another Arabidopsis MT gene, *MT2a*, is induced in roots by copper and oxidative stress. Combined analysis of T-DNA insertions in the *MT2a* promoter and promoter deletion-GUS transgenic plants has been used to identify promoter sequences required for expression of *MT2a* in response to 50 μM $CuSO_4$ or 10 mM H_2O_2. The results indicate that sequences from -193 to -131 nt. upstream of the transcriptional initiation site are essential for induction by both copper and hydrogen peroxide. Experiments are in progress to investigate whether two nearly identical tandem sequences located within this region constitute the *cis*-acting elements that mediate these responses.

496 Ambient temperature cycles or photoperiod diurnally regulate more than half of the Arabidopsis transcriptome

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Diurnal cycles of ambient temperature are an important, yet poorly understood developmental signal. In most organisms, temperature cycles of just a few degrees are sufficient to set the phase of the circadian clock. In plants, temperature cycles applied opposite of the natural conditions (i.e. cold days and warm nights) have profound effects on cell elongation, chlorophyll biosynthesis and general growth. In order to isolate the specific effects of temperature cycles on diurnal gene expression in Arabidopsis, we monitored the transcriptome using Affymetrix microarrays (ATH1) under different regimes of temperature cycles, photoperiod or constant conditions. We developed a new model-based, pattern-matching algorithm (MBPMA) to identify genes that are diurnally regulated. This analysis shows that temperature and light/dark cycles diurnally regulate more than half of the Arabidopsis transcriptome. About ten percent of diurnally regulated genes only oscillate under temperature cycles, suggesting light/dark cycles antagonize their expression. An additional ten percent are diurnally regulated only under light/dark cycles and seven percent require both temperature and light/dark cycles. Utilizing the contrasts between environmental treatments, putative signaling components of the temperature-sensing pathway have been identified. Time of peak gene expression (phase) occurs throughout the day, with an increased number of genes peaking at either dawn or dusk. The phase of peak expression for most diurnally regulated genes depends on the specific regime of temperature cycles or photoperiod, supporting a distinct role for each environmental cue. To dissect underlying transcriptional networks, we developed a new technique to identify combinations of overlapping 3-8mers in the promoters of coordinately expressed genes. Our results provide new insights into how unknown, as well as known, light and circadian enhancers interact combinatorially to establish phase-specific diurnal expression.

497 *Arabidopsis* SUMO E3 ligase controls *CBF*-dependent low-temperature signaling and freezing tolerance

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Conjugation of the small ubiquitin-like modifier (SUMO) proteins to a substrate (sumoylation) provides cells the capacity for sensitive and rapid responses to hormonal and environmental stimuli. We present evidence that *Arabidopsis* AtSIZ1 is a plant SUMO E3 ligase, which controls low temperature signaling and adaptation. Plants harboring dysfunctional T-DNA insertion alleles of *AtSIZ1* (At5g60410) are more chilling and freezing sensitive and exhibit reduced capacity for cold acclimation. Quantitative RT-PCR analysis determined that the expression of *CBF* (particularly *CBF3*) and *CBF*-regulated genes is attenuated in *siz1* plants. *HOS1* and *ICE1* mRNA transcript levels in *siz1* plants are similar to wild type after cold treatment. Cold induces AtSUMO1 conjugation but to a lesser extent in *siz1* relative to wild-type plants. Recombinant AtSIZ1 can substitute for the yeast SUMO E3 ligase ScSIZ2 to mediate sumoylation in vitro. AtSIZ1-GFP was localized to subnuclear foci as are animal SIZ1 orthologs. Together, these results suggest that AtSIZ1 is a SUMO E3 ligase that controls *CBF*-dependent low-temperature signaling.

498 *short vegetative phase* (*svp*) mutation suppressed late flowering phenotype of *lhy cca1* under continuous light condition

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LHY and CCA1 are shown to be closely associated with circadian clock function in Arabidopsis. The *lhy cca1* mutation causes an extremely early flowering under short days (SD) (1). The early flowering phenotype of *lhy cca1* correlated with a phase shift of GI, a phase shift of CO and up-regulation of FT under SD. Based on the results, we have proposed that LHY and CCA1 may control flowering mainly through a transcriptional cascade GI-CO-FT in light/dark cycles (2).

We have recently found that *lhy cca1* flowered later than wild-type plants under continuous light (LL) and that *short vegetative phase* (*svp*) mutation suppressed the late flowering phenotype of the *lhy cca1* under LL. The *svp* in Ler flowered much earlier than *svp* mutants in Col and a region near the FLC locus on Ch5 has turned out to be responsible for the difference. FLC is a floral repressor and thought to negatively regulate gene expression of floral activators FT and SOC1. The *svp* was epistatic to the *co*. Both FT and SOC1 are downstream factors of CO and FT/SOC1 expressions were up-regulated in the *svp* suggesting that functional interaction between SVP and FLC may be important for the suppression of the late flowering of the *lhy cca1* under LL. Function of LHY and CCA1 in LL and light/dark cycles will be discussed.

(1) Mizoguchi et al., Developmental Cell 2002

(2) Mizoguchi et al., submitted

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499 Analysis of the light-induced inhibition of gravitropism in hypocotyl; the relationship between the random hypocotyl-bending by light and the auxin distribution

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In red-light, hypocotyls of *A. thaliana* exhibit a random growth pattern due to the inhibition of gravitropism. We refer to this phenomenon as random hypocotyl-bending by red-light. To understand the mechanism which induces the random hypocotyl-bending, we isolated an *A. thaliana* mutant, *flabby* (*fby*), which shows the enhanced random hypocotyl-bending in red-light. *FBY* encodes the AtMDR1 protein which belongs to the multi-drug resistance subfamily of ABC transporters involving the auxin distribution. *phyA phyB fby* triple-mutant did not exhibit the red light-induced random hypocotyl-bending. Polar auxin transport inhibitors, NPA and TIBA mimicked the red-light effect on the *fby* mutant, and the exogenous administration of auxins suppressed the random hypocotyl-bending. The activity of polar auxin transport was inhibited by red-light in both wild-type and *fby*, whereas the inhibition was transient in wild-type and prolonged in *fby*. Furthermore, *phot1 phot2 fby* triple-mutant showed the random hypocotyl-bending under blue-light. These results suggest that 1) the activation of phytochromes inhibits the polar auxin transport transiently, 2) the defect of AtMDR1 prolonging the inhibition of the polar auxin transport enhances the random hypocotyl-bending of *fby* and 3) cryptochromes also participate in the inhibition of the polar auxin transport.

500 Characterization of a family of remorin proteins in Arabidopsis thaliana

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Remorins make up a family of small, hydrophilic, plant-specific proteins so named because of their strong attachment to the plasma membrane, reminiscent of the remora fish. Previous work has established that *in vitro* phosphorylation of remorins from several plants species is stimulated by oligogalacturonic acid (OGA), and remorins are capable of binding polyanions, with a stronger preference for OGAs (Farmer, et al., 1991; Reymond, et al., 1996). More recently, remorins have been shown to form filamentous structures *in vitro* (Bariola, et al., 2004). Despite a number of possible implications, to date, a functional role in plants has not been identified for these proteins. To this end, we have undertaken a reverse genetic approach to study a small family of four remorin-related genes in Arabidopsis. In addition, we have begun characterization of the expression and localization of these genes.

501 HRB1, a ZZ-type zinc finger protein, regulates phyB-mediated red and cry-mediated blue light responses

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Plant photoreceptors that regulate photomorphogenic development include red/far-red light-absorbing phytochromes and blue/ultraviolet A light-absorbing cryptochromes. We have undertaken a genetic screen to identify additional components downstream of the photoreceptors in Arabidopsis. We identified a short hypocotyl mutant under red and blue light, hypersensitive to red and blue 1 or hrb1. Mutation in HRB1 also enhances the end-of-day far-red light response, inhibits leaf expansion and petiole elongation, and attenuates the expression of CAB3 and CHS. Double mutant analysis indicates that phyB is epistatic to hrb1 under red light and cry1/cry2 is epistatic to hrb1 under blue light for both hypocotyl growth and light-regulated gene expression responses. HRB1 localizes to the nucleus and belongs to a protein family of Drought induced 19 or Di19. HRB1 and all other family members contain a ZZ-type zinc finger domain, which in other organisms is implicated in protein-protein interactions between dystrophin and calmodulin, and between transcriptional adaptors and activators. HRB1 activity is also required for red and blue light-induced expression of PHYTOCHROME INTERACTING FACTOR 4 or PIF4. pif4 shows a very similar hypersensitive response as hrb1 to both red light and blue light, and is epistatic to hrb1 in control of light-regulated gene expression responses. Thus, the roles of HRB1 and PIF4 together in regulating both red and blue light responses may represent points where red light signaling and blue light signaling intersect.

502 LIR1, an Arabidopsis protein containing a SPX domain and an EXS domain, regulates cry-mediated blue light signaling

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Plants acquire their photosynthetic capacity through photomorphogenesis, which is mediated by red/far-red light-absorbing phytochromes, blue/ultraviolet A light-absorbing cryptochromes, and their downstream signaling components. We have isolated an Arabidopsis mutant with a long hypocotyl under red, far-red, and blue light, lir1-1 for light insensitive response 1-1. The long hypocotyl phenotype in lir1-1 is caused by an overaccumulation of LIR1 transcript, and is recapitulated by overexpression of LIR1 in transgenic Arabidopsis. However, lir1-2, a knockout allele of LIR1, exhibits a short hypocotyl phenotype only under blue light. Thus, LIR1 functions specifically in blue light signaling but overexpression of LIR1 expands its activity to modulate phytochrome signaling. Studies on both lir1-1 and lir1-2 also demonstrated that LIR1 acts either positively or negatively. The positive action of LIR1 signaling may involve HFR1, a basic helix-loop-helix protein, based on the opposite effects of lir1-1 and lir1-2 mutations on HFR1 expression and the overlapping phenotypes of lir1-2 and hfr1 under blue light. LIR1 localizes to the cytosol, and contains an N-terminal SPX and a C-terminal EXS domain found in members of the SYG1 protein family from fungi, C. elegans, fly, mammals, and Arabidopsis. Our studies suggest a specific function of LIR1 in regulation of cry-mediated blue light responses in Arabidopsis, but also a possible involvement of LIR1-like proteins in either cry-mediated blue light signaling or other blue light responses in other organisms.

503 The Arabidopsis WAVY GROWTH 3 protein harboring a RING finger motif regulates the gravitropic response of roots negatively

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Growth pattern regulation of roots is important for plant growth and production. To understand how root growth direction changes in response to environmental stimuli, we characterize an Arabidopsis mutant, *wavy growth 3* (*wav3*), which roots show a shorter-pitch wavy growth pattern on inclined agar medium. The *wav3* mutant shows an enhancement of root gravitropic response, and a reduction of its phototropic response. Double mutants crossed with the *wav2* mutant, which shows an abnormality in root bending through root tip rotation, presents an additive phenotype on the shorter-pitch wavy growth of roots, suggesting that WAV3 plays a role in root bending independently of WAV2. A positional cloning of *WAV3* reveals that it encodes a novel protein with a RING finger motif in its N-terminal region and vWA motif in its C-terminal region, which domains are involved in the protein-protein interaction. Northern blot analysis shows that *WAV3* is expressed in seedlings and roots, but not in leaves, stems, and flower buds. Yeast two hybrid analysis indicates that WAV3 interacts with all members of SINAT family proteins and these interactions are confirmed in vitro. Previous study reported that SINAT5 is involved in auxin signaling through an ubiquitin/proteasome pathway. These results suggest that WAV3 is involved in a signaling pathway regulating the environmental stimuli-induced bending in roots.

504 Arabidopsis *RCI2* genes have been structurally conserved during evolution but play different roles in response to abiotic stresses

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AtRCI2A and *AtRCI2B* are two homologous genes from Arabidopsis whose expression is induced in response to low temperature and also by ABA, dehydration and salt treatments. Both genes encode small, highly hydrophobic proteins with two putative transmembrane domains. In an attempt to isolate new genes involved in cold response, we have identified six new Arabidopsis genes (*AtRCI2C-H*) that show high homology to *AtRCI2A* and *AtRCI2B*. The expression of *AtRCI2* genes is differentially regulated during development and in response to abiotic stresses. When exploring the occurrence of *AtRCI2*-related genes in plant species other than Arabidopsis and in organisms other than plants, BLAST searches revealed that *AtRCI2*-related genes are widely spread among very different organisms, including different plant species, prokaryotes, fungi and simply organized animals. Most *RCI2* genes exhibit similar exon-intron organization, indicating that they have been structurally conserved during evolution. Moreover, RCI2 proteins show an elevated level of sequence similarity and seem to have evolved from a common ancestor. Nevertheless, in spite of their high similarity and common origin, the RCI2 proteins seem to have different functional roles. Thus, AtRCI2A, AtRCI2B and AtRCI2H, but not the other AtRCI2 proteins, are able to complement for the loss of the yeast *AtRCI2*-related gene *PMP3*.

505 The Role of Inositol-1,4,5 Triphosphate in Transcriptional Regulation of Gravitopism

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The response of plants to gravity involves a sensing mechanism, initiating a signal transduction pathway, resulting in changes in gene expression, which ultimately leads to directional growth. Using whole genome microarray technology, we have identified genes that respond exclusively to gravity stimulation but not to mechanical stimulation in the Arabidopsis thaliana root apex (Kimbrough et al. 2004). The fastest responses occur within less than 1 min after gravistimulation in the wild type plants but not in the transgenic Arabidopsis plants overexpressing the human type I inositol polyphosphate 5-phosphatase (an enzyme that specifically hydrolyzes inositol-1,4,5-trisphosphate (InsP3)). These transgenic plants have reduced levels of InsP3, a very early mediator of gravitropic signal transduction and also exhibit a delayed and slow response to gravity. Comparative microarray analysis identified clusters of differentially regulated transcripts in wild type and transgenic plants after gravity stimulation that indicate that InsP3 is mediating a) gravity specific activation of transcription; b) repression of specific genes; and c) stability of specific transcripts. Auxin-regulated genes showed the same pattern in transcript abundance changes in wild type and transgenic plants, indicating that auxin-regulated transcription is InsP3-independent. Our analysis indicates that gravitropic regulation of transcription is mediated by two separate and independent branches of the signal transduction pathway: an InsP3-mediated gravity-specific mechanism and an InsP3-independent hormone-regulated pathway. Interestingly, we found that some of the fastest gravity induced genes, respond also to light stimulation by transient increases in RNA levels with similar kinetics. This light response is also IP3-mediated (R. Salinas-Mondragon et al, 2005).

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506 Interactions of temperature and the circadian clock

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A circadian oscillator follows three rules. First, circadian oscillations are maintained in the absence of entraining cues from the environment. Second, the internal timing or phase of the oscillator is synchronized, or reset, by light/dark and warm/cold temperature cycles. Third, the free-running period is temperature-compensated, meaning that is relatively constant over a wide range of temperatures. The current model of the Arabidopsis clock calls upon 2 feedback loops, both originating from the 2 Myb-domain transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE and ELONGATED HYPOCOTYL (LHY). CCA1 and LHY target 3 members of the PSEUDO-RESPONSE REGULATOR (PRR) family with opposite effects. PRR1/TOC1 expression is repressed, whereas the expression of PRR7 and PRR9 is induced. We have focused on the role of PRR7 and 9 in the response of the clock to temperature. The loss of both PRR7 and PRR9 function in *prr7-3 prr9-1* plants results in loss of rhythmicity following thermocycles, indicating that these 2 proteins may be part of a temperature signaling module leading to the clock. Such an extreme phenotype is not seen in loss of function alleles for *CCA1, LHY, TOC1* or *ZTL*.

Plants have developed an intricate circuitry for cold signaling that leads to the induction of the C-repeat/DRE Binding Factors CBF1-3. Changes in membrane fluidity is thought to initiate the cold signaling cascade. In Neurospora, mutants with altered lipid composition affect free-running period and temperature compensation, suggesting that the clock may respond to changes in lipid composition. We are using a collection of mutants (*prr7-3 prr9-1* double mutant, *fad7* single and *fad7 fad8* double mutants) and overexpressing lines (*CBF1-3 OX*) to investigate the degree of overlap, if any, between the cold sensing and circadian temperature signaling pathways. Mutations in *FAD7* and *FAD8* would be expected to act upstream of both pathways. We found that, in contrast to the *prr7-3 prr9-1* double mutant, plants lacking FAD7 and FAD8, or plants overexpressing the *CBF* genes entrain properly to thermocycles. These results demonstrate that Arabidopsis contains at least 2 distinct mechanisms for temperature sensing. Other responses of the circadian clock to temperature in these genotypes and others are being investigated, in particular temperature compensation and entrainment during frequency-demultiplication protocols, the latter which proved very useful in the assignment of the role of the Neurospora FREQUENCY protein in the fungal clock.

507 *FCL3/MAF3* and *FCL4/MAF2* belong to an *FLC*-independent vernalization pathway

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Arabidopsis plants with null mutants of *FLC* maintain vernalization-responsiveness under non-inductive photoperiods, suggesting the existence of an *FLC*-independent vernalization pathway. Here we show that the two *FLC* homologs *FCL3/MAF3* and *FCL4/MAF2* are downregulated by vernalization. In the Col-0 background, *FCL3/MAF3-FCL4/MAF4* double knockout mutants flower slightly earlier than *FLC* null mutants under non-inductive photoperiods. Triple knockouts of *FLC*, *FCL3/MAF3*, and *FCL4/MAF2* flower significantly earlier than either single or double knockouts and have a minimal vernalization response under non-inductive photoperiods. This suggests that *FCL3/MAF3* and *FCL4/MAF2* repress flowering in parallel with *FLC* and are a significant part of an *FLC*-independent vernalization pathway.

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508 Cold activation of *EARLI1* is light dependent and involves sugar signaling

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EARLI1, a putative lipid transfer protein-encoding gene that partially protects Arabidopsis plants from freezing-induced cellular damage has a complex environmental regulation. *EARLI1* is activated by both cold and light. *EARLI1* is not a direct target of CBF/DREB and efficient cold activation requires photomorphogenesis. Soluble sugars activate *EARLI1* in the absence of light and cold, suggesting that the synergism between light and cold for *EARLI1* activation involves sugar signaling.

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509 Genetic analysis of the circadian clock in Arabidopsis

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Circadian clocks are endogenous 24 hour oscillators that are entrained to exogenous environmental conditions, most notably light and temperature cycles, and control a myriad of physiological outputs including gene expression (5-7% of the Arabidopsis transcriptome), leaf movement, ethylene production, cell elongation, and stomatal aperture. We performed genetic screens to isolate circadian clock mutants in order to identify genes with clock function. Hypocotyl elongation is a clock controlled process and was exploited as a high throughput primary screen followed by a circadian assay for reporter gene expression. EMS-mutagenized seedlings exhibiting long hypocotyls grown under light:dark cycles were selected and rhythmic bioluminescence from a circadian-regulated reporter (CAB2::LUC) monitored for 5 days. Mutants exhibiting altered clock-regulated bioluminescence were then recovered for further analysis. The strategy of pre-selecting clock mutants for hypocotyl phenotypes was immensely successful and from over 80,000 M2 seedlings, approximately 35 clock mutants were identified. From this population of 35 clock mutants, we have identified 6 novel alleles of a known clock gene EARLY FLOWERING4 (ELF4), and another 5 alleles of a novel clock gene named LUX ARRHYTHMO (LUX). Both mutants exhibit strong clock phenotypes in constant white light for multiple clock-regulated outputs, long hypocotyls under light:dark cycles, and early flowering under short days (8hrs. light:16hrs. dark). Incorporation of these genes into a model of the Arabidopsis circadian system will be presented.

510 The Arabidopsis *SPR1* gene encodes a novel plant specific microtubule plus-end interacting protein involved in directional cell expansion

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Although it is well established that cortical microtubules play a central role in plant cell expansion, the underlying molecular mechanisms remain largely unresolved. To learn more, we have cloned and molecularly characterized the Arabidopsis *SPR1* gene. Mutations in *SPR1* cause defects in directional cell expansion, with roots and etiolated hypocotyls twisting abnormally about their longitudinal growth axes. *spr1* roots also respond abnormally to touch stimulation with skewed growth on tilted agar surfaces. The *spr1* mutant growth defects are enhanced at lower temperatures and suppressed by the anti-microtubule drug propyzamide. We cloned the *SPR1* gene and found it belongs to a novel six-member gene family specific to plants. The predicted 12kD SPR1 and SPR1-like proteins contain highly conserved direct repeat amino acid sequences at both ends separated by a low complexity central region predicted to form a rod-like structure. Confocal microscopic analysis of transgenic plants expressing a SPR1:GFP fusion protein revealed GFP-related fluorescence emanating from microtubules in all four arrays. Interphase cortical microtubule localization of SPR1:GFP occurred predominantly at the growing microtubule plus ends, with label dissipating upon microtubule depolymerization. Thus, SPR1 is a microtubule plus end localized protein (a so-called +TIP) that may regulate microtubule growth dynamics and/or act as a linker protein assisting in the movement of proteins or structures around the cell on the ends of polymerizing microtubules.

511 PIF1 is degraded by light through ubiquitin-mediated proteasome pathway

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Light signals perceived by the phytochrome (phy) family of sensory photoreceptors control multiple developmental responses of a plant's life cycle by an unknown mechanism. Recently, we and others have shown that PIF1, a phy-interacting basic helix-loop-helix (bHLH) transcription factor, negatively regulates many facets of seedling deetiolation processes, including seed germination, chlorophyll biosynthesis and gravitropic responses by light. We have shown that PIF1 transcriptional activation activity is reduced by light in a phy-dependent manner. In an effort to understand the mechanism of this reduction, we observed that Luciferease (LUC):PIF1 fusion protein is rapidly and reversibly degraded by light, while the LUC only control was stable under the same conditions, suggesting that light specifically degrades PIF1. Fluence-rate response curves showed that PIF1 degradation is very sensitive to light quality and quantity. The half-life of PIF1 is ~15 minutes under red light. Light-dependent degradation of PIF1 is mediated by the ubiquitin-mediated proteasome pathway, as inhibitors of this pathway blocked degradation of PIF1. PIF1 is also degraded by light in the presence of cycloheximide suggesting that de novo protein synthesis is not required for this degradation. Taken together, these results suggest that the light signals perceived by phys induce degradation of PIF1 and other phy-interacting factors to optimize seedling photomorphogenic development.

512 The *LI*ght response *D*efective *3* mutant accumulates reduced anthocyanin content in response to sucrose- and phytochrome A-inductive conditions

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The reduced-pigment mutant *lid3* (*li*ght response *d*eficient 3) was identified in a screen designed to identify phytochrome A- and sucrose-signaling components regulating anthocyanin content in early development. This mutant accumulates significantly less anthocyanin than wild type under conditions that yield high levels of anthocyanins in young wild-type *Arabidopsis* seedlings: growth for 3 days in continuous far-red light on agar medium supplemented with 2% sucrose. Semi-quantitative RT-PCR shows that levels of the first major light-regulated anthocyanin biosynthetic enzyme, chalcone synthase (CHS), are at wild-type levels in *lid3*. However, chalcone isomerase (CHI) and dihydroflavonol reductase (DFR), downstream biosynthetic enzymes more specific to the anthocyanins, are down-regulated in *lid3* compared with wild type. Without sucrose, wild-type levels of transcripts corresponding to CHS, CHI, and DFR are significantly less abundant than those from plants grown with sucrose. In the absence of sucrose, *lid3* yields reduced CHS transcript levels compared with wild type, and both CHI and DFR messages are undetectable. These results suggest that while CHI and DFR transcript accumulation requires sucrose and a functional lid3 protein, sucrose and lid3 appear to be regulating different points in the metabolic pathway.

Plants bearing the *lid3* mutation appear to have normal physiological phenotypes in darkness and in red, blue, or white light. Also, other phytochrome A-mediated processes appear normal, including the far red-preconditioned block of greening, the inhibition of hypocotyl elongation, and cotyledon separation and expansion. Anthocyanin levels at other stages of development appear normal. We hypothesize that *lid3* is a mutant specific to the phytochrome A-regulated branch of a pathway involved in anthocyanin accumulation in the early development of *Arabidopsis* seedlings.

Rough mapping of *LID3* places the gene on the long arm of chromosome 1, between SSLP markers nga111 and nga280 in a region lacking known light- or sugar-dependent signaling components. Isolation of the gene is expected to yield important information regarding the integrated effects of light, sucrose, and development on anthocyanin accumulation.

513 Modulation of photosynthetic inhibitor herbicide activity by salicylate and other SAR agents

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Salicylic acid (SA) is a primary signal activating the induction of systemic acquired resistance (SAR), induced plant defense against pathogens. Paraquat (1,1'-dimethyl-4,4'-bipyridinium; methylviologen) is a widely-used, non-selective contact herbicide that rapidly stimulates free radical generation. We have found that addition of sodium salicylate (NaSA) to paraquat spray solutions significantly decreases the herbicidal activity. Protection against paraquat was also observed when NaSA was applied to tobacco plants, either to the foliage or as a soil application before paraquat spray. In contrast, the addition of NaSA increased the post-emergence herbicidal activity of atrazine on dicots. NaSA increased atrazine activity when applied as a tank mix or up to 96 h prior to atrazine application. We tested other SAR inducers, including acibenzolar-S-methyl (benzo-(1,2,3)- thiadiazole-7-carbothioic acid S-methyl ester) and probenazole (3-(2-propenyloxy)-1,2-benzisothiazole-1,1-dioxide), as welll as salicylate derivatives and found that these compounds also decreased paraquat herbicidal activity and potentiated atrazine activity. To determine the role of SAR in paraquat protection and atrazine potentiation, we tested the ability of NaSA to modulate the Arabidopsis *npr1-2* mutant's response to paraquat or atrazine. NaSA protected *npr1-2* from paraquat, while potentiating atrazine activity, as it had done in ecotype Columbia. Our data indicate that modulation of herbicide activity by salicylates is independent of disease resistance and suggest the existence of an SA-mediated pathway capable of protecting plants from reactive oxygen stress.

514 Genetic analysis of tocopherol functions in *Arabidopsis* at low temperature

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α-, β-, γ- and δ-Tocopherols (collectively termed vitamin E) are well-known lipid-soluble antioxidants that are synthesized only by photosynthetic organisms (all plants and algae and some cyanobacteria). Molecular dissection of the tocopherol biosynthetic pathway in *Arabidopsis* and *Synechocystis* has allowed the isolation of mutants containing different amounts and compositions of each tocopherol such that studies directed at elucidating the functions of tocopherols in photosynthetic organisms has become approachable. In *Arabidopsis thaliana*, the *vte2* (vitamin e 2) mutant which is deficient in homogentisate phytyl transferase (HPT) does not produce any tocopherols or pathway intermediates and provides a powerful genetic tool to study tocopherol functions *in planta*. *vte2* mutants have developmental defects during germination due to greatly elevated lipid oxidation (Sattler et al. 2004) but once established their growth is indistinguishable from wild type at normal light (120 μE m^{-2} s^{-1}) and temperatures (22°C/18°C during day/night). When *vte2* are transferred to low temperature (7°C), however, they exhibit a series of phenotypes including accumulation of soluble sugars, starch and anthocyanins, decreased carotenoid and chlorophyll levels, and reduced growth rate compared to wild type. These changes occur in the absence of any obvious oxidative stress symptoms suggesting that tocopherols are not simply acting as bulk-antioxidants.

In order to understand the basis of the low temperature sensitive phenotypes of tocopherol-deficient mutants, we have undertaken a screen for mutations that could suppress the *vte2* phenotype. The primary suppressor screening of approximately 6,000 EMS mutagenized M2 *vte2* plants has identified 100 putative suppressor lines. Secondary screening is currently being performed. Detailed biochemical analyses of selected suppressor candidates will be presented.

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515 Identification of a Novel Arsenic Tolerant Mutant and a Role for *PHYA* in Providing Tolerance

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A genetic screen was performed to isolate mutants showing increased arsenic tolerance using an *Arabidopsis thaliana* population of activation tagged lines. The most arsenic resistant mutant shows increased arsenate and arsenite tolerance at all stages of development, while at the same time accumulating higher levels of arsenic than wild type plants. X-ray absorption spectroscopy analysis indicates that the mutant accumulates higher levels of free arsenite than wild type plants. Genetic analyses of the mutant indicate that two loci contribute to arsenic tolerance, designated *ars4* and *ars5*. *ars4/ars5* contains a single activation tag that cosegregates with arsenic tolerance, *ars4*, and is inserted in the *PHYA* gene. When grown under far-red light conditions *ars4/ars5* seedlings show the same elongated hypocotyl phenotype as the previously described strong *phyA-211* allele. Both the *phyA-211* allele and an additional newly isolated T-DNA insertion allele of *PHYA* also show increased tolerance to arsenate, though to a lesser degree than *ars4/ars5*. This observation indicates that a second genetic component contributes to arsenic tolerance in *ars4/ars5*. Genetic analyses suggest that *ars5* is not linked to *ars4*. Supporting the observations that PHYA contributes to arsenic tolerance, wild type plants grown in the dark, which prevents PHYA from being activated, showed increased tolerance to arsenic compared to plants grown under normal lighting conditions. These results show that *ars4/ars5* exhibits an increased ability to tolerate arsenic and that PHYA negatively regulates a pathway conferring arsenic tolerance.

516 Proline biosynthesis in Arabidopsis: a model for stress responses

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Proline biosynthesis is controlled by the delta 1-pyrroline-5-carboxylate synthetase (P5CS) enzyme, encoded by two P5CS genes in *Arabidopsis thaliana*. T-DNA insertion mutants of *AtP5CS1* and *AtP5CS2* genes were studied to characterize their function in plant development and stress responses.*p5cs1* mutants accumulated lower proline upon salt or drought stress, had reduced relative water content (RWC) and had higher concentration of reactive oxygen species (ROS) than in wild type plants. Reduced enzyme activities of the glutathion ascorbate detoxification system were accompanied by higher catalase activity, enhanced lipid peroxidation and chlorophyll damage in *p5cs1* mutants, suggesting the importance of proline in scavenging of reactive oxigen species. Proline deficiency lead to stress hypersensitivity, as growth of *p5cs1* mutant plants was more inhibited by salt stress than wild type plants. Microarray analysis suggested the alteration of numerous stress responsive gene activities in the analysed mutants. The *p5cs2* insertion mutant showed embryo lethality at the torpedo stage. Lethality could be complemented and embryos could be rescued by externally added proline in sterile conditions. Homozygous *p5cs2* plants had serios growth defect and remained dwarf. High transcriptional activity of the *AtP5CS2* gene in immature seeds and embryos confirmed the importance of this gene during early stages of plant development. Use of *p5cs1* and *p5cs2* insertion mutants therefore confirmed that these two very similar genes had clear functional differences. The *AtP5CS1* gene controls enhanced proline biosynthesis during osmotic stress, which is important to withstand drought and high salinity. The *AtP5CS2* gene is a housekeeping gene which is essential for normal embryo and plant development. This study describes the first time the function of genes controlling key regulatory steps in proline biosynthesis and shows that they have different functions in plant development and environmental stress responses.

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517 Expression analysis of zinc excess and deficiency response of Arabidopsis and the related metal hyperaccumulator Thlaspi caerulescens

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Micronutrients are essential for humans, plants and animals. The micronutrient zinc has an important role in physiological and metabolic processes of plants as a cofactor for more than 300 enzymes including RNA polymerase, alkaline phosphatase, alcohol dehydrogenase, Cu/Zn superoxide dismutase, and carbonic anhydrase. Next to this essential role of zinc, it is very toxic when available to the plant in elevated amounts. Plants therefore need to keep very tight control over the internal zinc concentrations in a process called zinc homeostasis. Although the zinc homeostasis mechanism is supposed to be universal within plants, there are plant species that can tolerate and even accumulate large amounts of zinc without any sign of toxicity. *Thlaspi caerulescens* J. & C. Presl (Brassicaceae), a close relative of Arabidopsis, is one of these natural zinc hyperaccumulator species, which also hyperaccumulates cadmium and nickel.

We examined in detail the transcription profile of roots of Arabidopsis plants grown under deficient, sufficient and excess supply of zinc. By cDNA hybridization to the Agilent vs. 3 oligo DNA microarray, we could detect the expression of previously non-annotated genes, including miRNAs, as well as all relevant genes with a presumed role in mineral uptake and translocation. A total of 335 zinc-responsive genes with at least a threefold difference in expression level were detected in Arabidopsis in response to changes in zinc supply. In a comparable, heterologous, analysis we identified 231 genes differentially expressed in *T. caerulescens* between any of the three zinc supply conditions. Only 12% of these genes were also differentially expressed in Arabidopsis. Genes with a remarkably different expression between Arabidopsis and *T. caerulescens* appear to have a function in metal membrane transport (ZIPs, PIB-type ATPases, NRAMPs, MTPs, FRD3) and metal chelation (nicotianamine synthases); abiotic stress response and so far unknown processes. The progress in the functional analysis of several of these genes will be discussed, as well as the latest data on the response of both species to cadmium exposure.

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518 The *anthocyanin-impaired-response-1 (air-1)* mutant in the *sos3-1* background of *Arabidopsis thaliana* is deficient in the accumulation of anthocyanins in response to salt stress

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One of the key phenotypes of the *salt overly sensitive3-1 (sos3-1)* mutant is its propensity to hyper accumulate anthocyanins when subjected to high levels of NaCl (120 mM). The *anthocyanin-impaired-response-1 (air-1)* mutant is unable to accumulate anthocyanins under salt stress. The *air-1* mutant was discovered in a screen of second site suppressors of *sos3-1*. We are currently investigating *air-1* and other mutants in order to understand the connection between regulation of anthocyanin accumulation and the salt stress response.

The *air-1* mutant shows a defect in anthocyanin (flavinoid) production, specifically in response to salt stress. When exposed to other stresses such as high light, low phosphorous, Paraquat, temperature or drought stress, it accumulates anthocyanins normally. This indicates that *air-1* is involved in the regulation of anthocyanins in response to salt stress and not simply anthocyanin biosynthesis. TAIL PCR analysis of this mutant has revealed a T-DNA insertion of the promoter region of the *Arabidopsis thaliana* gene encoding for the PSOF21 protein. The sequence of PSOF21 encodes for a basic leucine zipper transcription factor. Although previous efforts have revealed that PSOF21 is expressed in plants, no function has yet been attributed.

We have performed a series of RT-PCR analyses on the various genes in the anthocyanin biosynthetic pathway; leucoanthocyanidin synthase, leucoanthocyanidin dioxygenase, O-methyltransferase, rhamnosyl transferase, dihydroflavanol 4-reductase, and flavone 3-hydroxylase. This information, combined with reporter construct experiments are enabling us to develop an understanding of how PSOF21 acts in the regulation of anthocyanin biosynthesis specifically in response to salt stress.

519 Targeted degradation of Arabidopsis HFR1 desensitizes light signaling

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Arabidopsis seedlings undergo photomorphogenesis in the light and etiolation in the dark. HFR1, a bHLH transcription factor, is required for both phytochrome A (phyA)-mediated far-red and cryptochrome 1 (cry1)-mediated blue light signaling. We report that HFR1 is a short-lived protein in darkness and is degraded through a 26S proteasome-dependent pathway. Light, irrespective of its quality, enhances HFR1 protein accumulation via promoting its stabilization. We demonstrate that HFR1 physically interacts with COP1 and that COP1 exhibits ubiquitin ligase activity toward HFR1 in vitro. In addition, we show that COP1 is required for degradation of HFR1 in vivo. Furthermore, plants overexpressing a C-terminal 161 amino acid fragment of HFR1 (CT161) display enhanced photomorphogenesis, suggesting an autonomous function of CT161 in promoting light signaling. This truncated HFR1 gene product is more stable than the full-length HFR1 protein in darkness, indicating that the COP1-interacting N-terminal portion of HFR1 is essential for COP1-mediated destabilization of HFR1. These results suggest that light enhances HFR1 protein accumulation by abrogating COP1-mediated degradation of HFR1, which is necessary and sufficient for promoting light signaling. Additionally, our results substantiate the E3 ligase activity of COP1 and its critical role in desensitizing light signaling.

520 Temperature sensing and signaling in the non-extreme range

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Temperature is an important environment factor that regulates plant growth and development. Significant progress has been made to reveal the molecular mechanisms of responses to extreme temperatures. However, how temperature is sensed and how the signal is transduced to modulate various developmental and physiological processes is still not well understood.

We are investigating plants' responses to non-extreme temperatures to identify components in the temperature sensing and signaling. We have isolated a number of genes responsive to non-extreme temperature variations. With these marker genes, we asked whether plants use the same or different mechanisms to respond to extreme and non-extreme temperatures. Preliminary study shows that temperature fluctuations in these two ranges up-regulate both shared and distinct maker genes. One of the shared marker genes is *COR15a*, which is up-regulated by a decrease in temperature both from 22! to 4! and from 28! to 22!. Transgenic plants with a series of *COR15a* promoter deletion fused to the *GUS* reporter gene have been used to test whether the same *cis* element is utilized to mediate the responses to non-extreme and extreme temperature stimuli. In addition, mutants with abnormal responses to extreme temperatures are assayed for their responses to non-extreme temperatures to determine whether these genes are shared between the two responses.

In parallel, we are taking a genetic approach to dissect the temperature signaling pathway. Transgenic plants with *pMarker:GUS* reporter gene have been mutagenized and screens for mutants defective in responding to non-extreme temperature fluctuations are now underway.

521 Identification of the temperature signaling components in *Arabidopsis thaliana*

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Temperature, with its daily fluctuation and seasonal change, is one of the major environmental factors that regulate plant growth, distribution, and survival. Previous study has revealed some of the molecular mechanisms by which plants respond to extreme low temperatures (e.g. vernalization and cold acclimation) and extreme high temperatures (e.g. thermotolerance). The mechanisms of responses to non-extreme growth temperature which is strongly affected by current global climate change are largely unknown.

Recently, the identification and characterization of the *BON1* gene that modulates growth and defense in a temperature-dependent manner, provides us a tool to identify components in the non-extreme temperature signaling pathways. Lost of function *bon1* mutant shows dwarf phenotype due to abnormal activation of defense response at 22! and this dwarf phenotype can be completely rescued by 28!. A genetic screen on either T-DNA or EMS mutagenized *bon1* plants was carried out to look for mutants that are insensitive to high temperature rescue of the *bon1* phenotype. These mutants are named *int* (insensitive to temperature). Several *int* mutants have been isolated and confirmed to be *bon1* dependant. These mutants show dwarf phenotype at 28!, possibly due to defects in either temperature sensing or temperature modulating on defense response. Map-based cloning is carried out to isolate these *INT* genes. We expect the cloning of these *INT* genes will lead to further understanding of non-extreme temperature signaling in the Arabidopsis.

522 post-translational regulation of ACS5

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Ethylene is an important modulator of various plant growth and development processes, including seeds germination, root hair development, flower senescence, abscission, fruit ripening and the response to a wide variety of stresses (Wang et al., 2002). ACC synthase(ACS), which converts S-AdoMet to ACC, is the first committed and generally rate limiting step in ethylene biosynthesis. ACC synthase is encoded by a mutigene family in Arabidopsis (Mehta et al., 1999).ACS5, one member of the ACS family, is regulated post-transcriptionally.ACS5 protein stability is negatively regulated by the C-terminal domain and treatment of cytokinin relieves this negative effect on protein stability (Chae et al.,2003).

Using a yeast two-hybrid screen, we identified a number of clones that encoded proteins that interact with the C-terminal domain of ACS5. One of these was a member of the leucine-rich receptor like Ser/Thr kinase family in Arabidopsis that we have called ACS5 Interacting Kinase1 (AIK1). This gene has a close homolog in Arabidopsis genome, which we call AIK2. We found both AIK1 and AIK2 interact with ACS5 using a yeast two hybrid assay. However, an ACS5 mutation that disrupts ACS5 turnover in vivo, eto2, eliminates the interaction with AIK1 and AIK2. AIK1 and AIK2 also interact with a subset of other Arabidopsis ACS proteins. Analysis of ACS5 protein complexes from related Arabidopsis seedlings using gel filtration chromatograpy reveals a shift in the distribution of ACS5 in aik1aik2 double mutants, confirming this interaction is physiologically relevant. Promoter Gus fusions show the two genes express ubiquitously throughout the plant.

523 The *Arabidopsis* SUMO E3 ligase, AtSIZ1 is involved high temperature tolerance independent of heat shock proteins

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The AtSIZ1 gene of Arabidopsis encodes a SUMO (small ubiquitin-like modifier) E3 ligase that is an ortholog of PIAS-type proteins, which are necessary for conjugation of SUMO to substrate target proteins (sumoylation). In vertebrates, sumoylation of HSF1 (heat shock transcription factor 1) controls the up-regulation of HSPs (heat shock proteins) facilitating thermal adaptation. Arabidopsis plants with T-DNA insertions in AtSIZ1 (*siz1-2* and *siz1-3*) exhibit a heat shock (HS) sensitive phenotype and a decrease in heat shock-induced SUMO conjugation compared to wild type. However, the expression of heat shock induced genes including HSPs, APX1 and APX2 (ascorbate peroxidase) in *siz1* plants is similar to that in wild type after heat treatment. Interestingly, AtHSF1-GFP is localized in nuclear speckles under heat shock condition in wild-type, but not in *siz1*. These results indicate that HSF regulon gene expression is unaffected by sumoylation but the peptide conjugation process may control HSF1 function in thermal adaptation through a mechanisms that is independent of HSPs.

524 Characterizing the relationship between magnesium deficiency and aluminum toxicity in plants

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The acidification of soils resulting in the release of ionic aluminum (Al^{3+}) from inert aluminosilicates is a major agricultural problem throughout the world. Al^{3+} is toxic to most organisms, but the mechanism of toxicity is not well established. Recent work on the *Saccharomyces cervisiae* magnesium (Mg^{2+}) transport gene ALR1 has shown that overexpression of this gene confers increased tolerance to Al^{3+}. In addition, Al^{3+} has been shown to block the uptake of Mg^{2+} in yeast and in plants. Together, these findings have led to the hypothesis that Al^{3+} toxicity may arise as a result of an induced Mg^{2+} deficiency in both organisms.

Our work focuses on the relationship between Al^{3+} and Mg^{2+} using a combination of molecular, biochemical and physiological approaches in *Arabidopsis*. Using different concentrations of the two metals we have looked at changes in growth capacity, recovery and Mg^{2+} content and shown that an increase in available Mg^{2+} overcomes Al^{3+} toxicity, while the symptoms of Al^{3+} toxicity closely resemble those of Mg^{2+} deficiency. We are currently extending these experiments to include microarray analyses looking at the genomic responses seen in Mg^{2+}-starved seedlings treated with different combinations of Al^{3+} and Mg^{2+}.

We have also generated transgenic *Arabidopsis* lines to determine whether heterologous expression of an Mg^{2+} transport gene in plants is able to alter the plant Al^{3+} sensitivity in a way similar to that seen in yeast. Two microbial genes, ALR1 from *Saccharomyces* and CorA from *Salmonella*, have been expressed under either the 35S or AKT1 promoters. Transgenic lines have been analyzed for changes in cation uptake and sensitivity to Al^{3+}. To date no phenotype has been detected for lines expressing *ALR1*, however preliminary results from CorA-expressing lines suggest significant changes in Mg^{2+} content. These lines are currently being analyzed for alterations in Al^{3+}-sensitivity. Results to date will be presented.

525 RSTK1, an Arabidopsis Receptor-like Ser/Thr Kinase, Positively Regulates Cell Death and Resistance against Pathogens

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Protein kinases play important roles in relaying the message from perception of the signal to the effector genes in all organisms. The genome sequence of Arabidopsis has revealed at least 610 receptor-like protein kinases (RLKs), representing nearly 2.5% of the annotated protein-coding genes. However, function of very few RLKs is known. Here, we report molecular characterization of a *Receptor-like Serine/Threonine Kinase gene (RSTK1)* of Arabidopsis. This gene is induced rapidly (within 4 hrs) in response to avirulent [*Pst* DC3000 (*Rpm1*)] and virulent (*Pst* DC3000) bacterial pathogens. Induction in response to avirulent pathogen is quicker and stronger compared to the virulent pathogen. Analysis of transgenic plants expressing *RSTK1* promoter::GUS fusion revealed that the expression of *RSTK1* is regulated spatially and temporally. To investigate the role of *RSTK1* gene in regulating defense against pathogens, we analyzed growth of virulent bacterial pathogen (*Pst* DC3000) and expression of defense-associated genes in two T-DNA knockout insertion lines. The virulent bacterial pathogen was able to grow about 10-fold more in the knockout lines compared to the corresponding WT lines. The induction of *PR1* gene in response to avirulent pathogen [*Pst* DC3000 (*Rpm1*)] was suppressed in these lines. Our attempts to produce transgenic plants expressing *RSTK1* gene from the constitutive 35S promoter failed. Plants expressing this gene from a DEX-inducible promoter exhibited rapid tissue collapse in response to DEX treatment and accumulated high levels of transcripts of several defense-related genes including *PR1, PR5, PR2 and ICS1*. In addition, these plants accumulated high levels of H_2O_2 in response to DEX treatment. Furthermore, DEX treatment induced resistance against virulent bacterial pathogens in these plants. These results suggest that RSTK1, a novel receptor-like Ser/Thr kinase, positively regulates cell death and defense against pathogens and probably represents an early component of defense signaling pathway. Details of our findings will be presented.

526 Identification of regulatory elements involved in R/avr-mediated plant defense signaling

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Considerable progress has been made in identifying plant defense signal transduction components, and in characterizing *R* gene product structure/function, but many aspects of *R/avr*-mediated plant defense signaling remain unclear. In particular, the transcriptional control elements that are utilized for *R* gene-mediated defense activation are largely unknown. We used Affymetrix microarrays to generate gene expression profiles for four different *Arabidopsis-Pseudomonas R/avr* interactions to identify transcriptional control elements that mediate *R*-gene mediated defense responses. Two novel putative regulatory sequences were identified in the promoters of genes that are up-regulated in response to avirulent pathogens, and separately, promoters that are *R/avr*-regulated but which lack any previously described defense-associated *cis*-elements were also identified. *R/avr*-inducible promoter constructs fused to GUS have been generated to: a) Investigate the biological significance of the newly predicted *cis*-elements, and to b) Identify novel cis-elements that are utilized during *R* gene-mediated defense responses. Lastly, the microarray data is being utilized to identify and characterize plant genes that influence plant defense signal transduction.

527 Indirect activation of the Arabidopsis disease resistance protein RPS5 by the Pseudomonas effector AvrPphB

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Previous work has shown that the Arabidopsis RPS5 gene product mediates resistance against the pathogen Pseudomonas syringae carrying the avirulence gene avrPphB, and that recognition requires the plant protein PBS1 (Warren et al., (1998) Plant Cell 10: 1439-1452). We have shown that AvrPphB specifically cleaves PBS1, and that this cleavage is required to trigger RPS5-mediated resistance. How RPS5 is activated is still unclear however. We provide evidence that RPS5 functions as an oligomer, which is physically associated with PBS1 prior to the action of AvrPphB. Upon cleavage of PBS1, RPS5 is rapidly degraded. We provide evidence suggesting that the activation of RPS5 induces its degradation, rather than the protease activity of AvrPphB per se. PBS1 mutations that failed to induce disease resistance couldn't induce RPS5 degradation. In addition we show that the structure of PBS1 impacts its interactions with RPS5.

528 Gene Profiling of Geminivirus-Infected Arabidopsis thaliana

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Arabidopsis thaliana Col-O was agroinoculated with the geminivirus, Cabbage Leaf Curl Virus (CaLCuV), grown at 20°C under short day conditions (8h light/16h dark). Symptoms were observed as early as 8 dpi with 100% of the plants displaying symptoms by 12 dpi. The levels of viral DNA and coat protein accumulation were monitored at 12 dpi by DNA hybridization and DAS-ELISA, respectively. High levels of viral DNA and coat protein were seen in leaves 6-10. For gene profiling experiments, total RNA was isolated from symptomatic leaves (7-9) of at least 6 plants at 12 dpi. RNA was also collected from equivalent leaves from control plants inoculated with *Agrobacterium* carrying an empty transfer vector. The RNA was used to prepare target cRNA, which was hybridized to Affymetrix ATH1 GeneChips. The microarray experiments were performed in triplicate using biologically independent RNA samples. The perfect-match hybridization data was evaluated using an ANOVA mixed model analysis (SAS Microarray Solutions). Using a Bonferroni correction for multiple comparisons, we identified 2102 genes that are expressed differentially in infected versus mock-inoculated plants. Data mining analyses have uncovered a variety of processes, including the host/pathogen response, senescence, the cell division cycle and plant development, that are altered by geminivirus infection.

529 Withdrawn

530 Functional evaluation of plant defence signalling against fusarium ear blight disease in arabidopsis

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Fusarium ear blight (FEB) infections of cereal crops cause considerable losses to grain quality and safety. The two main causative agents of this disease on wheat crops in the UK are *Fusarium culmorum* (*Fc*) and *F. graminearum* (*Fg*) (sexual stage *Gibberella zeae*). Floral infections by *Fc* and *Fg* also cause the developing cereal grains to become contaminated with various fungal mycotoxins, including the highly toxic trichothecene mycotoxin deoxynivalenol (DON). The molecular basis of resistance to FEB in cereal species is poorly understood but it is QTL based and fusarium species non-specific.

We have previously demonstrated that *Fc* and *Fg* conidia can infect the floral tissues of arabidopsis to cause disease symptoms on flowers, siliques and upper stem tissue, however leaf tissue is not penetrated [1]. DON mycotoxin production was detected in fusarium-infected flowers. This novel arabidopsis floral model seems likely to provide a tractable system for elucidating fundamental aspects of the plant-pathogen interaction with direct relevance to the significant problems resulting from these infections in cereal crops.

We are now undertaking a detailed analysis of the FEB infection phenotype in arabidopsis genotypes with defined mutations in genes involved in defence signalling, including those that disrupt the salicylic acid (*npr1-1*, *sid2-2*), jasmonic acid (*jar1-1*) and ethylene (*ein2-1*) signalling pathways. Novel infection data quantified by FAD values (fusarium-arabidopsis disease) will be presented.

Forward genetics experiments are also being conducted to select and screen EMS mutagenised and activation tagged arabidopsis lines in various scenarios involving fungal infection and / or DON mycotoxin.

We are in the process of obtaining / generating a selection of transgenic fusarium strains that contain different reporter contructs. These include constitutive expression of GFP (green flurorescent protein) or GUS (B-glucuronidase) as well as *TRI5* promoter mediated GFP expression to mark the onset of mycotoxin production. These strains will be used to aid the visualisation and understanding of the fusarium-arabidopsis pathosystem in both wild-type and mutant genotypes.

This research is supported by the BBSRC.

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531 Identification of *Arabidopsis thaliana* Genes Potentially Involved in Mediating AvrRpt2 Virulence Activity

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AvrRpt2 is a *Pseudomonas syringae* effector protein that is injected into the plant cell by the type III secretion system. AvrRpt2 enhances the growth of *P. syringae* and causes more severe disease symptoms on plants lacking a functional *RPS2* gene. Our lab is interested in understanding the mechanisms by which AvrRpt2 acts inside the plant cell to enhance *P. syringae* virulence. We hypothesize that AvrRpt2 acts by suppressing host defense responses, by modulating host physiology, or a combination of both to enhance bacterial growth.

By examining gene expression in *rps2 Arabidopsis* plants infected with *P. syringae* pv. *tomato* strain DC3000 in the presence or absence of AvrRpt2, we have identified several genes that are differentially expressed in the presence of AvrRpt2. These genes may provide insight into the mode of action of AvrRpt2. While the expression of these genes is unaffected by infection with DC3000 in the absence of AvrRpt2, several genes are either specifically induced or specifically repressed in the presence of AvrRpt2. We are currently identifying homozygous T-DNA insertion lines for each of these genes. To determine if these genes play a general role in disease susceptibility we will examine the effect of loss of function of each of these genes on disease development after infection with DC3000. To determine the role of these genes in the virulence activity of AvrRpt2, we will characterize the ability of DC3000 carrying the *avrRpt2* gene to grow to high levels and cause disease symptoms on the T-DNA insertion lines in an *rps2* background.

532 Hypersensitivity to ABA and reduced resistance to Botrytis cineria in Arabidopsis mutant of a zinc finger transcription factor protein with ankryn repeat domains

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Plants have a complex network of defense response pathways that mediate resistance to potential pathogens. Host resistance to host unspecific necrotrophic pathogens is poorly understood but appears to be multigenic i.e. maximum resistance requires the concerted action of a number of genes. This is in contrast to single gene resistance to biotrophic pathogens. Genetic studies in Arabidopsis have established the crucial role of the plant hormones ethylene and jasmonate in induced plant defense responses to necrotrophic pathogens such as Botrytis cinerea and A. brassicicola. Salicylic acid (SA) appears to have various roles in plant resistance to necrotrophic infection. Lack of SA accumulation resulted in loss of resistance at the site of infection; impaired SA signaling had no effect whereas elevated SA accumulation correlated with susceptibility. In the case of ethylene and jasmonate both their accumulation and signaling are important for resistance. Although, absiscic acid (ABA) is a well known stress hormone in the control of responses to abiotic stresses such as cold, drought and salt stress, its role in defense has been less studied. Recently, ABA has emerged as a player in plant defense responses with data that support its role as a positive and negative factor in different plant-pathogen interactions. We identified a number of Arabidopsis regulatory gene that showed increased gene expression in Botrytis infected tissues based on microarray experiments. One of these Botrytis induced genes encodes a putative protein that contains two distinct domains that are evolutionarily conserved: four consecutive Ankryn repeats and a zinc finger protein (C-X8-C-X5-C-X3-H type). The zinc finger domain was implicated in the regulation of transcription of genes involved in various cellular processes. Ankryn repeats are common protein-protein interaction domains. Interestingly, a T-DNA insertion allele in the gene resulted in local susceptibility to Botrytis infection. Although the Botrytis susceptibility phenotype is relatively weaker than the previously described Botrytis susceptible (bos) mutants, this mutation confers strong hypersensitivity to germination on ABA providing a clear link between ABA signaling and responses to Botrytis in Arabidopsis.

533 RESISTANCE TO FUSARIUM OXYSPORUM 1, a Dominant Arabidopsis Disease Resistance Gene, is not Race Specific

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Arabidopsis thaliana ecotypes differ in their susceptibility to Fusarium wilt diseases. Ecotype Taynuilt-0 (Ty-0) is susceptible to *Fusarium oxysporum* forma specialis (f.) *matthioli* whereas Columbia-0 (Col-0) is resistant. Segregation analysis of a cross between Ty-0 and Col-0 revealed six dominant *RESISTANCE TO FUSARIUM OXYSPORUM* (*RFO*) loci that significantly contribute to f. *matthioli* resistance in Col-0 relative to Ty-0. We refer to the locus with the strongest effect as *RFO1*. Ty-0 plants in which only the Col-0 allele of *RFO1* ($RFO1^{Col\text{-}0}$) was introduced were resistant to f. *matthioli*. Surprisingly, $RFO1^{Col\text{-}0}$ also conferred resistance to f. *raphani*, demonstrating that *RFO1*-mediated resistance is not race specific. Expression of resistance by *RFO2*, *RFO4* or *RFO6* was dependent on $RFO1^{Col\text{-}0}$. Map based cloning of $RFO1^{Col\text{-}0}$ showed that *RFO1* is identical to the previously named Arabidopsis gene *WAKL22* (*WALL-ASSOCIATED KINASE-LIKE KINASE 22*), which encodes a receptor-like kinase that does not contain an extracellular leucine-rich repeat domain. Consistent with these results, a Col-0 *rfo1* loss-of-function mutant was more susceptible to f. *matthioli*, f. *conglutinans* and f. *raphani*. Thus, RFO1 encodes a novel type of dominant disease resistance protein that confers resistance to a broad spectrum of Fusarium races.

534 Dissecting regulatory mechanisms controlling the plant defense transcriptome by functional genomics, proteomics and chemical genetics

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Transcriptional re-programming is a key step of the plant defense response. However, details of regulatory processes controlling the plant defense transcriptome are largely unknown. Previous studies mainly focused on individual defense genes. We designed an approach to systematically identify *cis*-elements and transcription factors regulating defense gene clusters in *Arabidopsis thaliana* (Arabidopsis). Using microarray data we identified several clusters of genes responding by coordinated expression to pathogen recognition. Short sequence motifs conserved in their promoters are likely to constitute *cis*-elements responsible for their co-regulation. We found that some of these conserved motifs interact with nuclear proteins in gel shift assays. We purified candidate proteins corresponding to these DNA-binding activities by DNA-affinity chromatography. Mass-spectroscopy is being performed to reveal their identity. In addition we are conducting yeast one-hybrid screens to clone transcription factors interacting with conserved promoter motifs. *In vivo* roles of candidate factors will be examined using T-DNA mutants and over-expression. Furthermore, we are screening chemical libraries for organic compounds activating reporter genes fused to pathogen responsive promoters in Arabidopsis plants. Molecules identified by these screens may interfere with components of the defense-signaling network. Using known mutants we will determine if candidate compounds specifically interfere with certain signaling branches and at what hierarchical level regulatory mechanisms are affected. Microarray analyses will allow us to further select compounds that specifically affect expression of defined defense gene clusters. Such compounds are likely to interfere/interact with distinct transcription factors or other gene regulators. Molecular targets of promising compounds will be identified by affinity chromatography. The model Arabidopsis provides all tools required for such a systematic multi-component approach. Mechanisms controlling defense gene clusters uncovered in this study will facilitate designing new strategies to improve disease resistance in crops.

535 Electrophysiological characterization of bacterial *avrRpt2* gene-specific HR in Arabidopsis in the absence of other bacterial signals

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Physiological processes leading to the hypersensitive response (HR), defined as rapid cell collapse at the infection site that often accompanies plant resistance, are not well understood. In addition, when bacteria initiate an HR, they produce both non-specific elicitors that induce plant defense responses, and effectors that suppress plant defense responses. We used electrophysiology and conductivity measurements to characterize bacterial HR caused by a single avirulence gene in the absence of other bacterial signals. We found that membrane depolarization in dexamethasone- (dex) inducible transgenic Arabidopsis plants containing the *avrRpt2* gene from *Pseudomonas syringae* pv. tomato began 1 to 1.5 h after dex application, hours before electrolyte leakage. Progressive depolarization was a sensitive early indicator of HR that occurred only in Arabidopsis leaf cells expressing both *avrRpt2* and a functional *RPS2* gene. Both rate and amount of depolarization were reduced in *RPS2* plants treated with the calcium channel blocker, $LaCl_3$, at various times after the initiation of depolarization. In contrast, the potassium channel blocker, tetraethylammonium chloride (TEA), inhibited neither rate nor amount of depolarization. Electrolyte leakage was blocked when $LaCl_3$ was applied for 1.5 h beginning one half hour after dex. The window during which $LaCl_3$ is an effective blocker of electrolyte leakage and the effect of TEA on electrolyte leakage are being investigated. This experimental system is very sensitive and will allow a dissection of physiological processes leading to HR in plants.

536 Functional characterization of the Arabidopsis *RPS4* disease resistance gene

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RPS4 belongs to the large family of NBS-LRR resistance proteins, and has an N-terminal Toll/interleukin-1 receptor (TIR) domain. Like other TIR-NBS-LRR resistance genes, *RPS4* produces alternative transcripts with truncated open reading frames encoding TIR-NBS proteins. Alternative *RPS4* transcripts are predominantly generated by retention of introns 2 and/or 3. To investigate the biological significance of these alternative transcripts, we removed introns 2 and 3, either individually or in combination, from a functional *RPS4-Ler* gene, and generated stable transformants with these intron-deficient transgenes. Removal of one or both introns abolished the function of the *RPS4* transgene, whereas expression was not affected. 3'-truncated *RPS4* transgenes that mimicked the open reading frame present in naturally occurring alternative transcripts was not sufficient to confer resistance, suggesting that the combined presence of regular and alternative *RPS4* transcripts is required for function. This hypothesis was further confirmed by the reconstitution of *RPS4* function in double transgenic plants expressing both intron-deficient and 3'-truncated *RPS4* transgenes. To explore the function of *RPS4* alternative transcripts further, we characterized *RPS4* transcript ratios during the resistance response and found that the relative amount of certain alternative transcripts increases rapidly. Also, *RPS4* expression as a whole was significantly induced during the resistance response. These data support our hypothesis that in the native system truncated RPS4 proteins are required for activation of the disease resistance signaling cascade. Transcript quantification and biochemical characterization of truncated RPS4 proteins is ongoing.

537 Characterization of *srfr1* and *srfr3* suppressor mutants that reactivate *avrRps4*-induced resistance in Arabidopsis

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RPS4 is a TIR-NBS-LRR disease resistance gene in Arabidopsis that specifies the disease resistance response to *Pseudomonas syringae* pv. tomato expressing *avrRps4* and was cloned based on the identification of RLD as a naturally occurring susceptible accession. We have isolated two RLD suppressor mutants, *srfr1* and *srfr3* (suppressors of *rps4-RLD*), that show reactivated *avrRps4*-specific resistance by screening EMS-mutagenized RLD plants. Based on genetic analyses, the *srfr1* and *srfr3* mutations are recessive and not allelic. Both show *avrRps4*-specific resistance in the absence of constitutively activated basal resistance. We have mapped the *SRFR1* and *SRFR3* genes to the bottom of chromosome 4 between the SSLP markers AP22B and T28I19B and have further narrowed down the position of both genes to a 350-kb interval on two BAC clones by high resolution mapping. Now, we are in the process of constructing a cosmid library to cover this region and also of subcloning candidate genes within this interval for complementation tests. In addition, we have begun epistasis analyses between the *srfr* mutants and known pathogen resistance signaling mutants. The finding that resistance in *srfr1* and *srfr3* plants is a recessive trait suggest that SRFR1 and SRFR3 function as negative regulators in an *avrRps4*-triggered resistance response in Arabidopsis. Continued analysis of *srfr* mutants and characterization of the corresponding genes will further help our understanding of the vital mechanisms regulating activation of plant gene-for-gene resistance signaling pathways.

538 Novel Arabidopsis proteins required for full RPS2-mediated resistance to Pseudomonas syringae pv. tomato

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Arabidopsis *RPS2* encodes a CC-NB-LRR (coiled-coil, nucleotide binding site, leucine rich repeat) protein. *RPS2* confers resistance against *Pseudomonas syringae* pv. *tomato* (*Pst*) that express the effector protein AvrRpt2. We seek to identify proteins that interact with RPS2 and contribute to RPS2-mediated disease resistance. A previous yeast two-hybrid screen identified twelve Arabidopsis proteins that interact with RPS2 in the yeast cytoplasm. For three of the RPS2-interacting proteins (RPIs) we now have data suggesting a role in *RPS2*-mediated disease resistance. *RPI4* encodes an ankyrin repeat and tetratricopeptide repeat-containing protein, *RPI6* encodes an HSP70-like protein, and *RPI11* encodes a protein of unknown function. For these RPIs, DC3000 expressing *avrRpt2* grew to higher levels in the leaves of T-DNA insertional mutation lines than in the leaves of wild-type Columbia. Growth of virulent DC3000 was unaltered in the mutant lines. In each case, disruption of *RPS2*-mediated resistance was partial in that DC3000 expressing *avrRpt2* still grew to lower population levels than DC3000. T-DNA insertion mutants for *RPI6* (HSP70-like protein) were also less effective at restricting the growth of DC3000 strains expressing *avrRpm1* or *avrPphB*, suggesting that *RPI6* is involved in the resistance mediated by at least two other Arabidopsis CC-NB-LRR proteins, RPM1 and RPS5. Relative to the wild-type parent, T-DNA insertion mutants for the HSP70-like protein exhibit slightly smaller rosettes, altered leaf morphology, sensitivity to low humidity, and a more rapid reaction to wounding. *RPI4* and *RPI11* mutant plant lines are normal in appearance. In yeast, *RPI4* and *RPI11* interact with RPS2 but not RPM1, interact with the CC-NB region of RPS2 and not the LRR domain, and interact with RPS2 products that carry NB mutations.

539 Inference of comprehensive network neighborhoods associated with phenotypes: a case study using *hin1*/*ndr1* gene family

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The recent availability of complete genomes of a large number of organisms and increasing use of high-throughput measurements of cellular constituents enable simultaneous and comprehensive view of cellular processes associated with and causal to a phenotype being studied. Continued improvements in computational, statistical and bioinformatic tools facilitate integration of diverse data with increasing confidence to infer networks, modules and regulatory nodes that constitute key processes contributing to a phenotype. Here, *hin1*/*ndr1* gene family of Arabidopsis is used as an example to demonstrate one such approach towards this goal. *hin1* was identified from Nicotiana as specifically expressed during HR elicitors and host-pathogen interactions. NDR1 was identified from Arabidopsis as necessary for certain types of resistance interactions and having homology to HIN1. Arabidopsis genome has at least 28 genes with significant homology to *hin1* and ndr1. An integrated analysis, using model based approaches based on information content in the genome, and expression patterns, will be used to sub-classify the members of this gene family to aid inference of their functions in different biological context. Further, a *de novo* inference approach will be used to extract network neighborhood associated with expression phenotype of representative members in this gene family during Arabidopsis-pathogen interactions. This neighborhood is expected to be reveal direct and cross-regulatory signaling molecules and modules, co-expressed and co-acting components, as well as components causally affected by the phenotype being studied. In principle, this approach can be extended to study many phenotypes, and would be expected to be further refined with integration of additional data.

540 A Novel Mutant Screen for Activators of Programmed Cell Death in Arabidopsis

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We have generated an activation tagging mutant population using the pOp/LhGR trans-activation system and Arabidopsis in order to identify novel regulators of plant Programmed Cell Death (PCD). This forward genetics approach was used to try to find possible activators of PCD that would otherwise be lost due to their inherent lethality. A population of 13 000 T_1 plants were generated, T_2 seeds were collected from pools of 10 transformants and screened. The overall scheme of the project will be presented along with examples of some exciting mutants identified so far.

The insertion in one of these mutants caused a spreading necrosis primarily along the vasculature of new leaves resulting in large brown necrotic patches distributed over the surface of the leaves, easily seen by eye and an overall yellowing which eventually engulfs and kills the whole plant (often before seed set). Trypan blue staining confirmed that the mutation caused localized cell death throughout the leaves.

TAIL-PCR from the insertion point revealed an insertion in the first intron of a member of a small gene family in Arabidopsis coding for a Perforin domain. Perforins in humans are secreted from Cytotoxic T-cells and form macromolecular pores in target cells resulting in destructive lesions and the induction of PCD. We are currently characterizing in more detail the cell death induction in this mutant.

541 Functional genomics to define mechanisms of defense against flea beetles in *Brassica napus* and *Arabidopsis*

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The crucifer flea beetle *Phyllotreta cruciferae* is a specialist insect that is the most chronic and damaging insect pest of canola (*Brassica napus* and *B. rapa*), Canada's premier oilseed crop. The model plant *Arabidopsis thaliani* is also fed upon by crucifer flea beetles. We have been investigating the molecular basis for plant defense against flea beetles, with the intent of enhancing weak or untimely defense responses in *B. napus*. One strategy has been to generate a collection of *B. napus* leaf and cotyledon ESTs that represent genes responding to flea beetle feeding. A putative auxin-repressor protein (bnARP) was strongly represented in FB-damaged leaf and FB-damaged cotyledon EST libraries and strongly induced on Northern blots of FB-damaged leaves. This gene was not represented strongly in any *B. napus* development-related or stress-related EST libraries. The full-length putative *ARP* cDNA was cloned and introduced into Arabidopsis. T2 lines expressing the transgene were delayed in development (ranging from very slow, non-flowering lines to moderately slow lines with dark purple vegetation at the pre-bolting stage). Moderately slow lines resumed the growth rate of non-transgenic controls after bolting, although they had fewer siliques. Primary root length and number of lateral roots was low in the ARP+ transgenic *Arabidopsis* plants. Application of 2,4-D to the transgenic plants improved lateral root formation, supporting the role of this gene in auxin repression. A role for auxin repression in plant response to insect feeding has not been previously reported.

542 A putative glutamate receptor protein involved in cell wall integrity signalling

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Signalling mechanisms coordinating interaction between the cell wall and the plant cell are crucial in several different biological contexts such as (a)biotic stress response, cell wall polysaccharide biosynthesis and cell morphogenesis. Our understanding of these mechanisms is still rudimentary. Over the last years evidence has surfaced implicating ethylene and jasmonic acid signalling elements while lignin and pectin biosynthetic processes are apparently downstream targets of these mechanisms. We have performed expression profiling experiments monitoring the transcriptional response to cell wall stress. This was caused by inhibition of cellulose biosynthesis through the highly specific herbicide isoxaben. Amongst the genes responding very strongly on the transcriptional level, one encodes a putative glutamate receptor protein (**GLR**). Homozygous mutant seedlings for this GLR exhibit cell wall defects and reduced lignification upon treatment with isoxaben. Analysis of the available expression data for ***GLR*** genes implicates them in biotic stress response. We will present our results regarding the biological function of this receptor.

543 Negative Regulation of Cell Death and Defense Responses by *BON/CPN* and *BAP* Genes

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Plants utilize an array of disease resistance (*R*) genes to specifically recognize various pathogens in defense responses, and the activation of *R* genes trigger a form of program cell death termed hypersensitive response (HR) to control the spread of pathogens. Emerging evidence indicate that *R* genes and defense responses are regulated by host plant proteins in addition to pathogen effectors. *BON1/CPN1*, an evolutionarily conserved copine gene encoding calcium-dependent phospholipid binding protein, is one such gene that negatively regulates defense responses through an *R* gene *SNC1*. Here we show that the *BON1* gene family and the *BAP1* gene family act together to repress cell death related to defense responses. *BON1* has two homologs in Arabidopsis: *BON2* and *BON3*. Analysis of double and triple mutant combinations among *bon1*, *bon2*, and *bon3* shows that these three copine genes have overlapping functions essential for the viability of plants. Some of the mutant combinations have cell death phenotypes resembling HR leading to seedling lethality. The lethal phenotype can be suppressed by mutations in *PAD4* or *EDS1*. However, the *bon1bon2bon3eds1* and *bon1bon2bon3pad4* mutants still exhibit a temperature-dependent growth defect, suggest a role of this gene family in regulating development in addition to defense responses. BAP1 is a BON1 associated protein isolated from two-hybrid screen. Genetic studies indicate that *BAP1* functions similarly to *BON1* in suppressing cell death and defense responses. The *bap1* loss-of-function mutant, like *bon1*, has constitutive defense responses mediated by *SNC1*. It also has an altered HR to some of the pathogens. *BAP1* overexpression renders plants more susceptible to fungal infection. Furthermore, *BAP1* has an overlapping function with its homolog *BAP2*. Like the *BON1* family, the *BAP1* gene family is essential for the viability of plants. The lethal phenotype of bap1bap2 can be suppressed by *pad4* or *eds1*. Therefore, we have identified an important role of *BON* and *BAP* gene families in the control of cell death and defense response. They both encode membrane associated calcium-dependent lipid binding proteins and they act together to suppress defense response-related cell death likely through negatively regulating multiple *R* genes.

544 Characterization of a Methionine Sulfoxide Reductase B Gene of Arabidopsis Involved in Pathogen Defense

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The generation of reactive oxygen species (ROS), especially under conditions of stress-both biotic and abiotic, is a central theme in plant defense. ROS readily oxidize Met residues to form the sulfoxide (MetSO), which can be in the R or S configuration. Methionine sulfoxide reducatses (MSR) are enzymes that have the ability to catalyze the thioredoxin [T(SH)2]-dependent conversion of MetSO to Met. In doing so, MSR is proposed to act as a last-chance antioxidant, repairing proteins damaged from oxidative stress. While MsrA, the enzyme that utilizes thioredoxin to reduce only the S stereoisomer back to Met is well characterized; its counterpart MsrB that acts on the R stereoisomer of the sulfoxide has been identified only recently. MsrB proteins occur in several organisms including bacteria, yeast, fruit fly, mammals and plants. *Arabidopsis* genome contains nine MSRB genes distributed on chromosomes 1 and 4. We have identified and cloned one of these genes, *AtMSRB1*, that is specifically upregulated during plant-pathogen interactions. Expression profile of this gene coincides with the peak ROS production during incompatible plant-pathogen interaction, suggesting its involvement in repairing proteins damaged during oxidative stress induced by pathogens. Expression of this gene is also induced during other biotic and abiotic stresses. To determine the function of AtMSRB1 protein, we tested and found that AtMSRB1 cDNA was able to complement a yeast mutant strain lacking MSRB gene. We will present detailed characterization of *AtMSRB1* gene, and expression analysis of the entire MSRB gene family of Arabidopsis unraveling its evolution, and distinct patterns and levels of expression.

545 *Pseudomonas viridiflava* induces the jasmonic acid defense pathway in *Arabidopsis thaliana*

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P. viridiflava is one of the most prevalent species of bacteria found in US-Midwestern populations of *A. thaliana*. By investigating the genetic basis of virulence and resistance in this interaction, we are able to better understand the ecological and evolutionary dynamics of plant-bacterial interactions in natural populations. Previous sequencing of the pathogenicity island (PAI) genomic region in *P. viridiflava* revealed two distinct PAIs of differing gene composition. Strains of *P. viridiflava* possess either the tripartite structure of the PAI found in *P. syringae* (T-PAI) or a single component pathogenicity island (S-PAI) containing only the conserved hrp/hrc gene cluster. To test whether differences in PAI affect the interaction with *A. thaliana* ecotype Col-0, we evaluated pectate lyase activity, type III secretion system (TTSS) pilus production, and the induced defense response following infection by each of two S- and T-PAI *P. viridiflava* strains. We used *PR-1* and *PDF1.2* as marker proteins in combination with *A. thaliana* mutants *eds16* and *fad3/7/8* deficient in salicylic acid or jasmonic acid/ethylene mediated resistance to investigate which resistance pathways are induced in *A. thaliana* upon *P. viridiflava* infection in comparison to virulent and avirulent *P. syringae* pv. *tomato* (DC3000). We found that *P. viridiflava* S-and T-PAI strains induced a TTSS pilus on minimal media, indicating both are capable of delivering avirulence/effector genes. All strains of *P. viridiflava* induced *PDF1.2* on the Col-0 wildtype within 24 h of infection. On day two post infection, *P. viridiflava* also triggered *PR-1* expression but at lower levels than DC3000. DC3000 triggered high *PR-1* expression, with earlier induction for avirulent DC3000 as expected from the SA mediated *AVRRPT2/RPS2* interaction. For both *P. viridiflava* and DC3000, we observed higher *PR-1* induction in the JA deficient *fad3/7/8* mutant than in Col-0, indicating negative cross-talk between the SA and JA/ET pathways. PAI differences in *P. viridiflava* did not affect *PR-1* or *PDF1.2* expression but S-PAI strains exhibited higher pectate lyase activity than T-PAI strains, and pectate lyase activity was positively correlated with bacterial growth in Col-0. Three of four *P. viridiflava* strains were more virulent in *fad3/7/8* relative to Col-0 while there was no difference in *P. viridiflava* growth between *eds16* and Col-0. These results indicate that *P. viridiflava* may be generally suppressed by JA mediated resistance.

546 Differential gene expression in response to infection by *Plasmodiophora brassicae* in a partial resistant ecotype of *Arabidopsis* using CATMA analysis

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Plasmodiophora brassicae is the causative agent of clubroot, a disease affecting nearly all species of *Brassicaceae*, including *Arabidopsis thaliana*. *P. brassicae* is a soil-borne, obligate protist with a two-phase infection cycle. The primary phase occurs in root hairs and is followed by a secondary phase in cells of the cortex and stele of the roots. During the latter phase, multinucleate plasmodia induce clubs on roots, leading to inhibition of nutrient and water transport. To provide insights into the kinds of physiological and biochemical changes associated with resistance mechanisms in *Arabidopsis*, variations in gene expression were monitored in infected and non-infected plants of a partial resistant ecotype (Bur0). Plants were collected at different times after inoculation (24 hours, 48 hours and one-week) to relate specific host responses to the life-cycle of the pathogen. Total RNA was isolated and hybridized on the Complete *Arabidopsis* Transcriptome Micro Array (CATMA) which comprises 24577 genes. In total, the expression levels of 849 genes were altered in response to clubroot infection. Of these, 349 were induced and 505 repressed. Host gene expression became increasingly complex over the time course and 98 genes were induced or repressed at multiple time points. At the earlier times after inoculation (24h and 48h) corresponding to the primary phase, ie. first contact between primary zoospores and the root hairs and development of primary plasmodia, 256 genes were differentially expressed. Most of them were defence and stress-response genes. The latter time point (1 week after inoculation) corresponds to the moving of the secondary zoospores to the cortical cells leading to cell enlargement and proliferation. At this point, 720 genes were differentially regulated by *P. brassicae* infection. These included genes associated with defence and stress-response, signal transduction, growth and cell cycle, carbohydrate metabolism and transcriptional regulation. The gene list that was generated provides now several candidate genes to test for roles in clubroot infection processes and resistance mechanisms in *Arabidopsis* and other *Brassicaceae*.

547 Transcription factor NtWRKY1 is phosphorylated by the MAP kinase SIPK and mediates HR-like cell death in tobacco

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The salicylic acid-induced protein kinase (SIPK), a tobacco MAPK, is activated by various biotic and abiotic treatments. Overexpression of SIPK is known to trigger cell death. In this study, we used a targeted yeast two-hybrid approach and identified the tobacco transcription factor WRKY1 as a potential substrate. SIPK phosphorylated WRKY1; this led to enhanced DNA binding activity of WRKY1 to its cognate binding site, a W box sequence. This enhancement of WRKY1 DNA binding activity by SIPK was inhibited by staurosporine, a general kinase inhibitor. Co-expression of SIPK and WRKY1 in Nicotiana benthamiana resulted in more rapid cell death than expression of SIPK alone, suggesting that WRKY1 is involved in the development of HR-like cell death.

548 Over expressing a fatty acid amide hydrolase compromises innate immunity in Arabidopsis

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N-acylethanolamines (NAEs) are a group of lipid mediators that have been shown to accumulate under a variety of neurological and pathological conditions in mammals. This led to the proposal that these fatty acid amides might have neuroprotective roles in animals. In mammalian tissues, NAE signaling is terminated by the action of a fatty acid amide hydrolase (FAAH), which hydrolyzes NAE into free fatty acid and ethanolamine. NAEs have been quantified in a variety of plant tissues and plants were shown to have the necessary enzymes involved in the metabolism/degradation of NAE including FAAH. Transgenic *Arabidopsis* lines overexpressing *AtFAAH* exhibited altered sensitivity to exogenous NAE and a variety of other abiotic stresses. Compared to vector controls, *FAAH* overexpressors were more susceptible to bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and *Pseudomonas syringae* pv. *maculicola* ES4326. *FAAH* overexpressors were highly susceptible to a nonhost pathogen, *P. s*. pv. *tabaci* and they produced disease symptoms. *FAAH* overexpressors were also partially susceptible to nonhost pathogen *P.s. pv. glycinea*. However, the *FAAH* overexpressors did not significantly compromise gene-for-gene resistance. Interestingly, *AtFAAH* is upregulated in wild-type *Arabidopsis* plants during pathogen infection and the expression of several pathogenesis-related (*PR*) genes were altered in *FAAH* overexpressors. Our data suggest that *AtFAAH* might play an important role in mediating plant responses to environmental stress by regulating NAE accumulation in plants. A detailed characterization of *Arabidopsis FAAH* overexpressors will be presented.

549 Transcriptome analysis of *Bemisia argentifolli* adult and instar feeding on *Arabidopsis thaliana*

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The silverleaf whitefly (*Bemisia argentifolii*) is a phloem-feeding pest that causes extensive crop damage throughout the United States. These insects are viral vectors, produce honey dew leading to fungus infection, reduce photosynthesis, and cause developmental disorders in several crop and horticultural plants resulting in decreased crop value. Plant responses to insect infestation are specific to the method of insect feeding, amount of plant tissue damage, and specific insect elicitors. Silverleaf whitefly-plant interactions are unique in that the insects cause very little tissue damage but yet, have a prolonged interaction with the leaf. Few studies have examined plant-defense responses to phloem-feeding insects and preliminary studies suggest that these interactions may provide insights into "crosstalk" between wound and pathogen signal transduction cascades. The goal of this study is to use the Affymetrix ATH1 Arabidopsis microarray chip to compare how Arabidopsis gene expression changes in response to whitefly feeding differs from other stresses and identify novel genes and pathways unique to plant-whitefly interactions. Robust microarray analysis (RMA) method was used for background adjustment, quantile normalization, and median summarization of probe intensities. Significance analysis of microarray analysis (SAM) was used for differential analysis of significantly up and downregulated genes. A delta value of 2.06 and false discovery rate of 3.917% identified 1269 genes, including 562 upregulated and 707 downregulated genes in response to whitefly infestation. The data was used for hierarchical clustering analysis with other microarray profiles from wounding, oxidative stress, salt stress, UV-A, UV-B, insect feeding (*Trichnoplusia ni, Myzus persicae*) and pathogen infection (*Pseudomonas syringae, Peronospora parasitica, Botrytis cinerea*, and *Erysiphe orontii*) data. These results have allowed the identification specific whitefly-induced genes that will be investigated in further experiments. Furthermore, the biological relevance of the results obtained from statistical methods, RMA/SAM and MAS 5.0, are being compared using Southern blots of semi-quantitative RT-PCR of 20 genes. This will test the biological consistency and accuracy of two commonly used microarray data analysis methods.

550 Systems Analysis of Natural Variation in Arabidopsis/Botrytis Interactions

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Botrytis cinerea is a necrotophic pathogen of fruit and vegetable crops throughout the world, causing both pre- and post-harvest grey mould. Plant resistance mechanisms against necrotrophs, and their associated signaling pathways, are poorly understood. In contrast to biotrophic pathogens, necrotrophs actively kill the plant cell and as such, programmed cell death actually generates susceptibility. Arabidopsis is believed to utilize other resistance mechanisms such as secondary metabolites to defend against Botrytis.

We are working towards identifying the specific mechanisms by which Arabidopsis defends against Botrytis. To allow for test if any given mechanism is general to the species or specific to the genotype, we are evolutionarily characterizing a collection of > 40 Botrytis isolates. These isolates vary for their ability to detoxify camalexin and glucosinolates both *in vitro* and *in planta*. Using Arabidopsis mutants, we have shown that this pathogen variation in secondary metabolite sensitivity functions to determine the isolates virulence on different Arabidopsis genotypes, supporting the idea that Arabidopsis uses secondary metabolites as a primary defense.

We are investigating if Botrytis actively represses plant secondary metabolite to evade their toxicity. Using high-throughput metabolomics, we have shown that camalexin is specifically induced immediately proximal to the lesion while phenylpropanoids and glucosinolates are repressed local to the lesion. This suggests a gradient of signals emanates from the lesion edge causing both distal and local responses. We have identified classes of genes that show different gradient responses with microarrays. The strength and pattern of the gradient depends upon the Botrytis isolate utilized suggesting that there is variation in signal transduction depending upon the pathogen genotype.

The Arabidopsis accessions Bay and Sha show differential sensitivity to a wide-range of Botrytis isolates. This allows using the Bay x Sha RILs to test for the functional the basis of this differential sensitivity. To identify the underlying mechanisms we are mapping QTL for sensitivity to five Botrytis isolates as well as using metabolomics to map secondary metabolite response QTL to each isolate. We are also mapping QTL that regulate the expression of Botrytis inducible genes in the same lines. We have been able to identify mechanistic linkages between QTL at all levels. This systems analysis of quantitative natural variation should improve our capacity to understand the Arabidopsis/Botrytis interaction.

551 Investigating the role of lipids in systemic acquired resistance

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We had previously demonstrated that mutation in the Arabidopsis SFD1 gene, which encodes a dihyroxyacetone phosphate (DHAP) reductase, affects lipid composition and the activation of systemic acquired resistance (SAR). The sfd1 mutant along with the sfd2 and sfd4 mutants was identified in a screen for mutations that suppress the ssi2-conferred growth and defense phenotypes. The ssi2 mutant constitutively expresses SAR. SSI2 encodes a stearoyl-ACP desaturase that catalyzes the conversion of stearic acid to oleic acid in plastids. Like the sfd1 and ssi2 mutants, lipid composition is also altered in the sfd2 and sfd4 mutants. Indeed, SFD4 (same as FAD6) encodes a fatty acid desaturase. Thus our studies implicate a role for plant lipids in SAR. We will present our progress on the characterization of these lipid metabolism mutants and their role in SAR.

552 The *Pseudomonas syringae* type III effector protein AvrRpt2 modulates host auxin physiology

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The bacterial plant pathogen *Pseudomonas syringae* utilizes a Type III secretion system to introduce bacterial proteins into the host cell cytosol. These proteins, also referred to as "effectors", are hypothesized to alter host cell signaling and physiology in order to promote disease susceptibility. The *P. syringae* type III effector protein AvrRpt2 functions inside plant cells to promote growth and symptom production of *P. syringae* strain DC3000 on *Arabidopsis thaliana* plants lacking a functional copy of the *RPS2* resistance gene (e.g. *rps2* mutant plants). To investigate possible physiological or metabolic changes induced by AvrRpt2, we examined the phenotypes of transgenic *A. thaliana* plants constitutively expressing *avrRpt2*. Three independent transgenic lines expressing *avrRpt2* exhibited seedling phenotypes reminiscent of *A. thaliana* mutants with altered auxin physiology. The transgenic seedlings also exhibited increased sensitivity to exogenous auxin, and accumulated slightly elevated levels of the naturally occurring auxin, indole acetic acid (IAA). Free IAA levels were significantly and reproducibly higher (~1.5 fold) in plants infected with DC3000 than in uninfected plants, and the presence of AvrRpt2, either when delivered by DC3000 or when expressed in transgenic plants, resulted in significantly larger increases in free IAA levels in the infected plants. These results indicate that AvrRpt2 alters *A. thaliana* auxin physiology. Exogenous application of the auxin analog 1-naphthaleneacetic acid (NAA) resulted in increased disease susceptibility to DC3000, suggesting that elevated auxin levels promote pathogenesis by DC3000. Thus, *P. syringae* may actively modulate auxin physiology to promote disease, and AvrRpt2 may be among the virulence factors that are involved in this process.

553 JIN1/AtMYC2-dependent jasmonate signaling is required for suppression of salicylic acid-dependent defenses during infection by Pseudomonas syringae

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Many plant pathogens are proposed to suppress basic anitmicrobial defenses using secreted virulence factors that modulate endogenous host defense signaling pathways. The *Pseudomonas syringae* phytotoxin coronatine (COR) is proposed to promote virulence by acting as a molecular mimic of one or more jasmonates, such as JA-Ile. This hypothesis is based on evidence that *COR-insensitive 1* (*coi1*) *Arabidopsis thaliana* and tomato mutants are impaired in jasmonate signaling and exhibit reduced susceptibility to *P. syringae*. To further investigate the role of jasmonate signaling in disease development, we analyzed several jasmonate-insensitive A. thaliana mutants for susceptibility to *P. syringae* pv. *tomato* strain DC3000 (*Pst*DC3000). The *jasmonate insensitive 1* (*jin1*) and *auxin resistant 1* (*axr1*) mutants exhibit both reduced susceptibility to *Pst*DC3000 and reduced sensitivity to COR, while the *jasmonate resistant 1* (*jar1*) mutant exhibits wild-type responses to both COR and *Pst*DC3000. A *jin1 jar1* double mutant does not exhibit enhanced jasmonate insensitivity, suggesting that *JIN1* and *JAR1* are not functionally redundant and act in the same signaling pathway affecting jasmonate-dependent responses. Reduced disease susceptibility in *jin1* mutants is correlated with elevated expression of *Pathogenesis related 1* (*PR1*) and is dependent on accumulation of salicylic acid (SA), suggesting that reduced susceptibility of *jin1* mutants results from increased expression of SA-dependent defenses. Thus, *Pst*DC3000 appears to utilize COR to manipulate JIN1/AtMYC2-dependent host jasmonate signaling to suppress SA-mediated defenses, better enabling the pathogen to colonize host tissue. We present a revised model for jasmonate and SA signaling during *P. syringae* infection that incorporates these and other recent discoveries. Work is underway to clarify this model and identify additional components of the pathway affecting *P. syringae* pathogenesis.

554 Identifying natural modifier(s) of a copine mutant bon1bon3

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Copine proteins are highly conserved among protozoa, plants, nematodes, and mammals. The calcium-dependent lipid binding activity of these proteins indicates their potential roles in membrane trafficking and signal transduction. However, the biochemical and biological functions of these proteins are not well understood. There are three members of copines in Arabidopsis thaliana, named as *BON1*, *BON2* and *BON3*. These genes have both distinct and overlapping roles in growth and defense and they are essential for plant viability. Analysis of mutliple mutant combinations indicate that they act as negative regulators of cell death pathway possibly through *R*-gene mediated resistance.

The overlapping function between *BON1* and *BON3* are accession dependent. *bon1bon3* double mutant in Col background is seedling lethal while it is wild-type like in Ws background. Previous study showed that the activation of a Col-specific resistance (*R*) gene *SNC1* leads to the growth defect phenotype of bon1 single mutant in Col, and the lack of *SNC1* in Ws leads to the wild type-like phenotype of *bon1*. However, *SNC1* is not the only natural modifier of *bon1bon3* because the snc1 mutation can only partially rescue the lethal phenotype of bon1bon3 in Col and the bon1bon3snc1 triple mutant eventually dies without bolting. To uncover the natural modifier(s) besides *SNC1*, which are probably common target of *BON1* and *BON3*, map-based cloning approach is carried out. *bon1bon3snc1* in Col background was crossed to *bon1bon3* in Ws background, and the F2 population was used for mapping. Phenotypic analysis of F2 population revealed cell death phenotype at different development stages with different levels and the potential natural modifier(s) is(are) named *LCD(s)*(*Lesion Cell Death*) according to the phenotype. Initial mapping revealed an association of *lcd* with a dominant locus in Col on chromosome 1. Both fine mapping and candidate gene approach are undertaken to clone the LCD(s). The identification of *LCD* will help us to further understand the regulation of defense responses as well as the function of *BON* genes.

555 Study of biochemical activity of *BON1* and *BAP1* in regulating plant growth and defense response

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The Arabidopsis *BONZAI 1 (BON1)* is a member of evolutionally conserved copine family. A *BON1* interacting protein *BAP1 (BON1 association protein 1)* was discovered from yeast-two-hybrid screening using the A domain of *BON1* at the C-terminus as bait. Our study shows that *BON1* and *BAP1* are negative regulators of plant defense response and required for the maintenance of plant growth homeostasis under normal growth conditions. The loss of function mutants, *bon1* and *bap1*, both show enhanced defense responses and compromised plant growth, with *bap1* being less severe.

BON1, like other copines, has two C2 domains and it binds to phospholipids in Ca2+-dependent manner. The A domain at C terminus has a putative kinase activity. *BAP1* is a relatively small protein with only one C2 domain at the N-terminus and a short fragment at the C-terminus. Site-directed mutagenesis is carried out on *BON1* and *BAP1* to study the biochemical activity that *BON1* and *BAP1* use for their biological function. To study the role of lipid binding activity in *BON1/BAP1* function, Asp to Ala mutations were generated on the conserved aspartates which are essential for calcium binding in the C2 domains of *BON1* (D63, D69, D122, D124, D209, D215, D269) and *BAP1*(D94). As for the putative kinase activity in *BON1*, Ala350 and Gly353 in the conserved glycine loop for ATP binding and the essential Lys391 for kinase activity are mutated into Val, Val and Ala, respectively. All the mutated version and wild type *BON1/BAP1* control were transformed into *bon1* and *bap1* mutants respectively to see if they can functionally rescue the mutant phenotype. If the mutated protein can rescue the mutant phenotype, the mutagenized site(s) is(are) not essential for the *BON1/BAP1* function. Otherwise, these residues are essential for the biological function of *BON1/BAP1*. This analysis should allow a further understanding of the biochemical activities of the evolutionarily conserved copine family.

556 Innate Immune Signaling Pathways Controlling the Response to General Elicitors

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Despite advances in the understanding of salicylic acid, jasmonic acid, and ethylene signaling and *avr-R* gene mediated responses, the signaling events regulating the innate immune response to elicitors or PAMPs (Pathogen-Associated Molecular Patterns) have gone largely unexplored. The most significant exception to this trend has been recent studies of the Arabidopsis response to flg22, a 22-amino acid epitope of bacterial flagellin. We are addressing whether a MAPK pathway activated by flg22 represents a flg22-specific signaling mechanism or a shared innate immune signaling pathway that is activated by a variety of PAMPs. Preliminary data indicates that the same MAPKs are activated in response to flg22 and oligogalacturonides (OGs). Microarray data show that although the transcriptional responses to flg22 and OGs are overlapping, the response to flg22 involves the activation and repression of many more genes than the response to OGs.

557 Auxin repression in *Arabidopsis* by a flea beetle damage-induced *Brassica napus* gene.

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The crucifer flea beetle *Phyllotreta cruciferae* is a chronic, voracious pest of canola rape fields and will also feed on *Arabidopsis thaliani* plants. We have been investigating the molecular basis of plant response to flea beetle feeding with the intent of enhancing weak defense responses or eliminating susceptibility factors in *B. napus*. One strategy has been to characterize a putative auxin-repressor protein (bnARP), which represents 2.5% of the sequences in a flea beetle damage-induced *B. napus* mature leaf subtraction library and 0.08% in a damage-induced cotyledon EST library. The gene is strongly expressed in undamaged, mature *B. napus* leaves, and barely detected in RNA from cotyledons, seedling leaves, stems, flowers or seed pods. The gene is also strongly induced in flea beetle-damaged leaves, moderately induced in *Sclerotinia*-infected or dehydrated leaves, strongly down-regulated in leaves after a 1-2 h cold treatment, and unaffected by mechanical wounding. A full-length cDNA was cloned and over-expressed in *Arabidopsis*. T2 lines included very slow-growing, non-flowering phenotypes and moderately slow-growing, dark purple phenotypes. The moderate phenotypes resumed normal growth after bolting, but had fewer siliques. Primary root length and the number of lateral roots were reduced in the transgenic *Arabidopsis* plants, while a normal root phenotype was partially (~70%) restored after application of 2,4-D. Tissue-specific and stress-induced patterns of gene expression will be complemented with expression patterns for auxin signal transduction and metabolism to develop a hypothesis on the role of the *B. napus* auxin repressor protein in plant response to insect feeding.

558 Below-Ground Influence of Arabidopsis on Its Rhizobacterial Community Structure Varies with Plant Ecotype

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The rhizosphere is the portion of soil that is under the influence of plant roots. Several factors have been implicated in shaping microbial communities in the rhizosphere. The selective influence of plant species on bacteria associated with their roots is regarded as being of major importance. This phenomenon has been linked to root exudates. The production and composition of exudates have been shown to vary with plant taxa, implying that taxonomic differences in exudate profiles are attributable to plant genetic variation. Bacterial communities growing in the rhizosphere of natural ecotypes Arabidopsis thaliana were investigated to determine whether closely related variants of a single plant species exert a distinct selective influence on bacteria associated with their roots. Eight Arabidopsis thaliana ecotypes were grown on a loamy soil and rhizosphere soil was collected just prior to plant flowering. Genomic DNA was extracted from rhizosphere and bulk soil and bacterial profiles for each ecotype were obtained by terminal-restriction fragment length polymorphism (T-RFLP) analysis. Bacterial 16S rDNA was amplified by PCR and digested with the restriction enzyme MspI. Distinct and reproducible ecotype-specific T-RFLP community profiles, which also differed from bulk soil profiles, were obtained. Multidimensional scaling and hierarchical cluster analysis on bacterial population profiles were performed to reveal relationships among ecotypes and bulk soil. Replicates for each ecotype and for bulk soil grouped into separate clusters, indicating that each ecotype rhizobacterial community is unique. This implies that rhizobacterial community composition is influenced by the plant genetic background at the subspecies level, possibly in response to ecotype-specific differences in exudate activity and root growth patterns. Moreover, we will present bacterial population differences resulting from changing soil conditions.

559 Green Leaf Volatile Action in Arabidopsis

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Plants respond to herbivore damage by emitting a complex pattern of volatile organic compounds from vegetative tissues. The release of volatiles is part of the defense mechanism since these volatiles can attract natural predators of the herbivores, a process indicated as indirect defense. However, in recent years it has become clear that also neighboring uninfested plants exposed to these volatiles respond with transcriptional and metabolic changes related to defense mechanisms. This led to the "plant-to-plant communication" hypothesis, according to which neighboring plants can exploit this volatile information to prepare themselves for future herbivore attack. However, to date the mechanism by which plants respond to volatiles is unknown.

To elucidate this mechanism we decided to isolate mutants that do not respond to volatiles in the model system Arabidopsis thaliana. We focused on the interaction between Arabidopsis (Ecotype Columbia) and Green Leaf Volatiles (GLVs). These are a specific class of six-carbon (C6) volatiles, induced upon leaf damage in several plants, including Arabidopsis. We observed that aerial treatment of Arabidopsis seedlings with micromolar concentration of a GLV, E-2-hexenol, inhibits the elongation of the main root. The same treatment also induces the expression of defense as well as secondary-metabolism genes. We showed that the inhibition of the root elongation by E-2-hexenol is not dependent on JA, SA or ethylene. We screened EMS mutagenised Arabidopsis seedlings and we isolated several mutants that do not respond to E-2-hexenol. We refer to these mutants as hexenol response (her) mutants. Using a combination of microarray and positional cloning, we mapped the mutation in one of this mutant (her-1) to a 200 Kb region of the chromosome 3. Cloning of the mutated gene is in progress.

Currently, we are determining the effect of GLVs treatment on gene expression, in both wild-type and her plants, by making use of whole genome DNA-microarray. This will provide information on plant genes that are transcriptionally regulated by GLVs as well as on genes that are differentially regulated in the her mutants compared to wild-type plants.

560 The *Arabidopsis thaliana* response to the bacterial pathogen *Pseudomonas syringae*: the search for novel plant defense signaling pathways

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Upon pathogen recognition, the plant must activate its defensive arsenal. Three signaling molecules play roles in the activation of plant defenses: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The levels of these compounds typically increase in plants after pathogen exposure and these compounds mediate the plant response to different pathogens. *Arabidopsis thaliana* mutants that are defective in the production of SA (*sid2, pad4*) or in responses to SA (*npr1*) show enhanced susceptibility to the bacterial pathogen *Pseudomonas syringae pv maculicola* ES4326 (*Psm*ES4326). The JA signaling mutant *coi1* and the ET response mutant *ein2* exhibit enhanced susceptibility to the fungus *Botrytis cinerea*. Gene expression studies provide evidence for additional plant defense signaling pathways. Previous microarray studies identified 641 *A. thaliana* genes that are upregulated in response to *P. syringae*. The expression of a subset of these genes was unaffected in any of a panel of 12 SA, JA and ET mutants, suggesting that this group of genes may function upstream of the mutations used or may participate in a parallel *P. syringae*-induced pathway.

A closer examination of genes that are regulated independently of known disease signaling pathways may yield information about novel pathways controlling plant defenses. To identify plant genes that are differentially expressed during defense against *P. syringae*, we have used the *A. thaliana* whole genome Affymetrix GeneChip. We analyzed transcriptional profiles of plants exposed to the virulent P. syringae strain *Psm*ES4326 at three time points after inoculation (9h, 24h and 32h). We identified differentially expressed genes at each time point, those that are predicted with 90% confidence to exhibit a >2.5 fold change in expression: at 9 hours post inoculation, 100 genes were induced; at 24 hours, 3165 genes were differentially expressed; at 32 hours, 2933 genes were differentially expressed. In total, 4020 genes are predicted to change expression in response to pathogen inoculation. We will identify a set of genes for which expression is unaffected in plant mutants with defects in known pathways: *sid2, coi1, ein2, npr1, pad2 and pad4*. We intend to use a reverse genetic approach, targeting genes that are induced independently of known pathways, to identify genes that are required for resistance against *Psm*ES4326.

As a complete understanding of plant defenses requires the identification of all pathways involved, these studies are crucial to the study of the plant defense response to microbial pathogens.

561 local adaptation in natural population of Arabidopsis thaliana

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All plant species suffer from a variety of abiotic stresses (e.g. soil moisture level, nutrient status of the soil, light availability), but also from interactions with herbivores or other plants. In many cases, genetic adaptation has occurred in response to stressful levels of these environmental parameters. In a transplant experiment Arabidopsis thaliana plants from a dune and inland habitat were studied with respect to morphological and life history traits and resistance to the specialist beetles Ceutorhyncus atomus and C. contractus (Curculinoidae). We investigated 1) whether differences were environmentally or genetically determined, 2) whether plants were adapted to their local environment and 3) the importance of specialist herbivores for plant adaptation. There was a significant site effect in all traits, such that all plants performed better in the inland site with respect to number of stem, height, rosette size and seed production. Fruit damage was less in the inland site as well. Genetic differences between population were observed for several life history and morphological characters and for percentage of fruit damage. In particular, plants from the inland habitat flowered earlier, produced more fruits, and experienced more fruit damage by weevils. No significant site * population interaction was found for rosette size, number of stems, fruit number before damage and percentage of damaged fruits. Instead, for stem height and intact fruit (the most direct estimate of fitness) an interaction did exist. Plants at their home site produced the highest number of intact fruits, which demonstrates adaptation to local environment. In this process high resistance to herbivory in dune plants appears to be an important mechanism responsible for greater relative fitness of plant in the sites.

562 Molecular and Genetic Analysis of the Transcription Factor ETHYLENE INSENSITIVE6

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The plant hormone ethylene regulates a variety of developmental and stress responses, including the triple response displayed by etiolated seedlings. We have previously identified *ETHYLENE INSENSITIVE6* (*EIN6*) in a genetic screen for mutants deficient in the triple response. Mutations in *EIN6* are epistatic to *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*) and therefore *EIN6* acts downstream of CTR1 in the ethylene signaling pathway. Further characterization of this mutant revealed that it contained a second recessive mutation, *ENHANCER OF ETHYLENE INSENSITIVITY* (*EEN*), which dramatically enhanced the *ein6* ethylene phenotype. In the absence of *een*, *ein6* plants display an ethylene insensitive root phenotype. Mutations in *EEN* show no ethylene phenotype on their own, but dramatically enhance the *ein6* ethylene insensitive root phenotype; the *ein6een* double mutants show a near complete lack of the triple response. *EIN6* was mapped to the bottom of chromosome 3. Positional cloning of *EIN6* revealed that it encodes a DNA binding protein, consistent with its downstream position late in the ethylene signaling pathway. Protein levels of the transcription factor EIN3 and its related family members are regulated by the EIN6. In the *ein6een* double mutant EIN3 protein levels are nearly abolished. This indicates that the ethylene signaling pathway works through *EIN6* gene and that the *EIN6* transcript must be intact for proper downstream ethylene signaling.

563 HSC70s interact directly with the Arabidopsis SGT1s cochaperones *in planta* and are required for *R*-gene-mediated and basal resistance

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SGT1 (Suppressor of G2 allele of Skp1) proteins have initially been identified as essential for cell cycle progression in yeast and are core components of the kinetochore. SGT1 proteins are conserved in all eukaryotes and have recently been proposed to be HSP90 co-chaperones. In plants, SGT1 proteins are required for several SCF-dependent responses and resistance to various pathogens. In order to investigate SGT1 functions *in planta*, we affinity purified *At*SGT1 proteins from Arabidopsis leaf extracts using a StrepII epitope. Using mass spectrometry, four heat shock cognates 70 (HSC70) chaperones were identified as the major stable interactors of *At*SGT1 proteins. Those observations could be confirmed by co-immunoprecipitation experiments with native *At*SGT1 proteins. Expression of several of these HSC70 isoforms is strongly up-regulated at the mRNA level by pathogen challenge. Miss-expression of HSC70 compromised basal and *R*-gene mediated resistance to fungal and bacterial pathogens. Our results implicate for the first time HSC70 chaperones as important modulators of disease resistance.

564 The pathogenic bacteria *Ralstonia solanacearum* directly targets the plant ubiquitin-proteasome pathway by injecting bacterial encoded F-box proteins into the plant cells

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Ralstonia solanacearum is a β-proteobacteria adapted to live in soil. It infects the root system and is responsible for the bacterial wilt on a wide variety of plants. The model plants used to study this interaction are mainly tobacco, tomato and *Arabidopsis*. Like many other pathogenic bacteria *R. solanacearum* uses a type III secretion system (or *hrp* system) to directly inject bacterial effector proteins into the plant cells. This secretion system has been shown to be the key determinant controlling the "gene for gene" mediated resistance and the virulence of this bacteria. Genome mining for type III effector proteins enabled us to discover a protein family of 7 genes encoding atypical bacterial proteins containing plant-like LRRs and more surprisingly an F-box domain. F-box motif containing proteins are a very diverse and large family of proteins mostly restricted to eukaryotes. In these organisms it has been shown that the F-box protein together with SKP1 and Cullin forms a complex (called SCF) which specifically targets protein substrates for degradation by the ubiquitin-proteasome pathway. Here we show that *Ralstonia solanacearum* indeed injects an F-box protein into plant cells where it is able to interact with the *Arabidopsis* SKP1 homologues in a plant-like fashion. This suggests that this F-box protein participates in a bacteria/plant composite SCF complex where it could specify for degradation of a plant protein. We are currently trying to identify the possible plant proteins targeted and assessing the role in virulence of this family of effectors.

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565 Identification and characterization of Arabidopsis thaliana ecotypes with contrasting responses to inoculation with a Brazilian isolate of Xanthomonas campestris pv. campestris

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To assess the response of *Arabidopsis thaliana* to a Brazilian isolate of *Xanthomonas campestris* pv. *campestris*, 33 different ecotypes of diverse geographical origin were syringe inoculated with 5 x 10^6 CFU/ml of the CNPH17 isolate of this bacteria. Symptom development was monitored macroscopically and rated according to a numerical scale 5 to 7 days post inoculation (DPI). To quantitate symptoms progression more precisely chlorophyll levels were monitored. Statistical analyses of variance (ANAVA) and Tukey test of means indicated significant differences in response to inoculation supporting the classification of ecotypes as susceptible or resistant. To investigate if resistance involved a hypersensitive response, plants were inoculated with a high bacterial concentration of 5 x 10^8 CFU/ml and symptoms development was monitored at between 12 and 48 h. Crosses between resistant and susceptible ecotypes have been performed to generate populations for genetic characterization of resistance.

566 Evaluation of Arabidopsis thaliana response to infection by the tospoviruses Tomato spotted wilt virus and Groundnut ringspot virus

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To assess the response of *Arabidopsis thaliana* to *Tomato spotted wilt virus* (TSWV), 20 different ecotypes of diverse geographical origin were mechanically inoculated with isolate BR-01 of the virus. Symptom development was monitored macroscopically and infection of plants with TSWV was detected with double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using polyclonal antiserum against the nucleocapsid protein of the virus. All 20 ecotypes tested were susceptible to TSWV infection. To investigate if *Arabidopsis* is a host to another member of the genus *Tospovirus* that also occurs in Brazil, *Groundnut ringspot virus* (GRSV), six selected *Arabidopsis* ecotypes were mechanically inoculated with isolate A5 of this virus. Based on DAS-ELISA results, it was concluded that *Arabidopsis* supports GRSV replication and, therefore, should be included in its list of hosts.

567 Genome-wide analysis of gene expression reveals function of AtMYB30 in lipid signalling for the control of disease resistance and hypersensitive cell death

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In plants, one of the most efficient resistance reactions to pathogen attack, is the so-called Hypersensitive Response (HR) characterized by a rapid and localized cell death at the inoculation site. To identify genes involved in the regulation of HR-related cell death programmes, we searched for genes early and specifically induced during the HR. Among them, *AtMYB30* is specifically, rapidly and transiently expressed during incompatible interactions between *Arabidopsis* and bacterial pathogens (Daniel et al., 1999). Its expression was also found to be deregulated in Arabidopsis mutants affected in the control of cell death initiation. Using sense *AtMYB30* tobacco and *Arabidopsis* lines (*AtMYB30*ox) we demonstrated that overexpression of *AtMYB30* (i) accelerates and intensifies the appearance of the HR in response to avirulent bacterial pathogens, (ii) causes HR-like responses to virulent bacterial pathogens, and (iii) increases resistance against different avirulent and virulent bacterial pathogens, and a virulent biotrophic fungal pathogen, *Cercospora nicotianae* (Vailleau et al., 2002). In antisense *AtMYB30* tobacco lines (*AtMYB30*as), the HR cell death was significantly decreased or suppressed. Taken together, these results strongly suggest that *AtMYB30* is a positive regulator of the hypersensitive cell death.

In order to further understand *AtMYB30* function, we searched for putative target genes of this transcription factor using Affymetrix whole genome arrays. This analysis allowed us to monitor transcriptionnal changes occurring in the wild-type, *AtMYB30*ox and *AtMYB30*as lines upon infection by an avirulent bacterial pathogen. About 50% of the *AtMYB30*-upregulated genes identified by this strategy, were related to lipid metabolism. A complementary gene expression survey by Q-RT-PCR using the same lines and a T-DNA ko line allowed to validate the microarrays data and to identify a subset of lipid-related genes tightly regulated by *AtMYB30*, all putatively involved in very-long chain fatty acid (VLCFA) synthesis. Biochemical and genetic approaches confirmed these molecular data, and we propose a model in which *AtMYB30* modulates cell death lipid signaling, by enhancing the incorporation of FA of the ER in VLCFAs, and promoting the use of plastidial FA for biosynthesis of some oxylipins.

568 Identification of genes contributing to nonhost resistance of Arabidopsis thaliana against Phytophthora infestans

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Nonhost resistance is a characteristic feature of most interactions between plants and microorganisms. One of numerous examples is the resistance of *Arabidopsis thaliana* against the oomycete, *Phytophthora infestans*, the causal agent of late blight disease on potato and tomato. In general, *Phytophthora infestans* spores germinate on Arabidopsis leaves and appressorium-like structures are formed. In most cases, penetration of the epidermal cell wall is averted by papillae formation. However, if the penetration attempt is successful, additional defense responses are activated, usually resulting in the death of the attacked epidermal cell. One of our approaches for identifying genes that are involved in this nonhost resistance is a mutant screen. Approximately 70.000 M2 plants of an EMS-mutagenized population have been scored with regard to their hypersensitive response phenotype after inoculation with *Phytophthora infestans* spores. So far, several mutants with increased number of hypersensitively reacting cells have been isolated using a fluorescence stereo-microscope.

569 The gene RPB1 confers resistance of *Arabidopsis thaliana* to the obligate biotrophic root parasite *Plasmodiophora brassicae*

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A monogenic resistance phenotype to the *Plasmodiophora brassicae* single-spore isolate 'e3' has been found in *Arabidopsis thaliana* ecotypes Tsu-0, Ze-0, Ta-0 and RLD. The dominantly inherited RPB1 gene mediated a hypersensitive response reaction. Crossing experiments with mutant lines of salicylic acid, jasmonic acid and ethylene revealed no influence of these hormones in the resistance reaction. The RPB1 locus has been localized to a region of approximately 71kb on chromosome 1. Within these region there are 14 coding sequences and three pseudogenes according to the sequence of ecotype Columbia. Six of the 14 genes have been demonstrated to coseggregate with RPB1. Two of these six genes (At1g32020 and At1g32040) are highly polymorphic, two other genes (At1g32010 and At1g32000) are duplicated on chromosom 5. None of the 14 coding sequences revealed similarities to previously identified resistance genes. Ten of 14 genes genes have been demonstrated to be expressed in at least one of the resistant or susceptible ecotypes. Microarray data revealed very weak expression levels of these genes in the susceptible ecotype Columbia except for At1g32050, but expression analysis by in silico in situ hybridization revealed tissue-specific expression.

570 The role of the GH3-gene family during clubroot infection of *A. thaliana*

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The obligate biotroph root pathogen *Plasmodiophora brassicae* is the causal agent of clubroot disease which is one of the most damaging among *Brassicacae*. This disease was analysed among others with a microarray experiment (Affymetrix 22k) using the model plant *Arabidopsis thaliana*. One interesting result were changes in auxin metabolism and homeostasis during the infection. So the GH3-gene family, of which some genes adenylate auxins to form aminoacid conjugates (Staswick et al. 2005), is upregulated by *Plasmodiophora brassicae* during colonization of host roots. To repress the expression we took the two most upregulated GH3-genes and made antisense constructs with a root specific promoter from *Arabidopsis thaliana*. The testing of transformants is in progress.

571 Transcription factor Dof15 as a candidate gene regulating glucosinolate metabolism in *Arabidopsis*

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Glucosinolates are secondary metabolites that function as defense compounds against herbivores and pathogens in the plant order *Capparales*. In addition, indole glucosinolates (IGS) are essential for plant growth and development serving as both a sink for indole-3-acetaldoxime (IAOx) and a source of indole-3-acetonitrile (IAN) during indole-3-acetic acid (IAA) biosynthesis. Different functions were assigned to *Arabidopsis* 37 members of the DNA-binding-with-one-finger (Dof) transcription factor family. In our studies genome-wide analysis of transcript levels in plants over-expressing Dof15 (35S:Dof15) revealed an induction of genes involved in biotic defense reactions, including genes of the glucosinolate/myrosinase system: myrosinase binding proteins, CYP79B2 and CYP79B3 - two enzymes converting tryptophan to IAOx. Targeted gene expression studies on genes from this pathway confirmed an increase of CYP79B2, CYP79B3, MAM1 and CYP83B1 gene expression. MAM1 catalyzes the condensing reactions of the first two methionine elongation cycles of aliphatic glucosinolate biosynthesis and CYP83B1 is a branching enzyme that shifts the IAOx pool towards IGS. Biochemical measurements revealed elevated levels of aliphatic and indole glucosinolates and also IAA. RNAi:Dof15 plants revealed decreased expression of CYP83B1, CYP79B2 and CYP79B3. Induction of Dof15 expression within hours after jasmonic acid treatment and wounding in wild-type *Arabidopsis* plants as well as in transgenic lines transformed with a promDof15:GUS construct further corroborate a role in plant defense. Our data suggest a function of Dof15 in regulating glucosinolate metabolism in Arabidopsis both under normal and biotic stress conditions.

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572 Recognition specificity in the FLS2/flagellin interaction

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As one form of disease resistance, plants initiate innate immunity against microbial invasion by recognizing pathogen-associated molecular patterns (PAMPs). Several bacterial compounds such as flagellins, lipopolysaccharides, cold-shock proteins, and Tu elongation factor have been identified as having the characteristics of PAMPs that can induce plant innate immunity. In *Arabidopsis*, the FLS2 transmembrane LRR-kinase was previously shown to confer flagellin responsiveness. To elucidate the recognition determinants of bacterial flagellins, we cloned *fliC* genes from a set of diverse *Xanthomonas campestris* pathovar *campestris (Xcc)* strains. Clustal analysis and the resulting phylogenetic tree of these *fliC* genes indicated that these *Xcc* strains belong to two distinct clades, consistent with the data from virulence assays. His6-tagged flagellins were purified and tested for elicitation of plant defense responses. We discovered within-pathovar polymorphism for the defense-eliciting activity of flagellin. Through use of domain swapping and site-directed mutagenesis, we determined that the flg22 region of *Xcc* flagellins is the sole flagellin region that is responsible for elicitation of Arabidopsis defense responses. A single amino acid polymorphism in the flg22 region was identified that is critical for elicitation activity. Reciprocal substitutions of Val43 and Asp43 completely reversed the eliciting or non-eliciting nature of flagellins from *Xcc* strains B305 and B186. *Xcc* B186 gene replacement strains expressing these flagellin variants are now being tested for virulence on plants. Previous results indicated that *Ralstonia solanacearum* flagellins have no elicitation ability to induce plant defense. Amino acid residues that may have allowed *Ralstonia solanacearum* flagellin to avoid plant detection were also determined. In additional work, we identified polymorphisms between *Arabidopsis* and *Brassica* FLS2 LRR domains. Alanine-scanning mutagenesis of AtFLS2 LRR domain and several Brassica FLS2 LRR domains are being used to identify key residues required for flagellin recognition. Overall, we hope to learn to identify and manipulate key pathogen and plant structural features that determine function in this segment of the plant immune system.

573 Regulation of Plant Defense Responses in Arabidopsis by EDR2, a PH and START domain containing Protein

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We have identified an Arabidopsis mutant (*edr2*) that displays enhanced disease resistance to the biotrophic powdery mildew pathogen *Erysiphe cichoracearum*. Necrotic lesions are formed in *edr2* leaves upon *E. cichoracearum* infection. The inhibition of fungal growth in *edr2* mutant was occurred at late stage. Double mutant analysis revealed that the *edr2*-mediated powdery mildew resistance is suppressed by mutations blocking the salicylic acid (SA)-induced defense pathway, including *npr1*, *pad4* and *sid2* mutations, demonstrating that *edr2*-mediated disease resistance is SA dependent. In addition, the *edr2* mutant displayed enhanced susceptibility to a necrotrophic fungal pathogen *Botrytis cinerea*. However, *edr2* showed normal responses to bacterial pathogen *Pseudomonas syringae pv. tomato* strain DC3000. EDR2 expresses in various tissues and organs and encodes a novel protein, consisting of a putative pleckstrin homology (PH) domain and a StAR (Steroidogenic Acute Regulatory protein)-related lipid-transfer (START) domain. The PH and START domains are implicated in lipid binding, suggesting that EDR2 regulates defense responses through lipid signaling.

574 Indirect defense of Arabidopsis against herbivorous insects

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Many plant species are known to defend themselves against herbivorous insects indirectly, by producing volatiles in response to herbivory. These volatiles attract carnivorous enemies of the herbivores, such as parasitoids. Research on the model plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) has contributed considerably to the unravelling of signal transduction pathways involved in direct plant defence mechanisms against pathogens. We have demonstrated that Arabidopsis is also a good model plant for studying signal transduction pathways involved in *indirect* defence mechanisms, by showing that: (a) Arabidopsis plants infested by *Pieris rapae* caterpillars (Lepidoptera: Pieridae) emit volatiles from several major biosynthetic pathways, including terpenoids, methyl-salicylate (MeSA), nitriles and green leaf volatiles; (b) Adult females of *Cotesia rubecula* (Hymenoptera: Braconidae), a specialist parasitoid wasp of *P. rapae*, were attracted to *P. rapae*-infested Arabidopsis plants; and (c) Genes from major biosynthetic pathways involved in volatile production were induced by caterpillar feeding. Additionally, we have studied the role of the octadecanoid and the salicylic acid pathways in the induced attraction of *C. rubecula* by *P. rapae*-infested Arabidopsis, using exogenous application of jasmonic acid (JA) or salicylic acid (SA), transgenic Arabidopsis plants that do not accumulate SA (NahG), downstream signaling mutants (*npr1-1* and *jar1-1*) and measuring endogenous oxylipin levels. These studies demonstrate that JA and SA are indeed involved in the herbivory-induced production of volatiles plant and suggest additional role of the hormone 12-oxo-phytodienoic acid. Moreover, these data indicate that MeSA and the terpenoid (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene are important parasitoid attractants. We have also shown that herbivore species with a different way of feeding compared to P. rapae caterpillars - aphids and spider mites - induced no or less attraction of *C. rubecula* when infesting Arabidopsis. This difference in parasitoid attraction may be the result of different induction of JA and SA signalling pathways by different herbivore species. In conclusion, this work demonstrates that combining parasitoid behaviour and chemical analysis with a molecular genetic approach can be highly valuable in unravelling signal-transduction pathways involved in indirect defence of plants.

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575 Functional Analysis of Pathogen-induced WRKY3 and WRKY4 Transcription Factors in Plant Defense Responses

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WRKY transcription factors are encoded by a large gene family in plants with more than 70 members in Arabidopsis. WRKY proteins are so called because all these proteins contain one or two highly conserved WRKY DNA-binding domains that recognize the TTGACC/T W boxes present in the promoters of many plant genes. Many Arabidopsis WRKY genes are induced by pathogen infection, suggesting an important role of the gene family in plant defense. The Arabidopsis WRKY3 and WRKY4 genes encode two structurally closely related WRKY proteins with two WRKY domains. Both WRKY3 and WRKY4 genes have been previously shown to be induced by a virulent strain of the bacterial pathogen *Pseudomonas syringae* and plant defense signal molecule salicylic acid. To further determine the roles of these two pathogen-induced WRKY genes in plant defense responses, we have analyzed expression of the two genes in responses to other pathogens and defense signal molecules. The DNA-binding properties and sub cellular localization of the two proteins are also being characterized. In addition, we have generated transgenic overexpression lines and T-DNA insertion lines for the two WRKY genes. To overcome possible functional redundancy between the two closely related WRKY genes, we have also generated double insertion lines for the two WRKY genes. These overexpression lines and knockout mutants are currently been investigated for possible altered responses to a number of bacterial and fungal pathogens. The results from these experiments, including the characterization of these gain-of-function and loss-of-function mutants, will be presented and discussed.

576 Analysis of *pmr6* - why is this mutant resistant to powdery mildew?

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Plant cell walls have a very complex structure. We do not fully understand the molecular arrangement of their components and even less do we understand the necessity and function of this complexity. In recent years, several genetic studies have provided new lines of evidence implicating cell wall polysaccharides as important factors in host-pathogen interactions. In this context, the *pmr6* (powdery mildew resistant) mutant is very fascinating as it provides a link between the cell wall and pathogen resistance. The mutation lies in a gene with homology to pectate lyases and although we have not yet been able to show pectate lyase activity, the analysis of the cell wall composition has identified alterations that point to such a function for PMR6. The mutant raises two questions. First, what is the biological function of PMR6 and, secondly, what is the mechanism behind the resistance phenotype. The growth phenotype of *pmr6* and the analysis of plants expressing *PMR6* promoter::GUS constructs suggest that PMR6 activity is required for proper cell elongation. The second question regarding the resistance mechanism is more difficult to explain. However, we have evidence that *pmr6* can sense the changes in the cell wall and translates them into a constitutively activated defense response.

577 Combining transcript profiling and reverse genetics to find novel regulators of the defense response against Pseudomonas syringae

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In response to pathogen attack, the plant activates several different signaling pathways and initiates changes in gene expression. While some transcriptional changes are well characterized and used as markers for the defense response, questions remain about the timing and significance of all the transcriptional activation and repression events induced by infection. Using microarray technology, we sought to characterize the changes in gene expression that resulted from infection with the bacterial pathogen *Pseudomonas syringae*. At five different time points, we infected half of an *Arabidopsis* leaf with either virulent *P. syringae* or avirulent *P. syringae/avrRpt2*. The uninfected half of the leaf was then collected and prepared for microarray analysis using the Affymetrix GeneChip® *Arabidopsis* ATH1 Genome Array. We found that over 1,000 genes showed a 2-fold change in expression level at two consecutive time points after at least one type of infection. Therefore, careful consideration was required to determine which of the induced genes are good candidates for reverse genetic analysis. To our advantage, examination of the transcript profile of nearly every *Arabidopsis* gene at several time points after infection enabled us to compare each gene's expression pattern over time. Such comparisons allowed us to find genes most likely to be novel regulators of the defense response. In our search for the best candidates, we focused on groups of similarly induced genes that encode putative transcription factors as well as on genes that have a similar expression pattern to known regulators of disease resistance.

578 Dissecting the jasmonate signaling pathway in Arabidopsis

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Jasmonic acid (JA) and derivatives, such as the volatile methyljasmonate (MeJA), are collectively known as jasmonates. Jasmonates are important signalling molecules which have a developmental role, as well as a role in insect defence and disease resistance. However, no receptor for JA has been found and how the JA signal is transduced to affect gene expression is largely unknown.

A cDNA encoding a thioglucoside glucohydrolase, *PYK10*, was isolated using the differential display technique in an attempt to isolate MeJA-induced genes in *Arabidopsis thaliana*. Thioglucoside glucohydrolyses catalyses the breakdown of glucosinolates into glucose and sulphate and releases toxic byproducts which play a role in plant defence against insects and pathogens. *PYK10* induction by MeJA was found to be inhibited by ethylene and by the JA mutants, *coi1* and *jin4*. We are now using this gene to investigate JA signalling mechanisms.

Gene expression is regulated by transcription factors that bind to specific *cis*-elements. To date, the G-box, the C-box, the T/G-box and the GCC-box-like motif have been found to confer JA responsiveness to promoters. Several other motifs have been identified in JA-regulated promoters, but none of these have been shown to be JA responsive. In Arabidopsis only the JA-induced transcription factor, AtMYC2, has been recently isolated, with its homolog in tomato interacting with a JA-responsive element. Two JA-induced transcription factors, called octadecanoid-responsive Catharanthus AP2-domain proteins (ORCAs), isolated in periwinkle also interact directly with a JA-responsive element.

We have designed promoter deletion constructs around putative JA-responsive *cis*-elements in the *PYK10* promoter region and identified a 700 bp region responsible for enhancement of JA gene expression via luciferase assays. We are now in the process of delineating the JA responsive element further and will then use these cis-elements as bait in the yeast 1-hybrid assay to isolate interacting JA-induced transcription factors in Arabidopsis.

579 Pathogen induced AtWRKY48 functions as a negative regulator in plant defense response

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A number of studies have suggested that plant WRKY transcription factors function as both positive and negative regulators in plant defense responses. A positive role in plant defense has been demonstrated for several WRKY genes based on the phenotypes of their transgenic overexpression lines and/or loss-of-function mutants. However, information is still missing about specific WRKY transcription factors that function as negative regulators in plant defense responses. In the present study, we have analyzed the roles of pathogen-induced Arabidopsis WRKY48 transcription factor in plant disease resistance. Expression of WRKY48 is repressed by defense-inducing molecules salicylic acid, ethylene and jasmonic acid but is strongly induced by the bacterial pathogen *Pseudomonas syringae* and this induction is enhanced in mutants with enhanced disease susceptibility (eds) to the bacterial pathogen. WRKY48 protein recognizes the TTGACC W-box sequences with high affinities in vitro and its translational fusion with green fluorescent protein is nuclear localized in onion (*Allium cepa*) cells. Constitutive expression of WRKY48 in transgenic Arabidopsis plants leads to reduction in pathogen-induced PR gene expression and enhanced susceptibility to both bacterial and fungal pathogens. By contrast, a T-DNA insertion mutant for WRKY48 has stronger PR gene expression and develops milder disease symptoms than the wild type plants after infection by *P. syringae*. These results strongly suggest that pathogen-induced WRKY48 is a negative regulator of plant defense responses.

580 Spatial and temporal analysis of host gene expression in viral disease development

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Previous studies demonstrate that viral infection of susceptible plants results in coordinated changes in host gene expression. However, typically whole leaves were sampled for gene expression assays resulting in a lack of spatial resolution and dilution of interesting changes in host gene expression. To gain a more precise understanding of the spatial and temporal nature of host gene expression as viral infections proceed, a GFP-tagged virus (turnip mosaic potyvirus; TuMV-GFP) was used to provide a visible marker for virus infection. TuMV-GFP infection foci and corresponding regions of mock-inoculated tissues were dissected into four zones, which represented progressive stages of viral infection. RNA extracted from these zones was amplified and hybridized to the Arabidopsis ATH1 genome oligonucleotide array. Statistical analyses were applied and over 400 genes were identified that displayed differential expression in TuMV-GFP-infected tissues across the four zones with a 5% false discovery rate. As expected, genes encoding heat shock proteins and defense-related genes were induced, which was previously observed in infections by diverse viruses. We also observed that suites of genes encoding ribosomal proteins, proteasomal subunits, RNA processing proteins, translation factors, amino acid biosynthetic enzymes, and calcium homeostasis were up-regulated. Two interesting subsets of down-regulated genes include suites of genes associated with photosynthesis/chloroplast functions and cell wall biogenesis. Quantitative RT-PCR was applied to specific genes representing some of the functional groups listed above to further examine the expression of these genes in various tissues over the course of systemic infection and accompanying symptom development.

581 Roles of Structurally Related WRKY 20, WRKY25, WRKY26 and WRKY33 Transcription Factors in Plant Defense Responses

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WRKY transcription factors, found mainly in plants, are encoded by a large gene family. In Arabidopsis, there are more than 70 WRKY genes and many of them respond to pathogen infection and salicylic acid treatment, suggesting an important role of the gene family in plant defense responses. Arabidopsis WRKY20, WRKY25, WRKY26 and WRKY33 encode four structurally related WRKY proteins that belong to the first group of the protein family with each protein containing two highly conserved WRKY domains. We have shown that the WRKY25, WRKY26, and WRKY33 protein are all localized in the nucleus and specifically recognize the TTGACC/T W box sequences. To analyze the biological functions of these WRKY genes directly, we have also constructed transgenic overexpression lines for the WRKY25, WRKY26 and WRKY33 genes and isolated T-DNA insertion lines for all these four genes. Transgenic plants constitutively expressing WRKY25, WRKY26 and WRKY33 were more susceptible to the bacterial pathogen, while single knockout mutants for WRKY20, WRKY25, WRKY26 and WRKY33 responded normally to the bacterial pathogen. To overcome possible functional redundancy among these related WRKY proteins, we are currently generating double, triple and quadruple knockout mutants for these four WRKY genes. Further comparative analysis of these single and composite knockout mutants should reveal both distinct and overlapping functions of the four WRKY genes.

582 An Anti-Insect Protein from Arabidopsis

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Indirect evidence suggested that Arabidopsis vegetative storage protein (VSP) plays a role in defending its host against herbivorous insects. To test this hypothesis, we bacterially expressed an Arabidopsis VSP2 (AtVSP2), a wound-, jasmonate- and insect feeding-induced gene. The recombinant protein exhibited phosphatase activity in the acid pH range. When incorporated into the diets of three coleopteran and dipteran insects, recombinant AtVSP2 significantly delayed development of the insects and increased their mortality. To further determine the biochemical basis of the anti-insect activity of the protein, the nucleophilic Asp119 residue at the conserved DXDXT motif was substituted via site-directed mutagenesis. This single amino acid alteration did not compromise the protein's secondary or tertiary structure, but resulted in complete loss of its acid phosphatase activity as well as its anti-insect activity. Collectively, we conclude that AtVSP2 is an anti-insect protein and that its defense function is correlated with its acid phosphatase activity.

583 Flavonol synthases in Arabidopsis: isoform-specific responses to developmental and biotic signals

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Flavonols are ubiquitous plant secondary metabolites that participate in processes as diverse as UV protection, defense, symbiosis, male fertility, and the regulation of auxin transport. In Arabidopsis, relative levels of two flavonols, quercetin and kaempferol, can vary substantially among tissues and during development; however the biochemical and genetic basis for these differences remains unknown. The enzyme flavonol synthase (FLS), which converts dihydroflavonols to flavonols, is the only enzyme that is encoded by more than one gene in this species. To test the hypothesis that differential expression of the Arabidopsis *FLS* genes determines the types and amounts of flavonols produced in response to developmental and environmental cues, promoter:GUS fusions for five *AtFLS* genes were analyzed in transgenic Arabidopsis plants. Expression patterns as revealed by histochemical staining of plants at different developmental stages were confirmed by semi-quantitative RT-PCR. These experiments indicated that *AtFLS1* is expressed in seedlings, trichomes, and reproductive structures, while *AtFLS2* is also expressed in reproductive structures and *AtFLS3* and *5* are expressed predominantly in young roots. *AtFLS1* and *5* also appeared to be induced by wounding, similar to *AtCHS*, which encodes the enzyme at the entry point of the flavonoid pathway. However, in contrast to *AtCHS*, the *AtFLS* genes were not expressed in response to infection of roots with the plant parasite, *Orobanche aegyptiaca*. Interestingly, activity assays and yeast two-hybrid experiments indicate that *AtFLS2*, *3*, and *5* encode proteins that are catalytically-inactive yet are still able to interact with other flavonoid enzymes. These findings suggest that some FLS isoforms could influence metabolic flux by playing a purely structural role in the flavonoid enzyme complex.

584 The Role of Prenyltransferase Stimulating Proteins (PSP) in *Arabidopsis thaliana*

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Isoprenoid polymers are a diverse group of natural products that are involved in plant growth, development, defense signaling, and cell wall biosynthesis. These polymers are made up of repeating units of the 5-carbon isopentyl diphosphate (IPP). IPP is synthesized via the 2-C-methyl-D-erthriol-4-phosphate pathway in the plastid and by the mevalonate pathway in the cytosol, endoplasmic reticulum, and mitochondrion. Condensation of IPP units produces farnesyl diphosphate (FPP) geranyldiphosphate (GPP), and geranylgeranyl diphosphate (GGPP). These short chain isoprenoids can then be used to prenylate proteins or serve as precursors of isoprenoid alcohols. Prenyltransferases are either trans-or (E)-prenyltransferases (found primarily in the plastid) or cis-(Z)-prenyltransferases (found primarily in the cytosol).

Previous studies in citrus led us to the identification of a seed-specific gene, *Citrus reticulata p*renlytransferase *s*timulating *p*rotein (*CrPSP*). *CrPSP* encodes a protein that is highly related to *Hevea brasiliensis* rubber elongation factor (HbREF) and small rubber particle protein (HbSRPP), which stimulate the prenyltransferase-mediated condensation of the isoprene units. The *CrPSP* homologues include a cold-induced gene in grape (*VrPSP*), a wound- and metal-induced Phaseolus gene (*PvPSP*), and at least one gene in rice (*OsPSP*). In *Arabidopsis thaliana*, there are 3 *PSP*-like genes (*AtPSP1*,*AtPSP2*, and *AtPSP3*). Unlike the citrus *PSP*, *AtPSPs* have a more ubiquitous expression program.

Although the importance of HbREF and HbSRPP in stimulating rubber biosynthesis is well established, the function of non-rubber producing PSPs is less understood. We are investigating the key metabolic activities influenced by PSPs and how altered regulation of these pathways affects plant isoprenoid metabolism. Transgenic Arabidopsis plants that ectopically express or silence *AtPSP* have been compared to wild type plants. The impact of isoprenoid biosynthesis inhibitors, fosmidomycin and mevinolin on *PSP*-KO mutants will be presented.

585 Transcriptional control of flavonoid biosynthesis: importance of the regulatory feedback loop controlling TT8 expression

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The accumulation of proanthocyanidins (PA, flavonoid polymers) in the Arabidopsis seed coat is induced by the coordinated action of TT2 (MYB factor), TT8 (bHLH factor), and TTG1 (WDR regulatory protein). These three proteins interact to form a ternary complex that controls directly the expression of the *BAN* structural gene (encoding an anthocyanidin reductase essential for PA biosynthesis). In the complex, TT2 is responsible for the specific recognition of the promoter, but its interaction with TT8 is required for DNA-binding in vivo. Although TTG1 can participate in this transcriptional-activating complex, it would not be necessary for the specificity of target recognition. Potentially, TTG1 might act as a co-factor, through the post-translational control of TT8 activity.

The mechanisms controlling the expression of these regulatory factors are also of particular interest, because they constitute the primary step in the determination of the specific pattern of flavonoid accumulation in planta. For instance, there is a strong correlation between the activity of the *BAN* and *TT2* promoters in PA-accumulating cells. Additionally, when *TT2* is constitutively expressed in planta (70S promoter), *BAN* ectopic activation is restricted to specific cellular domains corresponding to the domains in which *TTG1* is expressed.

Control of *TT8* expression remained to be investigated. Previous results have suggested an interesting interplay between TT2 and the expression of this bHLH factor. In order to precise this mode of action, we have investigated *TT8* regulation by a set of genetic and molecular approaches, both in yeast and in planta. Our results support that TT2, TT8, and TTG1, in addition to the control of the structural genes of the PA pathway, might participate in an auto-activated feedback loop directly regulating *TT8* expression in planta. The importance of this regulatory mechanism in the strong and specific induction of flavonoid accumulation in Arabidopsis seed coat will be discussed, with regards to the mode of action of other known MYB-bHLH-TTG1 regulatory modules.

586 A novel isoform of glucan water dikinase is required for the breakdown of starch

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Many key enzymes responsible for starch biosynthesis have been identified. Degradation of transient starch in plant leaves is beginning to show the picture of an equally complex system. Novel activities are still discovered, a recent one being the starch-phosphorylating enzyme, α-glucan water dikinase GWD1, which phosphorylates starch by a dikinase-type reaction mechanism in which the β-phosphate of ATP is transferred to the glucan substrate (Ritte et al. 2002). The studies of plants with downregulated GWD1 activity have revealed a close link between starch phosphorylating activity and starch breakdown in transient leaf starch, as exemplified by the Arabidopsis *sex1* (starch excess) mutant (Yu et al. 2000). We have characterized a novel GWD isoform, termed GWD3, which binds and phosphorylates only phosphoglucans (Baunsgaard et al. 2005). This narrow substrate selectivity is a novel property for a glucan water dikinase. Our data also support the presence of an enzymatic starch phosphorylating pathway mediated by consecutive specific glucan water dikinase activities, which might lead directly to the first attack by degradative enzymes on a starch granule in the plastid. The molecular dissection of this starch phosphorylating protein GWD3 shows a conserved carbon binding module in the N-terminus, belonging to the carbon binding module family 20 (CBM20), which is unique for the GWD3-subgroup. The analysis of the *in vitro* activity of the enzyme and the regulatory role of this activity *in planta* will be presented.

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587 WRINKLED1 of Arabidopsis Regulates Sugar Metabolism in the Seed and Seedling

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The *wrinkled1* mutant of *Arabidopsis* was previously identified in a screen for seeds defective in seed oil (triacylglycerides, TAG) accumulation. The *WRINKLED1* gene of *Arabidopsis* has been cloned and identified through a map based approach. We have shown *WRI1* to encode a protein in the AP2/EREB class of transcription factors (Cernac and Benning, 2004). Analysis of the expression pattern of *WRI1* has indicated it to be primarily expressed in the developing embryo, germinating seedling, root and flowers, tissues where glycolysis and or gluconeogenesis would be especially active. Characterization of developing seeds in a *wri1* background has shown that the primary defect of *wri1* is in carbon metabolism, in particular the activity of five enzymes involved in glycolysis (Focks and Benning, 1998). The five enzymes for which activity is down regulated in *wri1* are hexokinase, pyrophosphate dependent phosphofructokinase, enolase and pyruvate kinase with a minor reduction in aldolase activity. Hexokinase and pyruvate kinase are thought to play key roles in the regulation of glycolysis. Transcript for the genes encoding the corresponding enzymes is also reduced in a *wri1-1* background. Subsequent microarray analysis of developing seeds of *wri1-1* and wild type have indicated that the *wri1-1* mutation results in the down regulation of transcript accumulation of genes encoding enzymes involved in all stages of carbon metabolism in the developing embryo (Ruuska et al., 2002). Overexpression of *WRI1* results in a sugar inducible accumulation of seed storage compounds, protein and TAG, along with a phenotype suggestive of a resumption of embryonic development in the seedling. The data indicate a central role for WRI1 in the regulation of development in response to sugar availability and suggest a mechanism by which metabolism and development are coupled.

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588 Metabolite profiling of PHB-producing Arabidopsis grown in three different illumination regimes

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Polyhydroxyalkanoates (PHAs) are a class of polyesters naturally produced by many bacteria. PHAs have a wide variety of plastic-type properties, which are dependent on the monomer composition. Plant-produced PHAs are biodegradable and have attractive industrial and medical applications. Polyhydroxybutyrate, a PHA, is produced from acetyl-CoA by the sequential action of three enzymes: 3-ketothiolase (*phaA*), acetoacetyl-CoA reductase (*phaB*), and PHA synthase (*phaC*). These three enzymes have been targeted to plastids of Arabidopsis using an inducible promoter system to minimize detrimental growth effects. Transgenic and wild type plants were grown in three different illumination regimes: short day (SD), long day (LD) and constant illumination (CI). PHB accumulation levels were determined, and metabolites profiles were examined by GC-MS and HPLC equipped with a photo diode array detector. PHB accumulates to levels as high as 15% of total dry weight. The changes in metabolite profiles are being examined to better understand the relationship between carbon utilization and illumination cycles in the presence of a foreign sink for plastidic acetyl-CoA.

589 Flavonoid metabolism in Arabidopsis seeds

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The two major end-products of the flavonoid pathway that are encountered in Arabidopsis seeds are proanthocyanidins (PAs; syn. condensed tannins) and flavonols. These polyphenolic compounds have an important impact on agro-industrial and nutritional qualities of seeds (e.g. protection against diverse biotic and abiotic stresses, strengthening of longevity and seed coat-imposed dormancy, antioxidant properties). Currently, there is a growing interest in the potential health benefits of flavonoids and more especially PAs, as natural antioxidants in diet. A European funded project named "FLAVO", coordinated by our laboratory, is starting on this topic.

In Arabidopsis seeds, PAs accumulate specifically in the seed coat (or testa), giving the mature seed its brown color after oxidation. On the other hand, flavonols (colorless to pale yellow products) are found not only in the testa but also in the embryo and endosperm. A genetic approach based on the isolation and characterization of seed color mutants has enabled to identify most of the presently known genes involved in the flavonoid pathway. They are named *TRANSPARENT TESTA (GLABRA)* or *TT(G), TANNIN DEFICIENT SEED (TDS), BANYULS (BAN)* and *AHA10*. Nevertheless, many unknown functions remain to be discovered, especially in relation to PA biosynthesis, condensation, compartmentation, and oxidation. We currently investigate the functions of TT10 and TT15, for which we have identified candidate genes. In parallel, we analyze the regulatory network driving *BAN* tissue-specific expression and PA biosynthesis in the seed coat, particularly focusing on the interplay between TT2 (MYB), TT8 (bHLH) and TTG1 (WD40). Transcriptomic analyses of mutants and transformants have also been initiated recently to identify functions not accessible to genetic approaches. Finally a comprehensive identification of all major classes of flavonoids occurring in wild-type and mutant seeds has been undertaken by LC-MS and acid-catalysed cleavage analysis. These metabolome analyses will pave the way to study the natural variability of seed flavonoid metabolism among various Arabidopsis ecotypes.

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590 Genetic, Molecular and Biochemical Studies of 3-Methylcrotonyl-CoA Carboxylase in Arabidopsis thaliana

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Biotin containing proteins play important roles in metabolic pathways. In plants, four biotin-containing enzymes have been characterized to date. These enzymes are: heteromeric acetyl-CoA carboxylase, homomeric acetyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase (MCCase) and geranoyl-CoA carboxylase. These enzymes are involved in a variety of metabolic processes, including fatty acid biosynthesis, amino acid metabolism and secondary metabolism. MCCase is a nuclear-encoded, mitochondrial-localized enzyme that catalyzes the carboxylation of 3-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA. MCCase is required for leucine catabolism in mitochondria, and it may also play important roles in the catabolism of isoprenoids and in the mevalonate shunt. In *Arabidopsis*, the two MCCase subunits (the biotinylated MCCA subunit and the non-biotinylated MCCB subunit) are each encoded by single genes (At1g03090 and At4g34030, respectively). To understand the physiological role of MCCase in plants, reverse genetic approaches are being used to isolate T-DNA knock-out alleles for each gene. Homozygous mutants of *MCCA* and *MCCB* genes have been recovered. The MCCase activity assays, RT-PCR and Western blot analyses indicate mitochondrial leucine catabolism is blocked in this mutant. Mutations in either *MCCA* or *MCCB* genes that block mitochondrial leucine catabolism result in an impaired reproductive growth phenotype. That is, many siliques of mutants undergo abortion; the average length of the siliques is reduced in comparison to wild-type plants; and seed yield per silique and per plant are also reduced. However, plants leucine catabolism can also occur via an MCCase-independent peroxisomal pathway. Therefore, we are biochemically characterizing the *mcca* and *mccb* mutant stocks to understand the relationship between the reproductive growth phenotype and leucine and / or isoprenoids catabolism and / or mevalonate shut. These characterizations are providing new insights into the role of catabolic processes in growth and development, an area of plant biology that is poorly understood.

591 The plastidic glucose 6-phosphate/phosphate translocator GPT1 is crucial for gametophyte development in Arabidopsis

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The glucose 6-phosphate/phosphate translocator (GPT) imports glucose 6-phosphate into plastids of non-green tissues as a substrate for starch and fatty acid biosynthesis and, in addition, as a substrate for the oxidative pentose phosphate pathway (OPPP). The genome of Arabidopsis contains two homologous and functional GPT genes, AtGPT1 and AtGPT2. Whereas disruption of the GPT2 gene had no obvious effect on growth and development under green house conditions, defects in the GPT1 gene resulted in lethality. The mutant lines gpt1-1 and gpt1-2 showed a distorted segregation ratio together with a reduced male and female transmission efficiency indicating profound defects on gametogenesis. Thus, import of glucose 6-phosphate by GPT1 into non-green plastids appears to be crucial for pollen maturation and female gametophyte development (1). Because glucose 6-phosphate serves as a substrate for the OPPP, the observed defect might be due to a reduced supply of the plastids with reducing power which, in turn, is required for fatty acid biosynthesis. Interestingly, one of the genes coding for a putative plastidic 6-phosphogluconate dehydrogenase (generating reducing equivalents within the OPPP), shows an expression pattern similar to that of the AtGPT1 gene and, in addition, a corresponding insertion mutant produced a high number of aborted pollen (as the gpt1 mutants do) and could not be established in the homozygous state.

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592 A Biochemical and Modeling Approach to Understand Inositol Phosphate Metabolism in Plants

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Inositol phosphates play many roles in plant metabolism and signaling. We are examining the genes involved in the synthesis of the precursor, myo-inositol, and breakdown of inositol phosphate second messengers. The genes encoding myo-inositol phosphate monophosphatase (IMP) and inositol polyphosphate 5-phosphatases (5PTases) reveal that these enzymes are encoded by gene families and result in diverse proteins. We are utilizing biochemical assays and molecular modeling of the predicted proteins to correlate substrate selectivity with modeled enzyme structures. For the IMP proteins, this approach is important because of the recent suggestion that the identified plant IMPs are also L-galactose-1-phosphate phosphatases, an activity required for Vitamin C synthesis in plants. To explore this relationship, we have manipulated expression of two IMP genes and found alterations in Vitamin C synthesis. With respect to the At5PTase enzymes, we have similarly focused on determining which inositol phosphate second messengers serve as substrates for the 15 gene products. We have found two types of substrate selectivities: At5PTase1, 2 and 3 encode enzymes that only hydrolyze the water-soluble substrates, I(1,4,5)P3 and I(1,3,4,5)P4. In contrast, the enzyme encoded by At5PTase11 hydrolyzes only lipid soluble substrates, PI(4,5,)P2 and PI(3,4,5)P3. Molecular modeling of these 5PTases reveals differences in hydrophobicity and potential membrane interactive surfaces that correlate with the substrate selectivities. To determine the function of the 5PTases, we have identified and characterized T-DNA knock-out mutants for several of these genes. Our results show that At5PTase1 and At5PTase2 loss-of-functions impact seed germination and/or early seedling growth. A loss-of-function in At5PTase1 leads to an increase in I(1,4,5)P3 levels, and no significant alterations in PI(4,5)P2 levels as shown by 3-H inositol labeling experiments. Loss-of-function At5PTase11 mutants also show growth alterations at the early seedling stage, but the phenotype is the opposite of the At5PTase1 and 2 phenotype. On-going analysis of substrate levels in At5PTase11 mutant plants has not yet revealed inositol second messenger substrate differences. Together, these data indicate that inositol phosphatase enzymes from plants play diverse roles in regulating inositol phosphate breakdown, and that proper regulation is required for early seedling growth stages.

593 Interactions between SHMT and GOGAT in photorespiration

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In addition to catalyzing the carboxylation of its substrate, ribulose-1,5-bisphosphate (RuBP), to initiate Calvin cycle (the C3 photosynthetic carbon reduction cycle), Rubisco also catalyzes the oxygenation of RuBP to initiate the photorespiratory pathway. The photorespiratory pathway is a complex series of reactions involving 16 enzymes and at least 6 transporters and occurring in three organelles: chloroplasts, peroxisomes, and mitochondria. Mitochondrial serine hydroxymethyltransferase (SHMT) activity is essential for photorespiration. In Arabidopsis, SHMT is encoded by a family of seven *SHM* genes. Arabidopsis *stm* mutants lack mitochondrial SHMT activity, accumulate glycine and are unable to grow at ambient CO_2 concentrations but grow normally at elevated CO_2 concentrations.

One *stm* allele was mapped to 95 cM on chromosome IV, where the *SHM1* gene (At4g37930) is located. A G to A transition at the 5' splice site of intron 6 of SHM1 was identified and overexpression of wild-type *SHM1* in this *stm* mutant rescued the photorespiratory phenotype; this mutant allele therefore has been renamed *shm1-1*.

However, a second allele, *stm-2*, that also lacks mitochondrial SHMT activity was mapped to the top of chromosome V, in a region that lacks any known *SHM* genes. Consistent with the mapping results, *stm-1* and *stm-2* complement in a test of allelism, indicating that they identify two distinct loci. Unexpectedly, the *stm* lesion mapped to the *glu1* gene (At5g04140), which encodes photorespiratory glutamate synthase (Fd-GOGAT). We identified a missense lesion in the *glu1* allele of *stm-2*, which we name *glu1-101*. Introduction of a wild type *GLU1* allele into *stm-2* (*glu1-101*) restored wild type levels of SHMT activity and rescued the photorespiratory phenotype while introduction of the *glu1-101* coding sequence driven by the 35S promoter failed to rescue the photorespiratory phenotype, confirming that the mutation in *glu1-101* confers the *stm-2* phenotype. This novel allele of *GLU1* results in loss of mitochondrial SHMT activity, yet allows retention of full GOGAT activity. We conclude that Fd-GOGAT plays a heretofore unsuspected role that is critical for expression of mitochondrial SHMT activity.

594 Plastidic lipases similar to DAD1 in *Arabidopsis thaliana*

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Oxylipins, such as 12-oxophytodienoic acid (OPDA) and jasmonic acid (JA), are important components in the regulation of plant development and in the interaction of plants with their environment (e.g. Weber, 2002; Schaller, 2001). OPDA is an intermediate of the JA biosynthetic pathway and it is derived from chloroplastic membrane lipids for example sn1-O-(12-oxophytodienoyl)-sn2-O-(hexadecatrienoyl)-monogalactosyl diglyceride (MGDG-O) by a lipolytic reaction (Stelmach et al., 2001). All genes from Arabidopsis encoding enzymes of JA biosynthesis have been cloned and some of them (lipoxygenase, allene oxide synthase and allene oxide cyclase) were shown to be localized in chloroplasts (e.g. Laudert et al., 1996). Based on the identification of the phospholipase DAD1 (*dad1 = defective in anther dehiscence1*) in flower buds from Arabidopsis (Ishiguro et al., 2001) as an enzyme catalyzing the initial step of the above mentioned biosynthesis, we decided to quest for a lipase releasing OPDA from MGDG-O in chloroplasts from Arabidopsis leaves. Using database search and information from chloroplast proteomics (Kleffmann et al., 2004), eight genes encoding proteins homologous to DAD1 according to their sequences were chosen to analyse.

We used different approaches to identify plastid-localized lipases expressed in leaf tissue: (1) localization of GFP-fusion proteins after transient expression of their genes in Arabidopsis epidermal cells and (2) analysis of tissue specific expression by RT-PCR. Two lipases, which were predicted to be localized in chloroplasts based on a proteomic approach (Kleffmann et al., 2004), could definitely be shown to be absent in plastids, while, for the remaining lipases, the predicted plastidic localization (ChloroP) could be verified experimentally. All plastidic lipases except of one lipase are detectably expressed in leaf tissue. These five lipases are candidates for further studies, e.g. enzymatic characterization or examination of knockout mutants.

595 *kidari1-1*, a Dominant mutation in Arabidopsis, confers hyper-Auxin phenotypes

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Auxin is well studied plant hormone involved in many aspects of plant growth and development. Here we report a fertile Arabidopsis mutant, *kidari1-1*, which has an auxin overproduction phenotype similar to *yucca*. The *kidari1-1* mutant was identified by activation tagging. The genomic DNA adjacent to the left border of the T-DNA insertion was cloned by Tail PCR. The CaMV 35S enhancer was inserted 8.6Kb downstream of the coding region of a flavin monooxygenease-like protein, which is among the yucca-like gene family. The transcript of this ORF was 9 fold increased in *kidari1-1* mutants. Genetic analysis showed it to be a single locus, dominant mutation. *kidari1-1* has typical auxin overproduction phenotypic alterations like epinastic cotyledons, increased apical dominance and curled mature leaves. Unlike other auxin overproduction mutants, *kidari1-1* does not have short or hairy root phenotypes. In addition, *kidari1-1* is very large and produces strong apically dominant inflorescences of one meter or longer. This mutant also displays delayed senescence and is able to survive for up to 5months longer than wild type. By using Microchip analysis, we found that expression of IAA-inducible genes, including *IAA/AUX, SAUR*, and *GH3* in *kidari1-1* is several fold higher than in wild type. We are now determining the precise role of *KIDARI* in auxin biosynthesis.

596 The QTL Epithiospecifier modifier 1 (ESM1) represses nitrile formation in combination with ESP during glucosinolate hydrolysis

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Glucosinolates (GS) are sulfur-rich plant secondary metabolites whose breakdown products play prominent roles in plant-herbivore interactions. In addition to aiding plant-pest interactions, glucosinolates are also important anticancer agents in the human diet. Arabidopsis is a model system for studying many aspects of plant biology and biochemistry, and produces over 40 different GS depending upon the genotype and environment. Hydrolysis by the Myrosinase enzyme in Arabidopsis produces the bioactive nitriles or isothiocyanates depending upon the complementary proteins present. This hydrolysis product difference is genetically controlled by variation at several loci. The major quantitative trait locus (QTL) on chromosome 1 affecting nitrile versus isothiocyanate formation is due to variation in the epithiospecifier protein (ESP) gene, which causes the formation of epithionitriles and nitriles instead of isothiocyanates. The chromosome 3 QTL epistatically affects nitrile formation in combination with ESP and has been termed Epithiospecifier modifier 1 (ESM1).

To map-base clone the ESM1 QTL, we generated a mapping population by crossing two near-isogenic lines (NILs), CS1945 and CS1995. Thus, we can map ESM1 without interferring variation at unlinked epistatic loci such as ESP. A simple GC-FID (Gas chromatography-flame ionization detection) protocol allows us to easily distinguish functional ESM1 from non-functional esm1 based on the nitrile to isothiocyanate ratio. Fine mapping with 1300 F_2 individuals show that ESM1 is a single semi-dominant locus located in a 120 Kbp interval. Affymetrix microarrays identified a single candidate gene. Sequencing and RT-PCR analysis shows that the likely cause of the phenotype is a promoter polymorphism leading to differential gene expression between Col-0 and L*er*. We have obtained homozygous T-DNA knockout lines and these show altered nitrile production, supporting that this gene is the ESM1 QTL. We are currently in the process of validating the biochemical function using *in vitro* protein expression and *in planta* transgenic complementation. Interestingly, this analysis shows that ESM1 is likely an isothiocyanate specifying protein as the functional protein enhances isothiocyanate production *in planta* in the presence of the nitrile specifying ESP. Thus, the regulation of glucosinolate hydrolysis is a more complex interplay of nitrile and isothiocyanate specifying proteins than previously believed.

597 α-Tocopherol oxidation products and metabolic analyses during high light stress in *Arabidopsis thaliana*

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Tocopherols (Vitamin E) are lipid-soluble antioxidants synthesized by all plants and many cyanobacteria. The tocopherol biosynthetic pathway has now been elucidated primarily by mutant analyses in *Arabidopsis thaliana*. The corresponding genes have been used to successfully engineer the content of tocopherols and biosynthetic intermediates in plants. Tocopherols play important roles in protection from oxidative stress. In animal systems α-tocopherol quenches and scavenges lipid peroxy radicals and can be oxidized and recycled in a redox cycle or oxidized further to α-tocopherolquinone, epoxy-α-tocopherolquinone and α-tocopherolhydroquinone. While these reactions have been characterized in animal systems, tocopherol oxidation and oxidation products in plants are still unclear. To understand the role that tocopherols play in protection against photo-oxidative damage, we have initiated studies of tocopherol oxidation and metabolic products in response to high light treatments.

Arabidopsis leaf tissues are used to detect the endogeneous tocopherol products during photo-oxidative damage. The *vte1* mutant, which accumulates the biosynthetic intermediate, DMPBQ, and the *vte2* mutants, which is deficient in tocopherols and all biosynthetic intermediates, were used in comparison to WT (Col) to identify tocopherol oxidation products. Col, *vte1* and *vte2* plants were exposed for 0, 2, 4 and 12 hours of high light (1800μmol) or normal light (90μmol) following by 12 hours additional darkness. To assess tocopherols and general metabolics, the extracts are analyzed by GC-MS systems and compared. For oxidation products of tocopherols and metabolic profiling, extraction and GC-MS analysis methods were modified from animal systems* and the Noble foundation methods**, respectively.

The time course experiments showed the degradation of α-tocopherol during the high light period and the subsequent recovery of tocopherol levels after 12 hours dark period. A variety of tocopherol oxidation products were observed. In contrast, under the low light condition α-tocopherol remained at a constant low level and was unchanged after the dark period. This indicates that α-tocopherol is consumed during high light stress presumably to aid in protection against photo-oxidative damage, and that tocopherol oxidation products are produced. Furthermore, metabolic profiling showed higher levels of free fatty acids following high light treatments.

* Liebler et al. Analytical Biochemistry (1996)

** http://www.noble.org/PlantBio/MS

598 A new type of peroxisomal acyl-coenzyme A synthetase from *Arabidopsis* has the catalytic capacity to activate biosynthetic precursors of jasmonic acid

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Arabidopsis thaliana contains a large number of genes that encode carboxylic acid-activating enzymes, including nine long-chain fatty acyl-CoA synthetases (LACS), four 4-coumarate:CoA ligases (4CL), and twenty-five 4CL-like proteins of unknown biochemical function. Due to their high structural and sequence similarity with *bona fide* 4CLs and their highly hydrophobic putative substrate-binding pockets (SBP), the 4CL-like proteins At4g05160 and At5g63380 were selected for detailed analysis. Following heterologous expression, the purified proteins were subjected to a large-scale screen to identify their preferred *in vitro* substrates. This study uncovered a significant activity of At4g05160 with medium-chain fatty acids, medium-chain fatty acids carrying a phenyl substitution, long-chain fatty acids, as well as with the jasmonic acid precursors, 12-oxo-phytodienoic acid (OPDA) and 3-oxo-2-(2'-pentenyl)-cyclopentane-1-hexanoic acid (OPC-6:0). The closest homolog of At4g05160, namely At5g63380, showed high activity with long-chain fatty acids and OPDA, the latter representing the most efficiently converted substrate. Using fluorescent-tagged variants, we demonstrated that both 4CL-like proteins are targeted to leaf peroxisomes. Collectively these data demonstrate that At4g05160 and At5g63380 have the capacity to contribute to jasmonic acid biosynthesis by initiating the β-oxidative chain shortening of its precursors. Current work aims at establishing the *in vivo* functions of both proteins by expression profiling and phenotypic analysis of the corresponding knock-out mutants.

References: Schneider et al. (2003) Proc Natl Acad Sci USA 100, 8601-8606; Schneider et al. (2005) J Biol Chem in press, DOI 10.1074/jbc.M413578200

599 The nucleoside diphosphate kinases, a small gene family involved in nucleotide metabolism

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Nucleotides have essential functions in a multitude of biochemical and developmental processes during the life cycle of a plant. They play an important role in dividing and elongating tissues as a component of DNA in the nucleus and DNA synthesising organelles. Furthermore as a component of RNA they are basic participants of metabolic processes. Nucleotides are also involved in many metabolic reactions; they provide cosubstrates and energy rich precursors for many biosynthetic pathways. Nucleoside diphosphate kinases (NDKs) are catalyzing the reaction: NDP+ATP->NTP+ADP and are therefore involved in the energy transfer from the adenosine into the uridine nucleotide system. Especially, in plants the pyrimidine nucleotides are of particular importance for sucrose and cell wall metabolism, because of the central role of the nucleotide sugar UDP-glucose.

From *A. thaliana* tissues we have isolated five different cDNAs of putative nucleoside diphosphate kinases. The NDK family in *A. thaliana* is highly conserved, all specific motifs are present. From AtGenExpress data we found that the nucleoside diphosphate kinases might have functions in rosette leave and seedling development. Our subcellular localization studies showed that NDKs can be found in different compartments of the plant cell: in plastids, mitochondria, in the cytosol and in the endoplasmic reticulum. We analysed the role of NDKs in stress response, and applied salt (NaCl) and reactive oxygen stress (H_2O_2) and measured expression levels using Real-Time RT-PCR. All NDKs reacted with a strong increase in expression on these stress factors up to 6-8 folds. In a functional approach using T-DNA insertion mutants and RNAi lines with different expression levels of individual NDKs, we are currently analysing the *in vivo* role of NDKs for plant metabolism.

600 Functional and Expresssion Analysis of Heteromeric Acetyl-CoA Carboxylase Subunit Genes in Arabidopsis

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De novo fatty acid synthesis in plants occurs mainly in plastids. Heteromeric acetyl-CoA carboxylase (htACCase) catalyzes the first reaction of this pathway converting acetyl-CoA to malonyl-CoA. This enzyme is composed of three nuclear-encoded subunits, biotin carboxyl-carrier protein (BCCP), biotin carboxylase (BC), α-carboxyltransferase (α-CT), and one plastome-encoded subunit, β-carboxyltransferase (β-CT). In *Arabidopsis*, single-copy genes encode all these subunits, with the exception of the BCCP subunit. The two BCCP-coding genes (At5g16390 (*CAC1-A*) codes for BCCP-1 and At5g15530 (*CAC1-B*) codes for BCCP-2) have similar spatial and temporal expression patterns during embryo development, but little is known about their individual functional significance. We have identified a T-DNA knockout mutant for the α-CT-coding gene (At2g38040 (*CAC3*)); the fact that the *cac3* mutant is recessive embryo lethal is consistent with the essential role of htACCase during embryogenesis. To test the potential redundant role of the two BCCP-coding genes, we have identified and characterized T-DNA knockout mutants for both *CAC1-A* and *CAC1-B* genes. While *cac1-a* mutant is recessive embryo lethal, the *cac1-b* mutant has no discernable phenotype. The composition and quantity of fatty acids in seeds, as well as the accumulation of the other subunits of htACCase, was not affected by *cac1-b* mutations. These results indicate that *CAC1-B* gene is dispensable for the function of htACCase. Plant htACCase holoenzyme complexes haven't been purified because they are labile and readily dissociate during purification. To understand the coordinate expression and organization of the subunits of htACCase, we have measured the expression of each subunit gene at both the mRNA and protein levels. Our data show that the accumulation of different subunits doesn't remain at a constant ratio across different organs, either at the mRNA or protein level. This suggests that the htACCase subunits are not tightly coordinately regulated and the structural quaternary organization of these subunits may be dynamic.

601 Phloem Transportation of Maltose in Maltose-excess Mutants

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Glucose and maltose are two major forms of carbon exported from chloroplasts at night. Maltose is exported by the maltose transporter (MEX1) and is metabolized in the cytosol by several enzymes, including disporportionating enzyme (DPE2). Inhibition of maltose export or maltose metabolism results in a 20-90-fold increase in leaf maltose content and a dwarf phenotype. What happens to the extremely high amount of maltose in the mutants is still a mystery. We studied whether maltose can be transported from source tissues to sink tissues via phloem transportation. Arabidopsis is a type 1-2a species by Gamalei's definition, which has high plasmodesmatal frequencies at interfaces involving phloem parenchyma cells. The predominant transport sugar in Arabidopsis is sucrose, although some raffinose is also translocated. Evidence shows that exogenous maltose serves as a transported substrate for the Arabidopsis sucrose transporter (AtSUC2). We assayed the carbohydrates in the phloem saps from Arabidopsis leaves. We found large amounts of sucrose, glucose, and fructose. We also found a large amount of maltose in *mex1-1* and *dpe2-1* phloem saps. The amount of maltose in phloem sap was higher in *dpe2-1* plants than in *mex1-1* plants. The ratio between maltose and sucrose was higher in *dpe2-1* plants than in *mex1-1* plants. After 8 h exudation under light, the amount of carbohydrates in the leaf decreased. We confirmed that the maltose transporter is upstream of DPE2 in transitory starch degradation pathway. We hypothesize that excess maltose in maltose-excess mutants could be transported through phloem via sucrose transporters. We also hypothesize that the large amounts of fructose and glucose in phloem sap is possibly the product of apoplastic invertase, which splits translocated sucrose into glucose and fructose.

602 Functional Importance of Diverse Hydroxyacyl-CoA Hydrolase Genes in A. thaliana

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In plants, the classical pathway for valine catabolism likely has multiple metabolic functions for disposal of both isobutyryl-CoA and propionyl-CoA from diverse sources such as valine, other amino acids, branched-chain fatty acids, and phytanic acid derived from chlorophyll. These functions appear to be matched by the presence of diverse beta-hydroxyacyl-CoA hydrolase isoforms. Eight distinct beta-hydroxyacyl-CoA hydrolase genes are present in the A. thaliana genome. Three of these genes have been reported to code for peroxisomal hydrolases and three have been reported to code for mitochondrial hydrolases. Only one of these genes, CHY1, encoding a peroxisomal hydroxyacyl-CoA hydrolase, has been functionally examined to date. We have examined functional importance of the three putative mitochondrial beta-hydroxyacyl-CoA hydrolase genes by characterization of sequence homology, T-DNA insertion mutations, and mRNA expression profiles during growth and development. We propose that only two of these three genes code for mitochondrial hydrolases, which we have designated M-CHY1 and M-CHY2. The third we propose as a chloroplast protein that we have designated Ch-CHY1. The proteins encoded by these genes show distinct differences in their N-terminal leader sequences. The leader sequence of Ch-CHY1 is structurally more consistent with a chloroplast targeting sequence. The M-CHY1 and M-CHY2 genes, unlike Ch-CHY1, are specifically expressed during germination and early development. T-DNA insertion mutations in either M-CHY1 or M-CHY2 appear to be lethal suggesting critical and non-redundant functions. Homozygosity in these mutations leads to defective seed development. Homozygous Ch-CHY1 mutants are viable, with no observed affects on germination, growth or development. These results indicate a surprising multiplicity in function for hydroxyacyl-CoA hydrolases in plants. This is in contrast to other higher eukaryotes which have only a single isoform in mitochondria devoted to valine metabolism.

603 The physiological role of glutathionylation of plastidic fructose-1,6-bisphosphate aldolase in *Arabidopsis thaliana*

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We have characterized plastidic fructose 1,6-bisphosphate aldolase (FBA), one of the glutathionylated proteins previously found in the suspension-cultured cells of *Arabidopsis* [Ito et al. (2003) Plant Cell Physiol. 44, 655-660]. We designated this FBA isozyme as FBA1 and other plastidic FBAs as FBA2 and FBA3. The activity of recombinant FBA1 was upregulated by conditional transition from pH 7 to 8, which was enhanced by glutathionylation of specific cysteine residues in the protein. This feature was not found in recombinant FBA2 or FBA3. Glutathione (GSH) inhibited FBA1 activity at pH 7, but the effect was the opposite at pH 8. This suggests that GSH effectively regulates the FBA reaction in accordance with the activation state of the Calvin cycle. The Calvin cycle is known to be activated by reduced thioredoxins. Unexpectedly, thioredoxin reduced by dithiothreitol (DTT) inhibited FBA1 activity at pH 7 and 8, this inhibition being reversed by not only glutathione disulfide (GSSG) but also by GSH. In addition, glutathionylation prevented the hydrogen peroxide-induced inhibition of FBA1. Based on these results, it was considered that the activation of FBA1 requires GSH to initiate and sustain the Calvin cycle and that glutathionylation of FBA1 facilitates the Calvin cycle under photooxidative conditions. These conclusions were not inconsistent with growth phenotypes of transgenic and knockout plants.

604 What is the role of the PII (AtGlnB1) protein in Arabidopsis?

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The PII (GlnB) protein is a highly conserved trimeric protein found in prokaryotes and plants. In bacteria and cyanobacteria it is involved in the carbon/nitrogen (C/N) ratio sensing and in the transduction of this signal to downstream target proteins. In general, the PII protein interacts directly with target proteins thereby modifying their activity. This interaction depends on the ATP and α-ketoglutarate levels within the cell as well as on the post-translational modification of the PII protein. Higher plants contain a PII protein homolog (AtGlnB1 gene) which is found in plastids but its precise function is not known. In Arabidopsis the expression of the PII gene and protein seems rather constitutive and its role in the sensing of the intracellular C/N ratio has been studied by analysing T-DNA insertion lines in this gene. These PII mutants showed an 80% (PIIV1 mutant) and 100% (PIIS2 mutant) reduced AtGlnB1 transcript level and no detectable PII protein. They did not display an altered growth or developmental phenotype when grown under non-limiting conditions. However, when grown hydroponically, the PII mutants showed small but reproducible differences in both C- and N-metabolites in response to NH_4^+ nutrition. Moreover, the PII mutants displayed also an altered sensitivity to exogenous nitrite. These data suggest the involvement of the PII protein in the adjustement of the C/N balance and in the regulation of some steps of NH_4^+ uptake and/or metabolism.

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605 Metabolomic analysis for understanding of disrupted nuclear-encoded chloroplast protein genes by FT-ICRMS in Arabidopsis thaliana

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To study function of nuclear genes involved in chloroplast development and photosynthesis, we have started to collect *Ds*-tagged knock-out lines that 2090 plastid proteins with a cTP predicted to be encoded by nuclear genomes in *Arabidopsis thaliana*.

To understand regulatory-networks of metabolite, we obtained metabolic profiles of *apg1*(*albino or pale-green*) (the 37 kDa inner envelope membrane polypeptide related plastoquinone biosynthesis), *apg2* (TatC homologue of a ΔpH-dependent protein transporter in thylakoid membrane) and *apg3* (translation system in chloroplast) using integration of metabolomics.

FT-ICRMS (Fourier Transform-Ion cyclotron Resonance-Mass spectrometry) is a nontargeted, high-throughput analytical system for metabolomics analysis in which crude by means of direct injection without prior separation metabolites by chromatography. We observed about 500 m/z values (mass peaks) from each analysis of 100% methanol extracts in 3-week-old apg mutants and the results were subjected to PCA (principal component analysis). We will discuss roles of metabolites with significant profiles.

606 Regulation of indolic glucosinolate and indole-3-acetic acid homeostasis by the Arabidopsis ATR1 Myb transcription factor

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Plants derive a number of important secondary metabolites from the amino acid tryptophan (Trp), including the growth regulator indole-3-acetic acid (IAA) and defense compounds against pathogens and herbivores. In previous work, we found that a dominant overexpression allele of the Arabidopsis Myb transcription factor ATR1, atr1D, activates expression of Trp-metabolizing genes including CYP79B2, CYP79B3 and CYP83B1, which encode enzymes implicated in production of IAA and indolic glucosinolate (IG) anti-herbivore compounds. Recently, we have dissected further the role of ATR1 in controlling Trp secondary metabolism using both ATR1 overexpression and loss-of-function strains. The gain-of-function atr1D mutation specifically increases flux of Trp into IGs by increasing expression of CYP79B2, CYP79B3 and CYP83B1. While a modest increase in IAA was observed, the elevated expression of CYP83B1 presumably directs IAOx preferentially into IGs. Analysis of the atr1D cyp79B2 cyp79B3 triple mutant showed that CYP79B2 and/or CYP79B3 are required for the atr1D mutant phenotypes. In addition, we found that the cyp79B2 cyp79B3 double mutant completely suppressed the cyp83B1 superroot phenotype suggesting that when IG synthesis is blocked, IAOx is shunted into the IAA branch of the pathway. Because a gain of function allele could lead to fortuitous activation of Trp metabolism genes, the atr1-2 loss of function allele was examined. atr1-2 mutants have reduced CYP79B2, CYP79B3, and CYP83B1 transcription, have approximately 30% reduced IG accumulation in adult leaves compared to WT. Consistent with ATR1 function being required for WT levels of CYP79B2/B3-mediated IAOx production, the atr1-2 mutation is able to partially suppress the cyp83B1 superroot phenotype.

607 The TRANSPARENT TESTA 10 Gene Encodes a laccase-like protein Involved in Oxidative Browning of the Arabidopsis Testa

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Whereas most of the biochemical steps of the flavonoid biosynthesis pathway are characterised, the mechanisms of proanthocyanidin (or condensed tannin) polymerisation, oxidative browning and compartmentation remain to be elucidated.

The *transparent testa 10 (tt10)* mutant of Arabidopsis exhibits a delay in developmentally-determined browning of its seed coat. Gene cloning by a candidate gene approach revealed that *TT10* encodes a putative laccase-type polyphenol oxidase. This result was confirmed by functional complementation of the *tt10* mutant and characterization of six alleles. TT10 protein sequence presents four typical consensus copper-binding domains. RT-PCR experiments revealed that the *TT10* gene is essentially expressed in developing siliques. *TT10* promoter activity was detected in the endothelium, the outer-integument cell layers and the chalaza of the seed coat. The *tt10* mutant seeds accumulate more of the flavan-3-ol epicatechin and more proanthocyanidins with lower degrees of polymerisation than wild-type seeds. They also exhibit a reduced amount of flavonol dimers. Histochemical analysis of enzyme activity in intact cells revealed an H_2O_2-independent browning in wild-type but not in mutant seed coat, in presence of flavan-3-ol flavonoid substrates. Together, these data suggest that TT10 may represent the first plant laccase-like protein involved in enzymatic flavonoid oxidation.

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608 Flavonol 3-*O*-glycosyltransferases in *Arabidopsis thaliana*

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Flavonol glycosides constitute a prominent class of secondary metabolites in *A. thaliana* as well as in several crop plants. They are mainly involved in protection against high light and UV irradiation as well in defensive processes and signaling. In Arabidopsis, mainly kaempferol and quercetin derivatives are present. Interestingly, a highly reproducible pattern of differentially glycosylated forms is found under constant environmental conditions. In all cases, sugar moieties are attached to the 3-OH and 7-OH groups of the flavonols. Arabidopsis harbors more than 100 UDP-sugar dependent glycosyltransferase genes (*UGT*). Two highly homologous UGT isoforms were identified via the metabolic profile of two knock-out mutants that indicated a role in the glycosylation at the 3-OH position as well as a discrimination of the sugars attached. In vitro studies using recombinantly expressed proteins confirmed that UGT78D1 was acting as a 3-*O*-rhamnosyltransferase, whereas UGT78D2 was a 3-*O*-glucosyltransferase[1,2]. Thus, both enzymes accept the same substrate yet distinguish between the activated sugar substrates. The *ugt78D1/ ugt78D2* double mutant was largely lacking any flavonol glycosides in leaf extract. Since no other intermediates including free flavonols accumulated in the single and double mutants, it is concluded that the glycosylation at the 3-*O*-position is the initial step of conjugation and a prerequisite preceding other modifications. Furthermore, a putative, so far unknown feed-back inhibition of biosynthesis prevents the accumulation of probably toxic amounts of free flavonols.

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2. Tohge et al. (2005) Plant J. 42, 218-235

609 Trehalose-6-phosphate in Arabidopsis

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Trehalose is the alpha1,1-glucose disaccharide. Genes encoding enzymes for its metabolism are ubiquitously found in plants. Synthesis of trehalose in plants, just like that of sucrose, occurs via its phosphorylated intermediate, trehalose-6-phosphate (T6P). Enzymes with homology to T6P synthases and phosphatases are surprisingly more numerous than those of sucrose synthesis. In contrast, plants generally contain a single or very few genes encoding trehalose catabolising trehalase. Radiation thus suggests a differentiation of the role of trehalose-6-phosphate (T6P) in plants and experimental evidence is accumulating that confirms this notion. tps1 mutants complemented with E.coli trehalose-phosphate synthase reveal that T6P is essential for embryo development, root growth and the transition from vegetative growth to flowering. Genetic manipulation of T6P levels indicate that T6P is further required for carbohydrate utilization and that it affects starch synthesis. Trehalose feeding experiments establish that accumulation of T6P in the absence of metabolisable sugar leads to seedling growth arrest. Accumulation of T6P in the absence of supplied carbon further leads to a specific stress response that protects Arabidopsis seedlings from infection with *H. parasitica*. Our research is now focused upon understanding the mode of action of this potent and ancient metabolite.

610 Starch content and starch-modifying-enzyme activity variations in α- and β-amylase insertional mutants of *Arabidopsis thaliana*

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Plants produce energy reserves through photosynthesis that are stored as starch. To recover the stored energy during periods of dark the plant degrades this semi-crystalline carbohydrate molecule, freeing sugars needed in various metabolic processes. The degradative process is thought to involve several types of enzymes that likely act in concert; the hydrolytic amylases are predicted to play a major role in starch catabolism. Amylases are divided into two broad classes, α and β based on functional characteristics and sequence comparisons. Both classes hydrolytically cleave α, 1-4 glycosidic bonds of amylose and amylopectin; β-amylases cleave exolytically to release maltose, while α-amylases cleave internally to produce branched and unbranched oligosaccharides of varying lengths. The *Arabidopsis thaliana* genome is predicted to code for three α- and nine β-amylase proteins, although the specific roles of all 12 are relatively unknown. To further investigate their enzymatic functions SALK T-DNA insertion lines were screened and homozygous mutant populations were isolated for all of the α-amylases and six of the β-amylases. The insertions were confirmed in each structural gene by PCR and checked for transcripts by RT-PCR. Starch contents were compared in mutant and wild type plants at the end of the day and end of the night. Native PAGE activity gels (zymograms) were used to analyze all starch modifying enzyme activities, including the amylases, in each mutant. Differences in starch staining or enzyme activities were detected in some of the mutants, and a few showed striking and intriguing changes that warrant further investigation. These data will lay the groundwork for future investigation into the functions of each amylase protein, singly and/or in concert with other isoforms, in order to further elucidate the dynamic processes of starch metabolism.

611 Light and carbon gene networks defined by genomic analysis of cli186: a carbon and light insensitive mutant.

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The integration of exogenous and endogenous signals are crucial for plant growth and development. Carbon and light are examples of two essential signals affecting all aspects of development. Previous genome-wide studies investigating the crosstalk between carbon and light identified 63% of the *Arabidopsis* genome and the biological process, metabolism to be affected by the interaction between carbon and light signaling interactions. To identify key players or regulators involved in carbon and light sensing and/or signaling interactions, a genetic screen was employed to identify carbon and light insensitive plants (*cli*). Here, one of the selected mutants, *cli186* is characterized on a genome-wide level to identify in an unbiased and undirected manner all the genes and gene networks affected by the mutation. A 'metric' classification scheme is used to classify genes on the basis of their expression profiles and to further identify genes that are mis-regulated by light, carbon or light and carbon in *cli186* when compared to wild-type. This classification scheme identifies 631 genes that are mis-regulated in *cli186*. Over half (54%) of these 631 genes show mis-regulation in carbon and light treatments only. To identify biological processes that are mis-regulated in the mutant, it was determined whether the genes involved in a biological process are over-represented among the 631 mis-regulated genes. Of 29 primary functional categories identified by the Munich Information Center for Protein Sequences, metabolism and energy are over-representated among the 631 mis-regulated genes. The secondary functional categories over-represented among the 631 genes include amino acid metabolism/biosynthesis, glycolysis and gluconeogenesis. Networks of genes affected by carbon and light interactions that are mis-regulated in *cli186* are visualized using software that integrates gene expression profiles with molecular interaction networks. An amino acid metabolism network and its regulatory factors possibly involved in controlling genes in this network are shown. This may be one of the first mutants isolated and characterized that specifically exhibits insensitivity to both carbon and light signals. Therefore, identification of the affected gene in c*li186* will provide insight into the crosstalk between carbon and light interactions.

612 The circadian clock gates sensitivity of *GIGANTEA* gene expression to light

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The photoperiodic response of flowering is mediated by the interaction between light signals and the circadian clock. *GIGANTEA (GI)*, *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* are key genes in inducing the floral transition of Arabidopsis in response to long days. Based on genetic analysis they can be arranged in the functional hierarchy GI-CO-FT. *GI* gene expression oscillates with an evening phase, and this increases the amplitude of the rhythmic *CO* transcription. CO protein is also stabilized by light at the end of the long light period, and this leads to activation of *FT* gene expression. Unlike CO and FT, GI is involved not only in the floral transition but also in other light signaling pathways and control of the circadian clock.

Here we report that *GI* transcription is induced by blue, red and far-red light. The light responsiveness is controlled by the circadian clock in a gated manner. So that the activation is 5-7 fold higher at the time of the circadian peak in *GI* expression than it is at the time of trough levels of expression. This means that *GI* expression can respond to light strongest at dusk, when light triggers *FT* expression via CO activation. The diurnal peak of *GI* expression occurs earlier in short than in long days and the acute light response followed the same pattern. Our results suggest that the role of GI in flowering may not be restricted to the transduction of the rhythmic signal from the clock toward *CO* and suggests the possibility that light induction and circadian clock regulation of *GI* expression could contribute to day-length measurement.

613 Differing roles for hydrolytic and phosphorolytic starch break down

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Transitory starch is formed in chloroplasts during the day and broken down at night. Two pathways are proposed for starch degradation: (1) hydrolytic, which converts starch into sugars for export, and (2) phosphorolytic, which provides phosphorylated intermediates for chloroplast metabolism. Net transitory starch breakdown occurs at night and is under circadian control. However, under photorespiratory conditions we observed transitory starch to breakdown to occur at a rate equal to or faster than breakdown at night. In bean under photorespiratory induced starch breakdown levels of both maltose and glucose-6-phosphate (G6P) more than triplede. Nonaqueous fractionation has shown that the increase in G6P is occurs mainly in the chloroplast. This provides evidence that regulation of starch breakdown occurs at a point preceding the division of the hydrolytic and phosphorolytic pathways. In an *Arabidopsis* mutant in which the plastidic starch phosphorylase enzyme has been knocked out, no phenotype was observed under standard growth conditions, indicating that starch phosphorylase is not required. In this mutant photorespiratory starch breakdown still occurs with an increase in maltose and G6P. However, the maltose increase in the mutant is higher than in WT. Taken together this data is consistent with our developing hypothesis that hydrolytic starch breakdown is used for export from the chloroplast while phosphorolytic starch breakdown is used for internal metabolism. This data also indicates that the hydrolytic products of starch breakdown may be phosphorylated and reimported into the chloroplast.

614 The Arabidopsis thaliana transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis

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Comprehensive functional data on the large family of plant R2R3-MYB transcription factors is still scarce compared to the manifold of their occurrence. We identified the Arabidopsis thaliana R2R3-MYB transcription factor MYB12 as a flavonol-specific activator of flavonoid biosynthesis. Transient expression in A. thaliana protoplasts revealed a high degree of functional similarity between MYB12 and the structurally closely related factor P from Zea mays. Both displayed similar target gene specificity, and both activated the target gene promoter only in presence of a functional MYB recognition element (MRE). The genes encoding the flavonoid biosynthesis enzymes CHS, CFI, F3H and FLS were identified as target genes. Hence, our observations further add to the general notion of a close relationship between structure and function of R2R3-MYB factors. HPLC analyses of myb12 mutant plants and MYB12 overexpression plants demonstrate a tight linkage between the expression level of functional MYB12 and the flavonol content of young seedlings. Quantitative realtime RT-PCR using these mutant plants showed MYB12 to be a transcriptional regulator of CHS and FLS in planta, the gene products of which are indispensable for the biosynthesis of flavonols. Furthermore we have first evidence that the members of R2R3-MYB subgroup 7, MYB12 and the closely related factors MYB11 and MYB111, are flavonol-specific regulators.

615 Regulation of Metabolic Networks in Arabidopsis

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Plant composition, form, and function are the ultimate consequence of gene expression. Such expression is regulated by multiple mechanisms. Recent technological advances enable the high-throughput detection and measurement of changes in the accumulation of tens of thousands of cellular components - RNAs, proteins, and metabolites. The challenge is to use these data to reveal the network of interactions occurring in the plant, such that we can predict the consequences of directed genetic or environmental changes. One major mechanism for regulation of regulation is at the level of RNA accumulation. To evaluate the rich global transcription datasets deposited in databases such as NASCArray and PlexDB, we have developed the open source software, MetaChip, which uses a combination of statistical approaches to identify patterns across 1000s of chips, and the textmining tool PathBinderAt, which suggests "hidden links" among the genes. The hypothesis we are addressing is that the expression patterns of committed steps in pathways tend to be co-regulated. and that corregulation under particular conditions can be used to define novel, but functional, pathways. A corollary is that as yet unidentified metabolic and regulatory genes can be revealed by expression patterns. Studies using these tools reveal details of the co-regulation of subsets of genes across multiple developmental and environmental contexts. We discuss the global structure of the regulation of gene expression in Arabidopsis at the level of RNA accumulation, with a particular focus on the primary metabolic network of lipid and carbohydrate metabolism. We identify several candidate genes for regulation of metabolic flux in the cell.

616 Control of Nuclear Genes Encoding Enzymes of Lipid Biosynthesis in Chloroplasts by a Protein Released from Mitochondrial Membranes

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Interorganelle signaling is essential to many subcellular processes, but is still poorly understood at the molecular level. Control of lipid homeostasis and exchange of lipids during the process of organelle biogenesis is an example for strict coordination between organelles. We have recently discovered an Arabidopsis protein (*dgd1*-SUPPRESSOR1, DGS1) integral to the mitochondrial membrane, from which a nuclear targeted transcription factor-like domain similar to the HOX domain of homeo-box transcription factors is released. In mutants carrying a mutation in DGS1, the expression of *MGD2* and *MGD3* encoding galactolipid synthases localized in the outer chloroplast envelope is affected. The *dgs1* mutation was identified in a suppressor screen in the *dgd1* mutant background. The *DGD1* gene encodes the galactolipid synthase responsible for the bulk of the digalactolipid found in chloroplast membranes. Under phosphate-limited conditions a DGD1-independent pathway of galactolipid biosynthesis is induced which leads to the production of plastidic and extraplastidic galactolipids due to the activity of galactolipid synthases in the outer chloroplast envelope encoded by *MGD2*, *MGD3* and *DGD2*. It is proposed that DGS1 is a component of a lipid sensing/signaling mechanism in mitochondria, which controls lipid homeostasis and membrane properties in this organelle, directing the synthesis of galactolipids in the chloroplast envelopes and their transfer to the mitochondrial membrane. Such a proposed system, while novel for plants, has its precedence in the mammalian ER-based sensing system controlling sterol biosynthesis. As such it provides an intriguing mechanistic paradigm for the coordinated interaction of the mitochondrion, the nucleus and the chloroplast in plants.

617 High-throughput enzymatic tests coupled with structural modeling identify an Arabidopsis farnesoic acid methyltransferase

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We have identified a new type of plant methyltranseferases that transfer a methyl group to a carboxyl or to a nitrogen. Products of the known enzymes from this family include secondary metabolites (such as caffeine) and signaling molecules (such as methyl salicylate and methyl jasmonate). The Arabidopsis genome contains 24 genes encoding enzymes of this family. To identify the catalytic activities of members of this protein family in Arabidopsis, we have carried out assays with a large number of potential substrates. Using a high-throughput screen for enzymatic assays with potential substrates, we identified the protein encoded by the *Arabidopsis thaliana* gene At3g44860 as a methyltransferase with high specificity to farnesoic acid (FA). This S-Adenosyl-L-methionine:farnesoic acid carboxyl methyltransferase (FAMT) has Km values of 41 μM for FA and 71 μM for SAM. Modeling of the active site of FAMT based on known crystal structure of the homologous enzyme SAMT (salicylic acid methyltransferase) from *Clarkia breweri* indicates that FA has a close fit in the active site, consistent with the observation that FA is the best substrate among all the substrates with structural similarity. The expression levels of At3g44860 are increased by several compounds known to induce plant defense. Our findings suggest that an Arabidopsis protein is capable of synthesizing methyl farnesoate, an insect juvenile hormone known to be synthesized in other plants as a defense compound but not yet identified in Arabidopsis. The use of the high-throughput enzymatic tests for potential substrates, coupled with structural modeling, may contribute to the identification of unknown enzymes and pathways.

618 Genetic analysis demonstrates functional overlap between starch synthases II and III in *Arabidopsis thaliana*

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Starch synthases (SS) catalyze reactions that build ˜-(1,4)-linked linear glucosyl chains in starch. At least five classes of SS are conserved in higher plants, although the functional specificity of each isoform that might lead to conservation is for the most part unknown. To address this question this project is comprehensively analyzing the effects of eliminated particular SS isoforms singly and in combination. Here we report on individual knockout mutants for SSII and SSIII, and the SSII/SSIII double knockout. Both SSII and SSIII are minor isoforms in terms of total activity, yet they each have significant effects on starch biosynthesis. *Atss3* mutations cause starch excess, suggestive of a regulatory role controlling another SS. *Atss2* mutations did not have a major effect on starch quantity. In contrast to either single mutant, the *Atss2 Atss3* double mutant showed a major decrease in starch content. *Atss2* caused abnormal granule shape, whereas *Atss3* mutant granules had normal morphology and the double mutant granules were more severely altered than the *Atss2* granules. With regard to starch structure, *Atss2* conditioned reduced frequency of chains of DP12-23, and a corresponding increase in chains of DP6-10. The *Atss3* mutants had frequency changes in the same chain lengths, but to a much lower extent than the *Atss2* mutants. The double mutant had an extremely severe shortage of chains in the range of DP12-23 or longer, chains of DP6-10 were much greater in frequency than either single mutant. The most straightforward explanation for these data is that SSII and SSIII can both participate in synthesis of chains of DP13-23 and thus that they overlap in function at least in this regard. SSIII may also have a regulatory role on starch production that is not shared by SSII.

619 GARNet

Ruth Bastow
GARNet

GARNet The **G**enomic **A**rabidopsis **R**esource **Net**work was established in 2000 to provide reliable, efficient, user-driven and publicly available functional genomics resources for Arabidopsis research. The original three year funding period for GARNet was extended in 2003 to allow the services provided by GARNet to move to full cost recovery. Coordination activities were funded for a further 5 years from 2005, to keep services up to date, provide an information resource (website http://garnet.arabidopsis.info/, newsletter, annual meeting) and point of contact for other UK plant communities and international plant genomics programmes. Resources initiated by GARNet include transcriptomics, proteomics and metabolomic . Insert clone libraries and a screening service are available from GeTCID and additional insertional mutagenesis populations generated in the first funding period are now available at the Nottingham Arabidopsis Stock Center, NASC. Data from GARNet-funded Affymetrix and proteomics are available at NASC (http://arabidopsis.info), and metabolomics results are available at http://www.metabolomics.bbsrc.ac.uk/. The annual GARNet Meeting will be at the John Innes Centre Norwich UK 5-6 Sept 2005 for more information see http://garnet.arabidopsis.info

620 Development of a Gal4-mediated tri-reporter expression system in *Arabidopsis*

Karen Fitzsimmons, Cawas Engineer, Melissa Curran, Robert Kranz
Washington University

Plasmids based on BASTA and hygromycin resistance have been constructed that allow for the trapping of enhancers using Gal4 and Gal4-mediated expression of three reporters. An *Arabidopsis* enhancer trap library with 10,000 events has been generated that contains Gal4 and 5X UAS upstream of luciferase, GUS and rsGFP. To ensure that a 5X UAS is optimum for expression, we compared GUS expression using a 1X, 2X, or 5X UAS in plants with and without Gal4. To further determine that luciferase (LUC) reporter expression is Gal4 dependent, e35S/Gal4 and m35S/Gal4 were compared for luminescent expression in plant lines. LUC is currently being used as the primary reporter because it allows for *in planta* screening and quantification; rs GFP and GUS are used as secondary reporters. Results on silencing frequency (over 4 generations) and the basis for silencing are presented. Examples of tissue specific expressors will be shown.

621 A Rapid Transformation System for Octoploid Strawberry

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Cultivated strawberry (*Fragaria* x *ananassa*) is a valuable crop, yet has benefited little from recent advances in biotechnology and genomics. Here high-frequency transformation and regeneration protocols are presented for the new genetic line 'Laboratory Festival #9'. Direct organogenesis has been achieved from all tissues tested, with rapidly-growing shoots discernable in as few as 14 days. The conditions for optimal shoot generation, transformant selection, root generation and plant acclimatization are presented, and allow progression from gene construct to a transgenic plant in soil in fewer than 60 days. The development of transformation protocols for this laboratory genotype allows high-throughput studies of gene function in the octoploid strawberry genetic background.

622 ResurfP: A response surface aided parametric test for identifying differentials in experiments involving multiple independent measurements of a parameter

Suresh Gopalan

Independent Investigator

The increasing use of high-throughput technologies in biological experimentation pose new challenges in data analysis. Many of these challenges arise due to the small number of replicates and high stochasticity inherent in many biological systems. A common paradigm in biological study is pairwise comparison of data from two experimental conditions (mutant vs. wildtype, treated vs. untreated etc.). Many downstream analysis and functional inference are directly dependent on the specificity of the differentials identified. Here a response surface assisted methodology is proposed for the evaluation of differentials in a parameter being studied with multiple independent measurements. The utility is demonstrated using GeneChip platform (Affymetrix, CA), where each gene or transcript is represented by eleven or more independent sequences (probes). A test dataset with known differentials was used to study the effect of the number of probe-pairs included at different statistical threshold used for selection of differentials between two datasets. A response surface was plotted by formulating an equation that captures the effect of varying threshold of probe-pairs and t-statistic on true positives and false positives identified. The toplology of the surface was used to define one form of cost-based approach to determine optimum threshold when comparing two datasets at the probe-level. In addition, a data scaling approach is proposed to study the impact of a selected threshold on the number of false negatives of differing magnitude of differentials in a given dataset. The results indicate that this response surface assisted approach (termed **ResurfP**) would be effective in determining optimal data-specific threshold when analyzing differentials between two datasets using probe-level data. Some properties of the proposed approach will be demonstrated using data from a published study of Arabidopsis-pathogen interaction. This method also has utility in sensitive identification of differentials in time course studies and in study of responses represented by multiple independent measurements of a parameter.

623 MapPlants: A Gene-Mapping Activity Optimized for the Undergraduate Laboratory

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We are creating plant-based tools for education that help instructors integrate classical genetics, molecular biology and bioinformatics. These laboratories provide opportunities for undergraduates to observe growing plants and see how molecular and genomic tools are applied to answer experimental questions about plant growth and development. MapPlants is a laboratory module in which students determine the chromosomal location of a mutant gene. During this four-week laboratory students learn the concepts of Mendelian genetics, probability, linkage, and polymorphic DNA. In addition, students get hands-on experience with bioinformatics resources (TAIR), DNA extraction, PCR amplification, restriction enzyme digestion and gel electrophoresis. The laboratory has been optimized for the undergraduate laboratory. The mutant allele the students map produces a glabrous phenotype visible one week after germination. The markers used for the mapping are CAPS all of which amplify a 0.8-1.0 kb region around an *XbaI* polymorphism. Seeds, detailed instructions and presentation materials are provided to instructors.

624 The Arabidopsis Information Resource (TAIR) – New developments

Eva Huala[3], Margarita Garcia-Hernandez[3], Leonore Reiser[3], Katica Ilic[3], Hartmut Foerster[3], Douglas Becker[3], Tanya Berardini[3], Rachael Huntley[3], Aleksey Kleytman[3], Suparna Mundodi[3], Neil Miller[2], Mary Montoya[2], Nick Moseyko[3], Jon Slenk[3], Julie Tacklind[3], Christophe Tissier[3], Dan Weems[2], Christopher Wilks[3], Iris Xy[3], Thomas Yan[3], Daniel Yoo[3], Peifen Zhang[3], Brandon Zoeckler[3], Seung Rhee[3]

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TAIR (http://arabidopsis.org) provides a comprehensive Arabidopsis data resource to researchers, integrating many kinds of data including genes, proteins, maps, clones, seed and DNA stocks, polymorphisms, germplasms, genetic markers, microarray experiments, biochemical pathways, community members and published literature. A variety of large-scale functional genomics data have now been integrated into TAIR, including data from sequence-indexed mutant collections, full-length cDNA clones, expression studies, protein localization studies, and polymorphism collections. TAIR has recently taken over the task of maintaining and updating the Arabidopsis genome annotation. A first release planned for spring 2005 includes approximately 400 new protein-coding and miRNA genes and updates to 6000 gene structures based on community submissions and new cDNA and EST data in GenBank. In the past year TAIR has also added gene expression profiling data from 1155 Affymetrix slides, including those of the AtGenExpress project. User interfaces for accessing microarray data have been revamped to improve usability. Genes and pathways in our AraCyc biochemical pathways database have been significantly updated. Large-scale functional genomics data resulting from microarray expression, metabolite or proteomic profiling experiments can be overlaid onto the AraCyc metabolic overview map. Tutorials describing (1) use of GO (Gene Ontology) annotations, (2) access to microarray gene expression data in TAIR and (3) use of the Aracyc Metabolic Pathway database are now available. We continue to associate Arabidopsis genes with GO terms using data from the published literature and have switched from using the TAIR anatomy ontology to using the Plant Ontology Consortium Plant Structure terms for annotating gene expression patterns. Data from TAIR can be viewed on detail pages for single objects, downloaded from search results pages or our bulk downloads page (http://arabidopsis.org/tools/bulk/) or downloaded from our ftp site. The community is encouraged to submit data on gene structure and function, microarray experiments, markers, protocols, gene families and seed and DNA stocks. Instructions and forms for submitting data and materials can be found at http://arabidopsis.org/info/data_submission.jsp. TAIR beginning and advanced workshops will be presented.

625 Analyzing and sorting *Arabidopsis* seedlings using the COPAS PLUS instrument

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COPAS instruments automate the analysis, sorting, and dispensing of "large" objects such as viable small model organisms, seeds, beads, and particles, measuring the object size, optical density, and the intensity of fluorescent markers. Once analyzed, objects are sorted according to user selectable criteria, and then may be dispensed into stationary bulk receptacles or multi-well microtiter plates for high throughput screening. COPAS instruments have been proven to analyze and sort large objects with a higher speed and precision than present manual techniques. By automating the current, time consuming manual processes, the time required for experiments is dramatically reduced, human error is eliminated, and new experiments that previously could not be considered are now possible.

Here we show that in addition to seeds, the COPAS PLUS instrument, which features a 1mm flow cell, can also be used for analyzing and sorting germinated *Arabidopsis* seedlings. We also tested the capability of Profiler, an add-on module for the COPAS instrument, in detecting the fluorescence signal along the longitudinal axis of a seedling. This module measures positional information of fluorescence peaks along the length of an object. With the Profiler, it is possible to distinguish localized fluorescence from the auto-fluorescence background and thus enabling sorting out objects with even weak fluorescence expression patterns.

626 Development of an Automated Petri Plate Pourer

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An Automatic Petri plate pourer has been designed by Scinomix Corporation in collaboration with the Monsanto Automation Group and Media Preparation Team to fill plates in order to reduce ergonomic strain associated with this task. Repetitive hand, back, and wrist motions required to dispense semi-solid medium into Petri plates can be a source of ergonomic injury. The equipment has successfully pumped media into Petri plates at a rate of 6-7 seconds per plate with no splashing or bubbles observed. Ergonomic, operational safety, and sterility issues were considered in overall design.

627 Novel plant tissue digestion methodology stream lines plant genomic DNA purification

Michelle Mandrekar, Susan Koller, Rex Bitner, Hemanth Shenoi
Promega Corporation

High-throughput systems for isolating DNA from plant tissue have been described that are readily adaptable to multiple sample types (e.g. leaf or seed) with required yields (50-500ng) and purity for amplification-based genotyping. However, high-throughput plant genomic DNA purification still suffers from a bottleneck at plant tissue preparation. We have developed a novel purification methodology that uses enzymatic digestion to release DNA directly into solution. This eliminates plant tissue grinding and centrifugation, allowing for the potential of full walk-away automation of plant genomic DNA purification from leaf to applications ready DNA (e.g. PCR and other amplification methods). These methods may also be performed manually with minimal equipment needs. All methods involve optimized MagneSil® paramagnetic particle technology to bind and purify DNA. An option for normalization of DNA yield is described using a specially designed MagneSil® particle that allows a standardized DNA yield from a given plant tissue. Together, these describe one of the first continuous automated processing methods to obtain plant genomic DNA in a high-throughput 96-well format.

628 Uncovering novel cell wall-related genes in Arabidopsis thaliana

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Mid-infrared spectroscopy provides a convenient, high throughput way to detect alterations in cell wall composition and architecture even when the mutant has no visible phenotype if compared broadly to wild-type. The infrared spectrum of a cell wall is not merely the sum of the spectra of its component molecules, but also contains information that reflects the local environment of molecular bonds, such as their hydration state, the conformations of the molecules, and interactions with other molecules. Bioinformatics approaches have identified over 1000 genes in Arabidopsis that function in wall biogenesis and disassembly. Robot-assisted, high throughput PCR protocols have been developed to obtain homozygous T-DNA tagged lines of Arabidopsis of many of these (see http://cellwall.genomics.purdue.edu and links therein). We have established a library of characteristic infrared spectra, spectrotypes, for many well-known cell wall mutants and individual members of cell-wall-related gene families. We have also identified several genes of unknown function that are implicated in the formation of secondary walls in xylem cells. For one gene family, we have obtained homozygous lines for four of the gene family members and we have established that these lines are cellulose-deficient. For limited numbers of mutant classes, we can apply chemometric analyses. For example, we can identify spectrotypes that are characteristic of cellulose deficiencies or alterations in xyloglucan or pectin structure. However, the potential diversity of mutant spectrotypes has encouraged us to consider algorithms that have the capacity to deal with multivariate data, very large numbers of observations (spectra), and large numbers of potential classes of observation within the data set. Therefore, we are applying fully-connected feed-forward artificial neural networks in order to refine the classification of spectrotypes. Cluster plots based on the output from neural networks are generated to show the relationship between mutant classes: if individuals of a mutant genotype are more similar to each other than to individuals of any other mutant or wild-type, then a new class is defined, and its characteristics may be inferred from the characteristics of its nearest neighbor classes. We continue to screen families of genes of unknown function to discover new proteins and enzymes that contribute to building the highly complex structure of the cell wall.

629 Functional Analysis of *Medicago* Genes Using *Arabidopsis EMB* Knockouts

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We describe here a strategy for analyzing the functions of putative *Medicago* orthologs of genes required for growth and development in *Arabidopsis*. The general concept was to use molecular complementation of *Arabidopsis* mutants defective in genes with defined cellular functions to identify genes with equivalent functions in the model legume *Medicago truncatula*. We reasoned that such an approach might be informative and beneficial as functional comparisons between *Medicago* and *Arabidopsis* genomes become more prevalent in the future. Two *EMB* genes with interesting knockout phenotypes were chosen for this pilot study: (1) *CYT1*, required for GDP-mannose formation, N-glycosylation of proteins, and vitamin C biosynthesis; and (2) *TTN5*, involved in B-tubulin folding and the regulation of microtubule dynamics. PCR-amplified, full-length *Medicago* cDNAs for two genes similar to *CYT1* (*Mt CYT A, Mt CYT B*) and one gene similar to *TTN5* (*Mt TTN 5*) were combined with two different promoters (35S, native *Arabidopsis*), transformed into wild-type *Arabidopsis* plants, and the resulting transgenic plants crossed with *emb* heterozygotes. Progeny plants heterozygous for both the *Arabidopsis* mutant allele and the *Medicago* transgene were then scored for defective seeds following self-pollination. Full complementation of the mutant phenotype was observed with *Mt CYT A*. Partial complementation with atypical embryo phenotypes more advanced than expected was found with *Mt CYT B* (native *Arabidopsis* promoter) and *Mt TTN 5* (native *Arabidopsis* promoter). We conclude that this strategy provides a sensitive experimental test for assaying and comparing the functions of *Medicago* genes with high sequence similarity to known *EMB* genes of *Arabidopsis*. Results presented here suggest that *Mt CYT A* is a functional ortholog of *CYT1*, because unlike *Mt CYT B*, with which it shares a high degree of sequence similarity, *Mt CYT A* can fully complement the *Arabidopsis cyt1* mutant. Future completion of the *Medicago* genome sequence should make such an experimental strategy applicable to the analysis of a wide range of genes with important cellular functions.

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630 Functional genomics of nuclear-encoded chloroplast proteins in *Arabidopsis*

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A chloroplast is the most important organella in plant involved in photosynthesis and production of various metabolites, but the most proteins are the nuclear-encoded. In *Arabidopsis*, a large-scale analysis of mutants is possible because many tag lines exist. For the functional analysis of the nuclear-encoded chloroplast proteins, we systematically collected these tag lines. Richly and Leister (2004) identified 2,090 proteins with a targeting signal to chloroplast by at least 3 of the 4 predictors (iPSORT, PCLR, Predotar, and TargetP). Base on database of RIKEN and SALK, 1,277 genes were disrupted by insertion of *Ds* or T-DNA, which is about 61 % of these 2,090 chloroplast proteins. This collection of tag lines become larger as numbers of tag lines increase. We have not only isolated albino or pale-green mutants (*apg*), but also collected homo lines without clear phenotypes (which means "cryptic mutants") when plated on agar medium. Using these homo lines, the screening of the unusual chlorophyll fluorescence and environmental stress phenotype will be performed to identify novel functions of chloroplast protein.

631 Metabolomics: A functional genomics tool for deciphering functions of Arabidopsis genes in the context of metabolic and regulatory networks

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A multi-institutional consortium of labs is developing metabolomics as a new functional genomics tool for elucidating the functions of Arabidopsis genes that are currently annotated as having an unknown function. Approximately one third of the Arabidopsis genes are so annotated. The consortium utilizes five distinct analytical platforms that couple different separations methods (GC, LC, and CE) to mass-spectroscopic detection systems. These analytical platforms are used in both non-targeted and targeted metabolomics analyses, which in combination detect approximately 2,000 metabolites, of which 900 are chemically defined. The consortium is applying these platforms to reveal changes in the metabolome associated with knockout mutations in genes of unknown function and comparing these to similar mutants in genes of known functions. We will discuss initial data generated from a small set of exemplar mutants. These data indicate that metabolomics can reveal metabolic changes in mutants that are otherwise silent in phenotype. In addition, these data are revealing that metabolomics can uncover distinct metabolic changes in mutants of paralogous genes. These metabolomics data are being interpreted via two strategies: 1) as a fingerprint of the metabolic consequence of each mutation, which can be used to functionally cluster genes; and 2) by mapping metabolite changes on metabolic and regulatory network maps, such as AraCyc and MetNetDB, to identify specific functions that are affected by each mutation. Thus, metabolomics, in combination with other -omics technologies promises to be a new resource for determining the functions of Arabidopsis genes.

632 AGRIS: A platform to establish cis-regulatory networks and to identify direct targets for transcription factors in Arabidopsis thaliana

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An emerging premise in regulation of gene expression is to identify the regulatory networks in which Transcription Factors (TFs) participate to categorize the temporal and spatial expression of all genes in an organism. This fact underscores that it is vital to establish the architecture of plant promoters to understand gene expression. The identification of direct target genes for selected TFs will act as a platform in establishing genome-wide regulatory networks to explain the expression of all Arabidopsis genes, simultaneously validating an Arabidopsis cis-regulatory element map developed in parallel. AGRIS (Arabidopsis Gene Regulatory Information Server) serves as an information resource for all Arabidopsis promoter sequences, TFs and their target genes and functions as a platform to establish regulatory networks. AGRIS currently houses three linked databases: AtcisDB (Arabidopsis thaliana cis-regulatory Database), AtTFDB (Arabidopsis thaliana Transcription Factor Database) and AtRegNet (Arabidopsis thaliana Regulatory Network). AtTFDB contains 1,500+ Arabidopsis TF sequences (protein and DNA) grouped into 41+ families with information on available mutants in the corresponding genes. AtcisDB consists of 25,800+ promoter sequences of annotated Arabidopsis genes with a description of putative cis-regulatory elements. AtRegNet links in direct (and indirect) interactions several hundred genes with the TFs that control their expression. AGRIS provides tools on Arabidopsis TFs and their experimentally identified binding sites on all genes to initiate the identification of networks in the model dicotyledoneous plant Arabidopsis thaliana. AGRIS can be accessed at http://arabidopsis.med.ohio-state.edu.http://arabidopsis.med.ohio-state.edu.

633 Plant-localizome markers: a versatile collection of fluorescent fusion proteins labeling plant cell compartments

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Whether one studies a single protein, a protein family or a proteome, a question that is always relevant to address is where that protein is localized. One of the commonly used techniques to investigate this is to fuse fluorescent reporters to the protein of interest enabling tracking in time and space. Probably the highest resolution that can be achieved by fluorescent techniques is targeting to the sub-cellular compartments.

We would like to present a tool, which we are in the process of developing, that should be of a great utility to the plant research community: We have PCR cloned into a gatewayTM pENTRY vector a variety of plant sequences encoding peptides that have been characterized in the literature to be targeting determinants for specific plant sub-cellular compartments. The gateway system enables us to then fuse any of the peptides to the fluorescent proteins of our choice (existing or available in the future).

These fusion proteins can then be used to label one or several cell compartments to do localization experiments and also to track specific cell bodies under developmental or stress conditions.

634 Oligonucleotide Arrays for Rapid Genotyping of Arabidopsis Inbred Lines

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Hsp90 plays a central role in the unfolding of genotype to phenotype. Modulation of Hsp90 activity in *Arabidopsis* by pharmacological inhibition or increased temperature leads to an array of altered morphological phenotypes. These morphological variants are typically dependent on underlying cryptic genetic variation. As the uncovering of Hsp90-dependent buffered variation may be environmentally induced, Hsp90 may influence the rate of evolutionary change or allow the appearance of morphological novelty.

Our lab is examining the prevalence and identity of Hsp90-buffered variation in *Arabidopsis*. We have created Columbia x Landsberg *erecta* (Col x L*er*) recombinant inbred lines (RILs) which are reduced in Hsp90 levels by RNAi. We are conducting comparative quantitative trait locus (QTL) analyses of numerous life history traits between an Hsp90-reduced and a control RI population.

Conventional methods of establishing high-resolution maps of RILs for QTL analysis are laborious and expensive. To vastly increase the speed and reduce the cost of this process, we have developed a microarray-based approach to map *Arabidopsis* RILs using 70-mer InDel (Insertion/Deletion) oligonucleotide sequences as markers. These markers have been identified by sequence analysis between the Col (www.arabidopsis.org) and the L*er* (www.arabidopsis.org/Cereon) genomes. Competitive hybridization of fluorophore-labeled RIL and wild-type genomic DNA to these InDel markers determines the genotype of the RIL (Col or L*er*) at each marker. By combining map data for these RILs with their respective phenotypic data, we can identify Hsp90-dependent QTL. Roughly 325 of our InDel markers are informative for genotyping purposes. The average marker spacing is approximately 3 cM. We are also developing methods to increase throughput of our array by printing a 325-element array in each well of a 96-well plate. This advance will allow high-resolution mapping of 96 RIL lines in a single hybridization experiment.

We plan to provide this mapping array to members of the *Arabidopsis* community at cost. We have shown that many of our InDel markers segregate at intermediate allele frequencies in 19 other *Arabidopsis* ecotypes tested. Thus we expect our marker array to be usable for genotyping any *Arabidopsis* RIL collection.

635 A gene expression map of Arabidopsis development

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In contrast to most animals, plants develop continuously, with new organs being initiated and elaborated throughout the life cycle of the organism. As a consequence, individuals consist of repeated units, such as leaves or flowers, which are present in many developmental stages at any given time of the life cycle. It follows that many transcriptional programs underlying the development of different organ systems are continuously active. Plants therefore provide an excellent opportunity to study how transcriptional programs control multicellular development. We have analyzed global gene expression during development of the reference plant *Arabidopsis thaliana* in 79 samples covering many stages, from embryogenesis to senescence, and diverse organs. Among several findings is the observation that the expression levels of transcription factor genes and signal transduction components are similar to those of metabolic genes. In contrast, genes encoding proteins involved in photosynthesis or protein biosynthesis are expressed at substantially higher levels, whereas genes in the replication category are underexpressed. Another focus of our study is the analysis of co-expression of genes throughout development. Examining the expression patterns of large gene families, we find that they are often more similar than expected by chance, indicating that many gene families have been co-opted for specific developmental processes. Also, physical location along the chromosomes affects gene activity. Using sliding window analysis, we find that genes in close proximity are much more likely to be co-expressed than expected by chance. There are a few small regions of very high correlation, which in most cases correspond to tandem duplications. Tandem duplications do not, however, explain all of the co-expression, since we find significant correlation even when tandem-duplications are excluded from the analysis. The dataset is a part of the AtGenExpress expression atlas, which aims at giving the community access to a large micro array dataset. All data is being made publicly available through several websites.

636 Expression profiling using Arabidopsis whole-genome regulatory gene oligo DNA microarray and production of Arabidopsis DNABook containing about 1000 RAFL cDNAs for transcription factors

Motoaki Seki[1], Junko Ishida[1], Kei Iida[1], Maiko Nakajima[1], Akiko Enju[1], Tetsuya Sakurai[2], Ayako Kamei[1], Youko Oono[1], Taishi Umezawa[3], Miki Fujita[4], Saho Mizukado[4], Taeko Morosawa[1], Kenji Akiyama[2], Yoshihiro Narusaka[5], Mari Narusaka[5], Mitiko Go[6], Masatomo Kobayashi[7], Jun Kawai[8], Yoshihide Hayashizaki[8], Kazuo Shinozaki[2]

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Regulatory genes, such as transcription factors, protein kinases and F-box proteins play critical roles in all aspect's of higher plant's life cycle. Although extensive studies have been carried out for functional analysis of individual regulatory genes, the function of only a small fraction of these regulatory genes has been revealed so far. It is valuable to learn how the regulatory genes are expressed and regulated at the whole-genome scale. Therefore, we prepared *Arabidopsis* whole-genome regulatory gene oligo DNA microarray containing all transcription factors, protein kinase and F-box protein genes in *Arabidopsis* genome recently. The 60-mer oligo DNA corresponding to 1979 transcription factors, 1060 protein kinase and 551 F-box protein genes are spotted on slide glass as a custom array of Agilent Co. In this meeting, we present expression profiles of regulatory genes in various stress and hormone treatments, and various plant tissues using the *Arabidopsis* regulatory gene oligo DNA microarray. We also produced *Arabidopsis* DNABook™ containing about 1000 RIKEN *Arabidopsis* Full-Length (RAFL) cDNAs for transcription factors (http://pfgweb.gsc.riken.jp/DNA-Book/). The DNABook™ offers the following advantages for delivering DNA: (1) simultaneous delivery of scientific information and associated DNA clones; (2) a cheap method of DNA distribution; (3) easy extraction of DNA by PCR; (4) stable preservation in the laboratory environment.

637 Systematic RNAi in Arabidopsis: the AGRIKOLA project

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The completion of the Arabidopsis genome sequence has provided an extremely important resource for scientists interested in comparative genomics. However, experimental proof of the function of less than 20% of the 27000 genes predicted by the Arabidopsis Genome Initiative has been obtained so far. Reverse genetics approaches give valuable information on gene function, but in plants, until recently, there was no feasible way of systematically making targeted gene-knockouts on a large scale. The situation changed when Peter Waterhouse (CSIRO, Canberra) developed GATEWAY®-based vectors for expressing hairpin RNA, a potent inducer of RNAi in plants. The EU-funded AGRIKOLA project is generating a genome-wide resource for reverse genetics in Arabidopsis by constructing 40000-50000 plasmids each designed to specifically induce gene-silencing by RNAi of a single target gene, either inducibly or constitutively. The project is employing gene-specific tags generated in the CATMA microarray consortium to produce hairpin RNA constructs. So far, over 20000 constitutive RNAi constructs have been generated and over 1500 of these introduced into Arabidopsis. Numerous transformed lines exhibiting potentiel knock-down phenotypes have been observed and are being characterized. The project will provide an unparalleled resource for Arabidopsis reverse genetics, allowing researchers to quickly and easily produce mutants at will in almost any Arabidopsis gene. The AGRIKOLA resources will be distributed by NASC from summer 2005 onwards.

AGRIKOLA : http://www.agrikola.org/

638 An Integrated Platform for Rapid Gene Expression Analysis by RT-PCR

Keming Song, Derek Douglas, Carol Kreader

Sigma-Aldrich

Scientists at Sigma-Aldrich have developed an integrated system that combines rapid RNA isolation with optimized RT-PCR reagents enabling medium to high throughput gene expression analysis using small amounts of plant materials. The system consists of three parts: 1) a proprietary lysis buffer that efficiently releases RNA from partially homogenized plant tissues processed without grinding in liquid nitrogen; 2) a high density oligo-dT coated PCR plate that captures Poly A+ RNA in presence of lysis buffer; and 3) two one-step RT-PCR mixes for conventional and real time RT-PCR, respectively. Using this system, 24 to 96 Poly A+ RNA samples from 10 – 100 mg plant tissue/sample can be isolated in 1 –1.5 hour and then RT-PCR can be directly conducted in the capture plate. The RNA isolated using the system has high purity and the amount recovered per well is sufficient for10 - 25 RT-PCR reactions, depending upon abundance of the target transcript. The system has been extensively tested using multiple plant species and multiple genes. The method is rapid, sensitive, convenient, and cost-effective.

639 A new TAP system for isolation of plant protein complexes and subsequent Mass-Spec analysis

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Tandem Affinity Purification (TAP) is becoming accepted as a preferred strategy for isolation of protein complexes and for analysis of protein-protein interactions. TAP technology involves incorporation of dual affinity tags into a known protein to pull down unknown endogenous proteins. The whole process involves a two-step purification scheme so that the protein complexes isolated usually have much higher purity compared to single purification and thus could be subjected to direct analysis using mass spectrometry. Existing TAP systems, used in mammalian and yeast, are not suitable for plants, mainly due to their poor specificity. Here, we report a new TAP system that incorporates a unique combination of FLAG and HA affinity tags in the bait protein to facilitate isolation of plant protein complexes. Major advantages for this system include: 1) short peptide sequences of FLAG-HA tags, which minimize interference to protein function; 2) superior specificity of the affinity tag-antibody combinations for a variety of plant species; and 3) mass spec compatible elution conditions without protease digestion or using EGTA. This new TAP system has been characterized using known protein-protein interaction models from Arabidopsis. The results indicated that the new TAP technology can efficiently isolate protein complexes with much less non-target protein contamination compared to other affinity tagging systems (e.g. calmodulin, Protein A, GST, 6xHis, streptavidin, etc.) and the protein complexes isolated are suitable for direct Mass spec analysis. In addition to the TAP protein purification system, we have also developed a novel universal cloning system that enables researchers to clone FLAG-HA tandem-tagged genes into any plant vector for bait protein expression.

640 A Novel Differential Expression Technology for Gene Discovery and Expression Profiling

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Sigma-Aldrich, Biotechnology Division

Scientists at Sigma-Aldrich have developed a novel differential expression system as a simple and effective tool for gene discovery and expression profiling. The system is a RT-PCR based differential display method using a proprietary ACP (Annealing Control Primer) technology. The ACP technology features a set of specially designed primers that comprise a tripartite structure with a polydeoxyinosine linker between the 3'end target core sequence and the 5' end non-target universal sequence. This unique structure prevents annealing of the 5' end non-target sequence to the template and thus facilitates primer hybridization at the 3' end to the target sequence at specific temperatures, resulting in a dramatic improvement of annealing specificity. Compared to existing differential display methods, the new system provides superior reproducibility and sensitivity. The amplicons produced by this new method can be displayed on regular agarose gel, making it easy for band excision, purification, and sequencing. Also, the whole process can be carried out in a high throughput fashion. We have conducted extensive validation of the ACP technology in Arabidopsis, corn, soybean, and mammalian systems. Comparison between our data with the data from microarray and/or real time RT-PCR indicated that the new system is a simple, reliable, and cost-effective tool for gene discovery and expression profiling, particularly useful for plant and animal species where genome sequence information is often limited.

641 Computational analysis of seedling development phenotypes demonstrated on Arabidopsis seedlings undergoing de-etiolation and gravitropism

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A computer algorithm for measuring changes in length and shape of Arabidopsis seedlings with high spatiotemporal resolution was developed for the purpose of quantifying the effects of mutations on the growth and curvature of stems and roots during development. The algorithm operates on a time series of electronic images of seedlings undergoing responses to light or gravity. An edge-finding routine defines the perimeter of the subject (root or hypocotyl). For each point within the subject, Delaunay triangulation finds the closest perimeter point and the distance between the two is determined by a distance transform. The midline of the subject is defined by the coordinates of the distance transform maxima. They are identified within a semicircular evaluating window that processes towards the local maximum to obtain the next midline point, iteratively until the end of the subject is reached. Each image in the time series is similarly analyzed and the sequential midlines stacked to produce an ordered coordinate point set describing the subject's spatiotemporal response surface. Length and curvature measurements are calculated from least squares bivariate tensor product splines fit to this surface. Seedlings undergoing de-etiolation and roots undergoing gravitropism were analyzed with the algorithm. Time courses of light-induced changes in hypocotyl growth rate are resolved at the level of microns per minute over time periods of many hours. When and where light-stimulated apical hook opening or gravitropic bending occurs was calculated and displayed on colored x-y sheet plots. Integrating the curvature values over the organ's midline produces plots of total angle versus time. The shoots of *cry1* mutants responding to blue light were found to the escape from inhibition after 30 min and grow at rate much faster than wild type for several more hours as previously found by laborious manual methods. A previously unknown negative effect of the *cry2* mutation on apical hook opening was found. Roots of *mdr1* and *mdr4* mutants (defective in acropetal and basipetal auxin transport, respectively) were analyzed as they responded to a gravitropic stimulus. An 80% reduction in acropetal transport did not affect gravitropism but reduced basipetal transport in the *mdr4* mutant was associated with faster gravitropism driven by curvature developing in regions different than the wild-type root. Prospects for high-throughput computation-based phenotyping will be discussed.

642 Development of Arabidopsis whole-genome arrays and their utilization for chromatin immunoprecipitation studies

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We have developed two long-oligonucleotide microarrays that will be useful for the analysis of genome features in Arabidopsis thaliana, in particular the high throughput identification of transcription factor binding sites. The first platform contains 190,000 probes representing the 2kb-regions upstream of all annotated genes at a density of seven oligonucleotides per promoter. The second platform is divided into three chips, each of over 390,000 features, and represents the entire Arabidopsis genome at an average density of one oligonucleotide per 91 bases.

In order to test the utility of the array for the discovery of transcription factor binding sites, cross-linked chromatin from plants treated with salicylic acid for 2 hours was immunoprecipitated with antibodies against TGA2. The immunoprecipitated DNA (ChIP) along with a raw (non-IP) chromatin sample were reverse cross-linked, randomly amplified, and hybridized to the arrays. The TGA transcription factors play an important role in the onset of systematic acquired resistance (SAR) in plants. However, the TGACG box of the gene Pathogenesis-Related 1 (PR1) promoter is the only confirmed binding site of these factors in Arabidopsis. High signal ratios of the ChIP vs. non-IP samples in 51 distinct regions of the genome provided evidence for putative TGA2 binding sites. The presence of the PR1 promoter among the regions enriched in the ChIP sample validated the utility of the arrays. Motif scanning revealed that 7 to 10mer motifs containing the TGACG box are significantly over-represented in the enriched regions. The palindromic octamer TGACGTCA, in particular, was found in 54% of these. Interestingly, eighteen of the regions enriched in the ChIP sample lie outside of presumptive promoter regions.

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643 Reverse Breeding

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Plant breeding operates through iterative cycles of recombination and selection in order to produce elite varieties optimally adapted to local growing conditions. Varieties of many crops are produced as F1-hybrids which are developed by an initial phase of line selection followed by a phase in which experimental hybrids, are tested for their agricultural or horticultural value. Technologies which enable a more efficient operation of the plant breeding cycle are of considerable interest for the commercial plant breeder. As the understanding of meiotic recombination at the molecular level rapidly increases, breeding tools will be developed which ultimately allow to control this process. We are exploring downregulation of meiotic recombination to develop a concept termed reverse breeding. Reverse breeding is a process in which the order of events leading to the production of a hybrid plant variety is essentially reversed. Initially, individual heterozygous plants are selected for their qualities and, subsequently, lines are derived from these plants which upon hybridisation reconstitute the original genetic composition of the selected plant from which the lines are derived. This process is pursued through downregulation of genes like DMC1 and SPO11 through RNAi, combined with doubled haploid technology. RNAi constructs are currently transformed to both model as well as crop species to determine their efficacy in suppressing meiotic recombination. This novel plant breeding approach offers clear advantages over existing methods due to the fact that in principle any heterozygous plant can now be commercially exploited through re-synthesis of its genetic constitution by the formation of an F1-hybrid.

644 A systematic RNAi screen for functional genomic analysis in Arabidopsis thaliana

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AGRIKOLA is a large scale European project aiming for genome-wide determination of gene function by generating hairpinRNA constructs for post transcriptional gene silencing of every Arabidopsis gene (see also related posters). As a proof of principle we are using a subset of these constructs for transformation into Arabidopsis thaliana, recording their phenotypes and creating a seed-collection of T2 RNAi lines. Here we describe the range and frequency of Rnai knock down phenotypes obtained with more than 1000 constructs targeting random genes many of which have unknown functions. To test the efficacy and specifity of our RNAi constructs we analysed the knock down levels of functionally described genes at the RNA and the protein level. We find that high efficacy of gene silencing producing phenotypes identical to knock out mutants can be obtained for non-essential genes, while various degrees of intermediate knock down levels are obtained with genes whose function is essential for plant growth and development. Our results show that our initiated RNAi-based screen is an efficient and powerful tool for functional genomic analysis in plants, which allows also the study of genes for which knock out lines are not available.

645 Gene trapping of the Arabidopsis genome with a firefly luciferase reporter

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Gene trapping has been developed to monitor gene expression profiles by random insertion of reporter genes into the plant genome. Addition to expression profiles, it also provides knockout mutations of genes for functional analysis. Experiments with gene-trap vectors containing the firefly luciferase (*LUC*) reporter genes were carried out with the aim of analyzing functions of the Arabidopsis genome. Studies with protein fusion-type trap vectors as well as an internal ribosome entry site (IRES)-assisted non-fusion-type vector revealed that both types of vectors were suitable for gene trapping in Arabidopsis. The established trap lines were subjected to analyses for environmental responses, demonstrating the powerful and unique applications of the *LUC*-trapping system. A systematic survey of the insertion sites of the T-DNAs in *LUC*-expressing lines revealed that IRES type trapping vector has highest trapping efficiency (41%), although traditional fusion-type vectors showed 12 - 20% gene-trapping efficiencies. Hence, we are planning large scale preparation of the IRES type-trap lines for functional genomics.

646 Preliminary Findings from a Proteomic Characterization of the Auxin Response in *Arabidopsis thaliana* using ^{15}N-Metabolic Labeling

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Auxin, the first plant hormone described, has been shown to be a factor regulating numerous plant behaviors involving cellular elongation and differentiation including gravitropism and phototropism. While many of its physiological effects have been well characterized, numerous aspects of the auxin response remain poorly defined at the molecular level. We have initiated a global proteomic characterization of the auxin response in *Arabidopsis* that allows differential quantification of proteins from seedlings that are either treated (1-napthalene acetic acid for 60 minutes) or untreated with exogenous auxin. By incorporating a universal stable isotopic label into either the experimental or control seedling populations, one can extract chemically identical peptide species through multiple purification steps that may be ultimately differentiated by their masses using tandem mass spectrometry. Labeling is accomplished *in vivo* by cultivating plants on defined media containing nitrate that is either natural abundance (mostly ^{14}N) or ^{15}N. This practice is commonly referred to as 'metabolic labeling' because it utilizes an organism's metabolism to incorporate the label, in contrast to strategies that employ chemical or enzymatic labeling that are typically accomplished *in vitro*. Based on observed isotope distributions, we achieved greater than 98% enrichment of ^{15}N in labeled samples after ten days growth. Through our initial round of data analysis we have identified close to 1,500 soluble fraction proteins that are expressed under control and experimental conditions. By comparing signal intensities between labeled and unlabeled peptides we have calculated relative levels of protein expression and/or abundance in sub-cellular fractions. Very few of the proteins show changes greater than two-fold in the soluble fraction. We do see a larger increase in soluble actin isoforms with auxin treatment that may be consistent with expected cytoskeletal re-arraignments. Other proteins putatively identified as changing upon auxin treatment include one histone deacetylase isoform, an 'ubiquitin carboxyl-terminal related protein' and several others. We are currently preparing samples from the organellar and microsomal fractions to extend our analysis.

647 Changes in the proteome of Arabidopsis during shoot development in tissue culture

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An analysis of the changes in the proteome during shoot development in tissue culture was conducted using the 2-Dimensional Difference In Gel Electrophoresis (DIGE) system. In *Arabidopsis*, shoots can be induced in tissue culture by preincubating root explants on an auxin-rich callus induction medium (CIM) and then by transferring explants to a cytokinin-rich shoot induction medium (SIM), during which time explants become committed to shoot formation and ultimately form shoots. To capture various landmark events, proteins were sampled in three replicates at various time points during the course of development. DIGE utilizes three Cy dye fluors permitting one to multiplex or run two samples on each gel along with an internal standard (pool of all the samples within an experiment). Protein samples were labeled with either Cy3 or Cy5, and the internal standard labeled with Cy2 allowing for reliable inter-gel comparison and quantification. The samples were grouped and analyzed following a loop design. Protein levels were profiled using DECYDER software and a mixed linear model. The mixed linear model took into account time effect, dye effect, time and dye interaction, random replication, random gel effect and replication and time interaction. For significant spots with respect to overall time effect, pairwise comparison of the protein levels were conducted for adjusted time points. The results were also compared to the results obtained from an analysis of the transcriptome of this time course.

648 A systematic proteomic approach to study early phases of root gravitropism in Arabidopsis thaliana

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Proteomic studies exploring global patterns of protein expression in plants have proliferated rapidly within the past few years following completion of the sequencing of the Arabidopsis genome. Although most of these studies have involved a characterization of the proteins present in specific tissues or organs, comparative proteomic experiments also led to the identification of proteins that change in abundance in response to treatment. In this study, a proteomic approach was used to identify Arabidopsis root tip proteins whose abundance, localization or modification changes early in response to gravistimulation. Root tips were excized from control, mechano and gravistimulated plants, and then proteins were extracted using a sequential extraction technique, then separated by two-dimensional gel electrophoresis. More than 2100 proteins spots could be reproducibly resolved and detected by silver staining. PDQuest analysis of at least 6 biological replicates showed that 56 proteins spots significantly changed in relative intensity upon mechano or gravistimulation. Of these, 51 were shown to change in intensity specifically in response to gravistimulation. Most of these protein spots were extracted and subjected to nano-LC-MS/MS or MALDI-TOF MS analysis. Using a Mascot search engine, we were able to identify 40 gravity regulated proteins. The corresponding genes were identified, and their expression is being analyzed. GFP translational fusions to the corresponding genes are being generated to analyze protein localization and responses to gravistimulaton in transgenic plants. Mutants are being analyzed to understand the role of these proteins in gravity signal transduction within the root apex. A summary of the identified proteins and their major functional categories will be presented.

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649 Withdrawn

650 Resources Available to Arabidopsis Researchers Through the Center for Eukaryotic Structural Genomics (CESG)

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CESG (http://www.uwstructuralgenomics.org/) is a member of the Protein Structure Initiative (PSI), an NIH funded project with the goal to develop new methodologies to dramatically reduce the cost and time required to determine three-dimensional protein structures. CESG has built high throughput cloning, expression, purification and structure determination pipelines for both *E. coli* cell-based and wheat germ cell-free expression systems. Over the past 4 years CESG has selected over 2700 ORFs from the annotated *Arabidopsis* genome, focusing primarily on targets for which 3-D structure information is not available. This effort has yielded a large number of constructs and products. These resources are freely available to the scientific community and include:

- Over 1500 GatewayT entry ORF clones (note that a TEV protease cleavage site has been engineered between the upstream recombination site and the 5' end of the target)
- GatewayT-based protein expression clones for most of the above entry clones (in most cases including cleavable His tag and MBP fusion sequences)
- Small amounts of purified and cleaved protein (not all targets have protein available)
- Over 40 3-D structures of *Arabidopsis* proteins (available through the Protein Data Bank (PDB))
- Optimized protocols for producing recombinant Arabidopsis protein in both cell-based and cell-free systems

A full list of available clones and proteins will be presented along with a summary of the performance of each selected target in the CESG pipeline.

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651 A subproteomic approach to apoplastic proteins involved in cell-wall regeneration in protoplasts of suspension cultured cells of Arabidopsis

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The plant cell wall is a dynamic structure that plays a critical role not only in determining cell shape and formation of the plant body, but also in interactions with environmental factors including those required for nutrition, response to abiotic stress and biological attack by other organisms. It is made of various types of macromolecules, and undergoes extensive construction and remodeling during cell division and differentiation.

To clarify the mechanisms of construction and modification process of the cell wall, we used a proteomic approach to investigate the proteins secreted into cell wall spaces during cell wall regeneration from the protoplasts of Arabidopsis suspension cultured cells Alex. We focused on cell wall proteins loosely bound to the cell wall architecture and extractable with 1 M KCl solutions from: (a) native suspension cultured cells, (b) protoplasts that had been allowed to regenerate their cell walls for 1 h, and (c) protoplasts allowed to regenerate their cell walls for 3 h. We adopted a non-destructive extraction procedure without disrupting cellular integrity, thereby avoiding contamination from cytoplasmic proteins. Using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and matrix-assisted laser desorption ionization-time-of-flight/mass spectrometry (MALDI-TOF/MS), we separated, mapped, and identified 71 proteins derived from the native cell wall, and 175 and 212 proteins derived from the 1 h and 3 h regenerated protoplasts, respectively. Quite different sets of proteins with differing status of their post-translational modifications, including phosphorylation and glycosylation, were identified in the three protein fractions. This indicated dynamic in muro changes in the cell wall proteins during cell wall regeneration in the protoplasts. The analysis revealed not only a set of enzymes involved in cell wall expansion and construction, but proteins possibly involved in defense and extracellular signaling.

Furthermore, This approach has also determined a set of cell wall proteins that had not been predicted to be localized in cell wall spaces.

Kwon, H-K., Yokoyama,R. and Nishitani K.(2005) A proteomic approach to apoplastic proteins involved in cell wall regeneration in protoplasts of Arabidopsis suspension cultured cell. Plant Cell Physiol (in press). Correspondence: nishitan@mail.tains.tohoku.ac.jp

652 An Overview of Arabidopsis Structural Genomics at the Center for Eukaryotic Structural Genomics (CESG)

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CESG was founded to develop high throughput and cost effective methods to determine the structures of eukaryotic proteins. CESG is a coordinated effort of 9 centers funded by NIH, and seeks to sample protein fold space to determine structure/function relationships and facilitate sequence/fold predictions. Initially focused on *Arabidopsis* proteins, since a high quality, thoroughly annotated genomic sequence was available, and a large percentage of the sequences are 'pioneer,' with no known clues on their function. Using semi-automated cloning methods we've cloned over 1500 *Arabidopsis* ORFs in Gateway entry vectors. These have been expressed in *E. coli* using custom-made expression vectors with amino-terminal tags to facilitate soluble expression, visualization, and purification of recombinant protein. *E. coli* strains have been developed to express these proteins in an inducing medium that also efficiently incorporates selenomethionine into the polypeptide chain to aid in X-ray diffraction analysis or ^{15}N followed by $^{15}N+^{13}C$ for NMR analyses. To date we have purified over 250 targets through semi-automated methods at average yields of more than 10 mg. In a more recently developed parallel pathway, *Arabidopsis* ORFs are expressed in a wheat germ cell free system. Currently, yields are sufficient only for NMR analyses, but we are testing chip-based crystallization technology that should enable small scale (100 ug) crystallization screening for X-ray analysis. Cell free translation reactions are automated using screening and preparative scale robotic platforms.

Up to 192 crystallization conditions are set and screened by a robot, and another robotic system optimizes protein crystallization conditions. Synchrotron data from well diffracting crystals are collected at Argonne National Laboratory. NMR data are collected at the National Magnetic Resonance Facility at Madison (NMRFAM) and the Medical College of Wisconsin (MCW).

CESG has solved the structures of 40 *Arabidopsis* proteins by NMR and X-ray crystallography. Most of these proteins were of unknown function. In several cases the structure suggested a biological or biochemical function. As a pilot study to establish the function of some of the unknown proteins CESG, in collaboration with Aled Edwards at the University of Toronto, has screened 82 targets with 14 showing esterase activity, 6 phosphodiesterase activity, and 3 phosphatase activity. Further case studies will be presented.

CESG is supported by the National Institute of General Medical Sciences Protein Structure Initiative (P50 GM64598).

653 Natural variation in *Arabidopsis thaliana* populations in response to differential availability of soil nutrients

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The evolutionary process that may lead to adaptation can be viewed as a dynamic interplay between selection and the genetic architecture of fitness traits. Over time, selection can erode genetic diversity, which can lead to local adaptation. However, selection in heterogeneous environments has been proposed to facilitate the maintenance of genetic variation. Adaptation to heterogeneous environments can also be achieved through phenotypic plasticity. We have recently started a series of experiments using wild populations of *Arabidopsis thaliana* to experimentally examine this dynamic between selection in heterogeneous soil nutrient environments and the species' genetic characteristics in a population. The goal of this research is to determine if and how selection history (soil nutrient environment) influences the genetic architecture of a population. We predict that more heterogeneous environments lead to populations of greater genetic diversity and greater expression of phenotypic plasticity. Furthermore, traits under relatively strong selection and having weak phenotypic correlations with other traits will show a stronger response to the local soil conditions. Here, I will report on the results of the first of these studies where plants from 11 populations from Michigan were grown in low or high soil nutrients (range based on natural sites). The population sites differed in their mean availability of N, P and K in addition to the extent of within-site heterogeneity found in these nutrients. Traits representing size, life history and fitness were measured throughout the life cycle on 10 genetic lines/population for 11 populations in the 2 nutrient environments. The nutrient environment significantly affected most of the traits. The populations, as well as the genetic lines within each population also significantly contributed to the variation for many of the traits. In a more limited set of traits, a significant expression of phenotypic plasticity was found. A limited but significant influence of selection history was found in reproductive traits. Thus selection history may structure the genetic architecture of a population. This effect of the selection history on genetic architecture has the potential to limit or facilitate the ability to adapt to different or changing environments. To further understand the genetic basis of these traits which may determine their response to selection, we have just completed growing recombinant inbred lines in several nutrient environments to allow for QTL analysis. Results from this analysis will also be presented.

654 Toward the cloning of nitrogen use efficiency related QTLs in Arabidopsis thaliana

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A better understanding of the mechanisms regulating nitrogen (N) metabolism is required to improve crops nitrogen use efficiency. *Arabidopsis thaliana* natural variation can be exploited to identify regulatory genes controlling N metabolism, which have not been identified so far through classical mutant approaches. Previous studies led us to the identification of a large number of QTLs (quantitative trait loci) for N, nitrate, free amino acids (AA), dry matter, water, anions and sugars contents, under two contrasting N environment (low/high nitrate concentration) in a Bay-0 (B) x Shahdara (S) F_6 progeny. We focused on four QTLs with major effects (14 - 48 %), each displaying different effects according to the N environment : two QTLs for free AA content (AA10.2 and AA3.4), one QTL for sulfate content (SO3.1), and one QTL for fructose content (FR3.4). For each QTL, F_6 lines displaying a residual heterozygosity only at the QTL were found and selfed to produce heterogeneous inbred families (HIFs). F_7 lines fixed for either the B or the S phenotype at the QTL but with otherwise stricly identical genetic backgrounds could thus be compared to confirm each QTL. The APR2 gene, presumably involved in sulfate reduction in *A. thaliana*, was chosen as a candidate gene for QTL SO3.1. The dominant B allele at this QTL was cloned and introduced in a S fixed HIF. Analyses are underway to determine whether transformants display B or S phenotype. A fine-mapping strategy was applied for the three other QTLs. Large HIF populations (400-900 individuals) were screened for recombinants inside the regions surrounding QTLs, and their phenotypes compared. QTL AA10.2 in particular could be assigned to a 600 kb genomic region, i.e. 1/10th of its original length, after a first recombinant screening. Our results demonstrate that HIFs are an adequate material for QTL fine-mapping. HIF use should quickly lead us to the isolation of genes underlying nitrogen use efficiency related QTLs in *A. thaliana*.

655 Progress toward the Cloning of Carotenoid QTLs on Arabidopsis Chromosome 4

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Carotenoids are a diverse group of pigments that are widely distributed in nature and present in all photosynthetic organisms. Carotenoids are synthesized and accumulated in plastids and play crucial roles in light harvesting, photoprotection and as precursors for abscisic acid (ABA) synthesis. In humans, carotenoids are essential components of the diet as a source of provitamin A and also play roles in decreasing human age-related-macular degeneration, some cancers and heart disease. Given the importance of carotenoids to agriculture and human health many groups have targeted the pathway for engineering. To complement these approaches we are studying the molecular basis of untapped natural variation between Arabidopsis ecotypes for seed carotenoid content and composition using quantitative trait loci (QTL) analysis. By this approach we hope to identify and clone novel genes or alternate alleles of known biosynthetic genes that positively impact seed carotenoid content. Using 100 recombinant inbred lines derived from a cross of Arabidopsis Columbia (Col) and Landsberg erecta (Ler) ecotypes and quantitative HPLC analysis, we identified four distinct QTLs that influence seed carotenoid levels in Arabidopsis. A QTL region (Ler) on chromosome 4 had the greatest impact on seed lutein and violaxanthin levels. To map this QTL, a chromosome substitution strain (CSS) containing chromosome 4 of Ler in the Col background was crossed to Col and F2s with recombination events across the interval were identified. Seed of homozygous recombinants were analyzed by HPLC and used to delineate the one chromosome 4 QTL to a 1.0 Mb interval and are currently fine mapping this interval. A second more complex QTL region is also present on chromosome 4.

656 Characterization of a light signaling quantitative trait locus in Arabidopsis

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LIGHT1 is a major effect quantitative trait locus (QTL) detected in all light environments controlling hypocotyl elongation in Arabidopsis. In white light, it explains 21 percent of the phenotypic variance between the Landsberg *errecta* (L*er*) and Cape Verde Island (Cvi) accessions. It is inherited semi-dominantly with the Cvi allele conferring elongated hypocotyls under all light conditions tested. Fine mapping of *LIGHT1* with near isogenic lines (NILs) reduced the candidate region to about 135 kb comprising 34 genes. We confirmed that there was only this region from Cvi present in the L*er* background of NIL189 using DNA hybridization to Affymetrix ATH1 arrays. In this interval there are no known light signaling, or previously characterized genes. To identify candidate genes, we extracted triplicate RNA samples at midday from 5 day-old seedlings of either L*er* or NIL189, and hybridized them to ATH1 arrays. Of the 67 genes that were significantly differentially expressed between NIL189 and L*er*, one gene fell in the *LIGHT1* interval. We named this gene *UNCHARACTERIZED LIGHT SIGNALING* (*ULS*) since this single copy gene, which is conserved in other plant species, encodes an uncharacterized molecular function. Expression of *ULS* decreased in NIL189 compared to L*er*. *ULS* is also the only gene in the *LIGHT1* interval that is circadian regulated. Among the other 66 genes, the gene encoding the DET1 interactor DDB1B is the gene with the strongest change in expression, suggesting *LIGHT1* may play a role in the DET1 pathway. We also tested homozygous T-DNA insertions of 22/34 genes in the *LIGHT1* interval, and coincidently *uls* was the only mutant to display an interesting light-related phenotype. There is only a single nucleotide difference in the coding sequence of *ULS* between L*er* and Cvi, causing a non-synonymous amino acid substitution, with the Cvi sequence at this position being the same as that of the Columbia (Col) reference strain. The Col and Cvi amino acid is conserved in both tomato and potato, while the L*er* amino acid change is found as a medium frequency allele in *Arabidopsis thaliana* accessions (10/96). Multiple lines of evidence thus suggest that *ULS* is responsible for the *LIGHT1* QTL. Quantitative complementation experiments for further confirmation are in progress.

657 Mapping Quantitative Trait Loci Modifying the *revoluta* Mutant Phenotype

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The Revoluta- (Rev-) phenotype is markedly suppressed in the Landsberg *erecta* background relative to that in the Columbia background, and this suppression is due to the effects of multiple loci (Otsuga, et al. 2001). To determine whether changes in the related HD-Zip III loci are responsible, in part, for the phenotypic differences and to identify additional loci involved in the pathways regulated by *REV*, we have mapped the Rev- modifier (*MUFFLER*) loci using QTL analysis. F_2 progeny and F_3 families were scored for several traits (frequencies of aberrant flowers and barren cauline leaf axils, numbers of axillary inflorescences, and leaf pigmentation), and linkage map was constructed. The Rev- phenotype was significantly affected by nlne loci, with the effects of five being highly signicant. Although the largest effect QTL mapped near one of the homologous genes, fine-scale mapping efforts for each of the highly significant QTLs are ongoing.

D. Otsuga, B. DeGuzman, M. J. Prigge, G. N. Drews, and S. E. Clark. 2001. *REVOLUTA* regulates meristem initiation at lateral positions. Plant Journal 25:223-236.

658 Genomic Architecture of Variation in Gene Expression in Bay-0 x Shahdara RILs

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Many naturally variable traits exhibit a quantitative phenotypic distribution, which is determined by factors including the number of loci controlling the trait, the importance of cis- versus trans-acting factors, and the degree to which epistasis contributes to the phenotype. Long-standing goals have been to characterize the genomic architecture controlling quantitative traits and to identify the genes that underlie this variation. This requires the ability to reliably measure numerous traits on the same segregating population that also exhibits segregation of candidate genes. The Affymetrix ATH1 microarray allows the simultaneous measurement of gene expression across the entire genome, thus enabling characterization of the genetic architecture of variation in gene expression in Arabidopsis. In addition, an increasing number of Arabidopsis QTLs are being found to be controlled by differential gene expression. Therefore, expression QTL (eQTL) analysis will also provide a rich set of candidate genes to facilitate QTL cloning.

We used the ATH1 microarray to measure gene expression in Bay-0, Shahdara and 150 RILs from a Bay x Sha RIL cross in the presence and absence of exogenously applied salicylic acid with two independent biological replicates per RIL per treatment. The microarrays were used to simultaneously genotype and phenotype the RILs. We will use a high-density map (38 microsatellites and > 100 expression level polymorphisms, ELPs) to conduct composite interval mapping on ~1,700 genes that showed significantly different expression between the Bay-0 and Shahdara parents. We hypothesize that an analysis of ELPs with the largest significant differences will show that the majority are determined by single loci that also map to the same position as the gene exhibiting the ELP. We are also estimating the number of ELPs controlled by two or more eQTLs (cis- and trans-acting) and the proportion of phenotypic variation explained by each.

659 eXtreme array fine mapping of QTL involved in Arabidopsis red light response

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Genetic analysis of natural variation among accessions of Arabidopsis thaliana can facilitate the discovery of new genes or allelic variants of previously identified genes controlling traits that may be important for adaptation to the environment. Wide variation in the photomorphogenic responses exist among different Arabidopsis accessions that correlates with latitude of origin. Using composite interval mapping and eXtreme Array Mapping (XAM), two quantitative trait loci (QTL) responsible for hypocotyl length under red light were identified in recombinant inbred lines (RILs) derived from the Columbia and Kashmir accessions. These RED2 and RED5 QTL were verified in progeny of specific RILs segregating for these two loci. Fine mapping is proceeding by first identifying recombinants across the QTL region from a collection of thousands of F2 seedling descendants from these RILs. Phenotypic analysis of recombinant progeny allows pooling according to QTL allelic state. The fine recombination events are assayed on high density oligonucleotide arrays to position the QTL in a very small interval. This method called eXtrememem Array Fine Mapping to resolve QTL is presented

660 Metabolite profile-based analysis of the effects of methionine sulfoximine on nitrogen assimilation and metabolism in Arabidopsis

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Nitrogen uptake, assimilation and utilization are important components of plant productivity. Glutamine Synthetase (GS) is the primary route for assimilation and recycling of ammonium in plants and its activity is severely inhibited by an analog of glutamate, methionine sulfoximine (MSX). The primary effect of GS inhibition is the accumulation of ammonium to toxic levels, however, the effect of MSX-induced reduction of GS activity on other metabolic pathways has not been well described. Metabolic profiling was used to investigate the inhibitory effects of MSX on primary nitrogen assimilation by GS and on downstream metabolism. WS plants were grown hydroponically and treated with two different concentrations of MSX. For each treatment, nitrate, ammonium, amino acids, and organic acids were analyzed. The results of metabolite profiling analysis on MSX-treated plants have yielded a better understanding of the effects of MSX in the nitrogen metabolism of Arabidopsis. A number of metabolic changes occur in response to MSX treatment. Components of the glutamate synthase cycle were clearly affected, as was the oxidative carbon (C2) pathway. Glycolysis intermediates and the citric acid cycle were found to increase, as were some amino acid groups. Levels of amino acids in the aspartate pathway, aromatic amino acids, and branched chain amino acids were seen to increase, while others remained stable. The responses of these groups of metabolites may be used as a signature for wild-type response to MSX treatment. Together, these signature metabolite trends might be used to screen for plants with altered nitrogen metabolism, and potentially for MSX resistance and sensitivity.

661 NARC - Norwegian Arabidopsis Research Centre - University of Oslo

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NARC is a national technology platform, sponsored by the Norwegian Functional Genomics initiative (FUGE), that will offer Norwegian plant scientists resources and technology in the *Arabidopsis thaliana* model system. FUGE is the result of an initiative taken by the Norwegian research establishment; the underlying process has been supported by the Research Council of Norway. FUGE represents a cooperative effort between Norway's universities and research institutions and the industrial sector. NARC will establish a solid basis for basic plant molecular biology, applied plant research and plant breeding in Norway. The centre partners provide the basic technologies to utilize the powerful tools of comparative genomics and thereby to promote the quality of plant science in Norway. The establishment of NARC will result in more efficient use of resources and equipment available in the Norwegian plant science community. By taking advantage of the tools already available, gene function and interaction of gene products can be studied in great detail. NARC is built on existing competence in *Arabidopsis* research at the Norwegian University of Science and Technology (NTNU), University of Oslo and the Norwegian University of Life Sciences (UMB), and has a close collaboration with other FUGE resource centres, e.g. The Norwegian Micro Array Consortium, imaging and proteomics facilities and bioinformatics expertise centres. At NARC - University of Oslo we focus on **In situ hybridisation** for cellular localisation and expression analysis of specific transcripts, and **Yeast two-hybrid analysis** for protein-protein interactions and identification of binding domains and interacting/assoaciated protein partners.

662 Histone deacetylase expression levels during life cycle of *Arabidopsis thaliana*

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Acetylation of chromatin plays an important role in the growth and development of eukaryotic organisms. Although the mechanism of altering chromatin structure is not understood, acetylation/deacetylation of chromatin has been implicated in developmental processes in Arabidopsis. The Arabidopsis genome contains 16 histone deacetylase (HDACs) genes. HDA19, HDA3 (AtHD2a) have been implicated in the transition from vegetative to reproductive development and embryogenesis respectively. The expression patterns of the remaining genes have not yet been investigated. Through semi-quantitative RT-PCR, the expression levels of all HDAC genes will be investigated throughout the life-cycle of the plant. Developmental series will include: leaf development in juvenile and adult plants, floral development, silique/seed development and a germination series. Marker genes whose expression is unique to each stage of development will be used to identify distinct stages of development. This study will allow for the analysis of all HDAC expression during important developmental phases. This may allow for the elucidation of developmental functions for the lesser studied HDAC genes as well as providing the context of histone deacetylase expression of HDA19, and HDA3 during reproductive development. The results of this study will be compared with microarray data from previous studies.

663 Regulation of organ formation in Arabidopsis thaliana

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During development, cell groups must be selected and committed to specific fates in a coordinated way to ensure correct pattern formation. In plants, this process is repetitively employed throughout postembryonic development indicating the presence of robust patterning mechanisms. How is the position of organs determined within a body plan?

In the ovule of *Arabidopsis thaliana*, the development of the two neighbouring domains, nucellus and chalaza, is spatially and temporally tightly coordinated to allow for successful reproduction of the plant. The integuments arise from chalaza cells next to the nucellus-chalaza boundary. Integument initiation requires expression of the *WUSCHEL* gene in the nucellus and suggests that the position of organ initation in ovules is determined by interregional signaling between nucellus and chalaza. Our aim is to characterize the signaling pathway and the mechanisms that provide positional information in the ovule.

We have already identified a candidate gene, *WUT1*, whose expression in the nucellus depends on *WUSCHEL* function and which plays a role in ovule development. This gene encodes for a small peptide. By further analyzing the function of the *WUT1* gene during ovule development we hope to understand the molecular mechanisms underlying pattern formation in the *Arabidopsis* ovule.

664 Variation in vernalisation requirement among natural Arabidopsis lyrata populations, and the role of candidate genes FRI and FLC

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Differences in flowering characteristics among populations are expected to be due to adaptation to local environment, eg. winter conditions, summer draught and length of growing season. Bolting in the plant molecular biology model species *A. thaliana* is known to be governed by external cues, such as long days and exposure to prolonged cold (vernalisation) at rosette or seed stage. We studied the effect of cold treatment on flowering in three populations of *A. lyrata*, a close perennial relative of the annual *A. thaliana*, in a growth chamber experiment. Vernalisation of rosettes reduced bolting time and leave number at bolting in the two northern populations, Spiterstulen (Norway) and Storsand (Sweden), but had no effect in the southern Plech (Germany) population, when compared with nonvernalised controls. Vernalisation of germinating seeds did not accelerate flowering. Sequence variation at two epistatically acting candidate loci, *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)* genes was studied in the same populations to detect potential associations with flowering phenotypes. There was a high level of nonsynonymous polymorphism in the coding region of the *FRI* gene in *A. lyrata* but we did not find evidence that this variation would contribute to naturally occurring flowering time variation

665 Characterization of an Arabidopsis Phosphatase Involved in Microtubule Regulation

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In plants, cortical microtubule (cMT) arrays generally determine the polarity of cell elongation. Many microtubule associate proteins are thought to be involved in formation and maintenance of the array organization. A dominant *phs1-1* mutant allele of an Arabidopsis MAP kinase phosphatase-like protein showed compromised cMT organization and stability. Transgenic plants that expressed the genomic *PHS1* gene fused to GUS reporter showed strong GUS activity in the elongating region of roots. A MAP kinase cascade involving PHS1 may be important for regulation of cMT arrays in the elongation zone of root. To isolate components in the signal pathway relating to PHS1, we mutagenized *phs1-1* by EMS and isolated mutants that suppressed or enhanced the *phs1-1* phenotype. More than 20 enhancer and suppressor mutants have been isolated and are being characterized.

666 'Bacterial-like' PPP phosphatases in plants

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In eukaryotes, PPP (protein phosphatase P) family is one of the two known protein phosphatase families specific for Ser and Thr. The role of PPP phosphatases in multiple signaling pathways in eukaryotic cell has been extensively studied. Unlike eukaryotic PPP phosphatases, bacterial members of the family have broad substrate specificity or may even be Tyr-specific. Moreover, one group of bacterial PPPs are diadenosine tetraphosphatases, indicating that bacterial PPP phosphatases may not necessarily function as protein phosphatases. We describe the presence in plants and other eukaryotes of three groups of expressed genes encoding "non-conventional" phosphatases of the PPP family. These enzymes are more closely related to bacterial PPP phosphatases than to the known eukaryotic members of the family. One group, found exclusively in land plants, is most closely related to PPP phosphatases from some alpha-Proteobacteria, including Rhizobiales, Rhodobacterales and Rhodospirillaceae. This group is therefore termed Rhizobiales / Rhodobacterales / Rhodospirillaceae-like phosphatases, or Rhilphs. A possibility of aquisition of Rhilphs by plants from symbiotic rhizobia will be discussed. Phosphatases of the other group are found in Viridiplantae, Rhodophyta, Trypanosomatidae, Plasmodium and some fungi. They are structurally related to phosphatases from psychrophilic bacteria Shewanella and Colwellia, and are termed Shewanella-like phosphatases, or Shelphs. Phosphatases of the third group are distantly related to ApaH, bacterial diadenosine tetraphosphatases, and are termed ApaH-like phosphatases, or Alphs. Patchy distribution of Alphs in plants, animals, fungi, diatoms and kinetoplasts suggests that these phosphatases were present in the common ancestor of eukaryotes but were independently lost in many lineages. Rhilphs, Shelphs and Alphs form PPP clades, as divergent from "conventional" eukaryotic PPP phosphatases as they are from each other and from major bacterial clades. In addition, comparison of primary structures revealed a previously unrecognised (I/L/V)D(S/T)G motif, conserved in all bacterial and "bacterial-like" eukaryotic PPPs, but not in "conventional" eukaryotic and archaeal PPPs. Our findings demonstrate that plants and some other eukaryotes possess diverse "bacterial-like" PPP phosphatases, the enzymatic characteristics, physiological roles and precise evolutionary history of which have yet to be determined.

667 A functional ERECTA-family signaling pathway is required for normal integument development in Arabidopsis

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Leucine-rich receptor kinases (LRR-RKs) have been shown to play important roles in numerous fundamental processes including development, stress responses, disease resistance and hormone signaling. The ERECTA-family of LRR-RKs (*ERECTA, ERL1, and ERL2*) control cell proliferation without disrupting tissue identity. Plants carrying mutations in this family of receptors have fewer, larger, and disorganized cortex cells suggesting they are required for coordinating the proliferation and growth of cells within tissues. *ERL1* and *ERL2* also play a specific role in regulating integument and gametophyte development. We have determined that specific mutant combinations of ERECTA-family kinases are completely sterile and display morphological abnormalities in the outer integument. Furthermore, these phenotypic effects are sensitive to the level of ERL1. In situ hybridization data indicate that *ERL1* and *ERL2* transcripts are specifically expressed in the chalaza and initiating integument primordia during early ovule development and support the hypothesis that this family of receptors are integral for proper ovule growth and differentiation. Work is currently underway to generate combination mutants with genes known to effect ovule development to further our understanding of role ERECTA-family genes play in ovule differentiation.

668 Correlated evolution of leaf shape and trichomes in *Begonia dregei*

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In both functional and phylogenetic analyses of leaves, the various aspects of size, shape and surface features are often considered independently of each other. It is likely that many combinations of characters do not occur at random due to either functional constraint or genetic correlation. The distribution of variation in leaf morphology in the highly variable *Begonia dregei* species complex was examined in natural populations and in F_2 offspring from a cross between plants from two populations. Leaf shape was quantified using several morphometric measures and trichomes on leaves were counted and measured. There were significant correlations between the shapes of leaves and the presence, number and size of trichomes among both populations and in hybrid plants. Deeply incised leaves had larger numbers of longer trichomes at the sinuses. Higher numbers of trichomes on upper leaf surfaces occurred together with trichomes at the petiole and on the abaxial surface on leaves with shallow lobing. The potential for independent evolution of leaf shape and trichomes in this group appears limited. The possible developmental processes for the observed correlations are explored in terms of background from model species.

669 Light-dependent microtubule and cytokinesis defects induced by the small molecule Chuboxypyr

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As one approach to identifying new factors involved in skotomorphogenesis we performed a chemical genetic screen for inhibitors of cell expansion (See posters for more details). This screen identified 750 compounds that inhibit hypocotyl growth by 20% or more. In order to determine if any of these compounds inhibit cytokinesis, plants expressing a nuclear localized GFP marker were grown on the 750 compounds and examined for the presence of multinucleate cells. This screen uncovered three structurally related benzoylpiperazines that cause multinucleate cells in the root tips of light and dark grown seedlings. In addition, they cause fragmented microtubules and morphologically phenocopy Oryzalin in that they induce short, swollen hypocotyls and roots and induce left-handed twisting of hypocotyl cells. Based on the phenotypic similarity to Oryzalin we suspect that the benzoylpiperazines target tubulin directly and are in the process of determining whether they perturb microtubule polymerization in vitro.

We also identified another molecule that phenocopies Oryzalin that we are calling Chuboxypyr. Intriguingly the effects of Chuboxypyr are light dependent which distinguishes the action of this molecule from other microtubule drugs we have examined to date. Like Oryzalin, Chuboxypyr causes fragmented microtubule polymers, however it differs from Oryzalin in that it causes right-handed twisting of etiolated hypocotyl cells. In addition, roots of light grown seedlings are resistant to Chuboxypyr and do not display inhibited growth or multinucleate cells after Chuboxypyr treatment. Based on these two features we favour the hypothesis that Chuboxypyr acts through a different mechanism than classic depolymerizers like Oryzalin, which do not display major differences in sensitivity between light and dark grown seedlings and cause left-handed cell file twisting. Thus, Chuboxypyr appears to define a new mechanism for chemical disruption of the plant microtubule cytoskeleton and should prove useful for genetic analysis of the microtubule cytoskeleton and skotomorphogenesis.

As a first step towards genetic analysis of the effects of Chuboxypyr we screened 60 Arabidopsis ecotypes in an attempt to identify natural variation in Chuboxypyr sensitivity. This study revealed that the RRS-7 ecotype from Indiana is hypersensitive to Chuboxypyr. We are currently investigating the genetic basis of this hypersensitivity. Classical EMS-based genetic analysis of Chuboxypyr action is also in progress.

670 MADS box genes and the evolution of herbaceous plants

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In contrast to annual plants flowering in perennial plants is initiated each year only in a subset of meristems, while others continue vegetative growth to build up the plant body. MADS box proteins have evolved into a large family of transcriptional regulators from which many play key regulatory roles in floral induction and flower development. The MADS box proteins SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) and FRUITFUL (FUL) are important flowering time regulators in annual Arabidopsis plants. SOC1 is a central flowering time pathway integrator, whereas FUL is required for carpel development and additionally for floral induction and floral meristem identity. Results will be presented which show that SOC1 and FUL not only play redundant roles in floral induction and floral reversion, but also for suppression of bract formation. However, the most striking phenotype of *soc1-3 ful-2* double mutants is a perennial growth habit and a drastically expanded life span, suggesting that *SOC1* and *FUL* have been important for the rapid evolution and diversification of annual herbaceous plants.

671 Chemical genetic dissection of cell expansion

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The coordinate regulation of cell expansion and cell division are responsible for generating plant form. Although classical genetic analysis has been powerfully applied to these processes, chemical genetics offers a complementary approach for dissecting these essential cellular processes. As part of an effort to identify new factors involved in cell expansion, we screened a 10,000-member small molecule library to identify compounds that perturb cell expansion, assayed using 4-day old etiolated Arabidopsis seedlings. This effort has led to the identification of 750 compounds that cause 20% or greater inhibition of hypocotyl elongation. As one approach to dissecting the mechanism-of-action of these compounds as well as their specificity, we are systematically analyzing the subcellular organization of panel of GFP marker-lines grown in the presence of this set of 750 active compounds. We will present data on two compounds of particular interest that we have named Golumpazine and Eroonazole. Golumpazine was initially identified by its ability to cause the golgi apparatus to form clumps in etiolated seedlings. Systematic analysis of the effects of Golumpazine on a panel of GFP markers has shown that Golumpazine also perturbs cytokinesis (induces cell wall stubs) and causes microtubule bundling, suggesting that it could target a protein that links golgi organization, cytokinesis and microtubule function. In addition, Golumpazine is lethal to S. cerevisiae under certain growth conditions, a feature we are exploiting in attempts to identify its target. Eroonazole induces dramatic alterations in the structure of the endoplasmic reticulum without dramatically altering its dynamic properties. Specifically, Eroonazole causes the cortical ER network to degenerate into large balloon-shaped vesicles and thus appears to block a function required for creating and / or maintaining the tubular, trigonal morphology of the ER. Since relatively little is known about the maintenance of ER structure we are actively characterizing this compound using genetic strategies. Collectively, our work has generated a set of new probes for manipulating and dissecting cell expansion chemically, genetically and biochemicaly and highlights the utility of combining systematic cell biological characterization of lead compounds using live cell imaging.

672 Development of automated scoring software for SNPWave and quality quantifier assays

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Recently we presented the SNPWave™ technology for multiplexed detection of single nucleotide polymorphisms (van Eijk *et al*., Nucleic Acids Research 32: e47, 2004). The SNPWave technology is suitable for genetic screening and diagnostic applications in plants, animals, human and micro-organisms.

To streamline these powerful diagnostic screening technologies, automated scoring is essential. Specifically for length-based detection systems, such as capillary electrophoresis systems, highly automated scoring methods are hardly available. This is due to the difficulty to specify the exact location in electropherograms where signals corresponding to diagnostic markers are expected. Solving this problem in an automated fashion requires bin definition of expected signals (defined by the assay) and correction factors for mobility shifts, signal intensity variation and other platform-related detection variables.

We present a user-friendly software application that combines automated scoring with the ability to flag suspect data for manual quality control. Currently, this software supports the MegaBACE capillary electrophoresis platform (GE, Amersham Biosciences) as well as polyacrylamide slab-gels. Additional support of other platforms is in progress. With this automated scoring software, streamlining of the SNPWave and QQ assay procedures is completed.

673 Comparative Genomics of Angiosperm MADS Box Genes

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MADS box genes encode key transcriptional regulators that have been implicated in the control of various aspects of floral development, primarily through work carried out in Arabidopsis. We have embarked on a series of analyses to examine the extent to which gene duplication, regulatory diversification and differences in protein interactions have been important in modifying the roles of MADS box genes during the evolution of flowering plant species. As a first step, we have been developing strategies to rapidly identify all MADS box genes from a given species in order to carry out comprehensive phylogenetic and functional analyses. Using these data, we have initiated analyses of the roles of tomato MADS box genes in flower and fruit development. In addition, we have been developing methods to carry out functional analyses in non-model basal eudicot species. By combining in depth sampling of the repertoire of MADS box genes in phylogenetically informative species with functional analyses in a variety of systems, we can trace the evolutionary history of these genes and define their ancestral and derived roles. In addition, we can assess the extent to which developmental modules controlled by MADS box genes have been redeployed to give rise to new morphologies. Together, these approaches are paving the way for a greater understanding of how these genes have diversified to specify different aspects of floral architecture.

674 The evolution of plant architecture in Brassicaceae

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Variation in the disposition of flowers in time and space can greatly affect plant fitness in different environments. Previous work in genetic model systems has suggested that the interplay of shoot and floral meristem identity genes might play a key role in regulating plant architecture and the placement of flowers within shoot systems. In particular, homologs of the shoot meristem identity gene TERMINAL FLOWER1 (TFL1) and the floral meristem identity gene *LEAFY (LFY)* have been hypothesized to play a central role in architectural evolution. Brassicaceae have a rather conservative architecture: almost all species produce flowers on inflorescences (elongated portions of stems with reduced or absent leaves). However, at least three lineages have switched to rosette-flowering, a condition in which solitary flowers on long pedicels emerge from the axils of rosette leaves. We used an interspecific transgenetic strategy to study the role of meristem identity genes in the switch to rosette-flowering in three lineages that independently acquired rosette-flowering: *Ionopsidium, Leavenworthia*, *Idahoa*. The *LFY* homologs from one rosette-flowering species of each genus were introduced with their native promoters into *lfy* mutant *Arabidopsis* plants. The resulting phenotypes suggest that significant changes have occurred at the LFY locus during the evolution of *Leavenworthia* and *Idahoa* and implicate changes in the interaction with TFL1. Specifically, it appears that the LFY transgenes are not properly repressed by TFL1 due to changes in their *cis*-regulatory regions. These data suggest that changes in the identity of axillary meristems associated with the evolution of rosette flowering were driven by alterations in the regulatory network linking *LFY* and *TFL1*. However, the full evolutionary transition almost certainly entailed changes at more than these two genetic loci.

675 The role of KNOX genes in shoot morphogenesis and compound leaf development

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The evolution of the KNOTTED-like HOMEOBOX (KNO1) genes suggests a history of gene loss, duplication and sub-functionalization. Of particular interest to us in the divergence into the Class 1 and Class 2 genes. The KNOX1 genes function in shoot morphogenesis. Analysis of the atypical leaf of Kalanchoe diagrementiana shows that KNOX1 genes (together with LEC1) play a role in ectopic shoot production. KNOX1 genes have also been shown by us to be utilized in the developmental cascade leading to compound leaves in most flowering plants. We extended this study to analyze the role of PHANTASTICA, a MYB domain protein that regulates KNOX1 genes. In tomato leaf development PHAN and KNOX1 share a dosage sensitive regulatory relationship and PHAN regulates leaflet placement in numerous compound leaves. We will present data on analysis of leaf development in the tomato and bean families, and in related species in the same genus (e.g. the genus Lepidium) or within the same species (e.g. the heteroblastic species Neobeckia aquatica) in the mustard family. These data have begun to give us tools to discriminate between the two conflicting hypothesis (the partial shoot and the leaf subdivision hypotheses) on compound leaf origins.

676 Molecular Evolution of LEAFY transcription factor in land plants

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Understanding how molecular mechanisms can be linked to evolution of organism form is the founding theme of "evo-devo" studies. Transcription factors, which regulate and coordinate the expression of thousands of genes during development, represent candidate of choice to address the question of how molecular evolution of a developmental regulator can result in changes in the repertoire of gene it controls and ultimately translate into morphological evolution.

The plant-specific transcription factor LEAFY controls general aspects of the life cycle in a basal plant, the moss *Physcomitrella patens*. In contrast, LEAFY has more specialized functions in angiosperms, where it specifically induces floral fate during the reproductive phase. This raises the question of a concomitant change in the biochemical function of LEAFY during the evolution of land plants. We have identified that the DNA binding domain of LEAFY, although largely conserved, has diverged in activity. On the contrary, other, more rapidly evolving portions of the protein have few effects on LEAFY activity[1]. In a broader perspective of functional evolution of LFY among land plants, two testable hypotheses can be proposed. LFY might control similar networks of genes in non-flowering and flowering plants, with co-evolution of target sequences and LFY DNA binding specificity. Alternatively, there may have been a complete change of LFY function between basal taxa and flowering plants, in which an initial, albeit gradual change in biochemical activity was the prerequisite for recruitment and/or intercalation of new targets, such as AP1.

Our results establish a framework for the study of the functional/morphological evolution of plant form and flower invention and tentative unifying scenario will be discussed.

1. Maizel, A. et al. *Science* 308, 260-3 (2005)

677 Evidence of genetic conservation of diverse nectaries within the eudicots

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Nectaries are secretory organs widely present in flowering plants that function to lure floral pollinators. Due to diversity in nectary positions and structures, they are thought to have originated multiple times during angiosperm evolution, with their potential contribution to the diversification of flowering plants and pollinating animals being considerable. Using CRABS CLAW (CRC), a gene required for nectaries in Arabidopsis, the genetic bases of diverse nectary forms in eudicot angiosperm species were investigated. CRC expression is conserved in morphologically different nectaries from several core eudicot species and CRC is required for nectary development in both rosids and asterids, major phylogenetic branches of eudicots. However, in a basal eudicot species, no evidence of CRC expression in nectaries was found. Combining a mapping of nectary positions onto an angiosperm phylogeny and CRC expression analyses in eudicots, we propose that diverse nectaries in core eudicots share conserved CRC gene regulation, and that derived nectary positions in eudicots have altered regulation of CRC. Since the ancestral function of CRC lies in the regulation of carpel development, it was likely co-opted as a regulator of nectary development in basal eudicots, which may have led to the association of nectaries with reproductive organs in derived lineages.

678 Abstract not submitted

679 The roles of genetic integration and constraint in adaptive evolution: a floral case study

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The evolution of phenotypic traits depends on the form and strength of natural selection as well as the genetic variances and correlations among the traits. Genetic correlations may constrain phenotypic evolution, or they may themselves evolve to increase functional integration among traits. Using floral traits as a model system, I will describe a combination of lab, greenhouse, and field studies designed to increase our understanding of the roles of integration and constraint in evolution. A comparative phylogenetic study suggests that a high correlation between the lengths of the stamens and corolla tube has evolved from a lower correlation at least twice in the Brassicaceae, and that this correlation is phylogenetically stable in the clade that includes radish and *Brassica*. This high correlation could be due to selection to maintain proper anther placement for effective pollination. Studies of this high correlation ($r = 0.85$) in wild radish indicate that it is stable across populations and environments and that it is caused by pleiotropy rather than linkage disequilibrium. Thus, this correlation is an extraordinarily strong candidate to cause a constraint. However, when we applied artificial selection to anther position, rapid independent evolution of the stamens and corolla tube occurred. This work should improve our understanding of how rapid adaptive evolution can occur, even in the face of possible constraints caused by genetic correlations.

680 The Nature of Intrinsic Postzygotic Isolation

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Crosses are often made within and between species to infer evolutionary relationships and to determine the nature of intrinsic postzygotic isolation. Here I analyze crossing data from more than 300 genera of plants and animals and present new empirical results from sunflower on the basis of reduced hybrid fertility. I show that contrary to conventional wisdom, plant species are more strongly isolated than animal species and more likely to correspond to reproductively independent lineages. Also, comparisons of F_1 and F_2 hybrid fitnesses indicate that postzygotic isolation in animals and perennial plants is caused mainly by gene incompatibilities, whereas chromosomal rearrangements are the predominant cause of isolation in annuals. This observation is supported by genetic studies of annual sunflowers, where reductions in pollen viability map mostly to chromosomal rearrangements. However, in some cases, pollen viability QTLs behave epistatically, indicating that gene incompatibilities cluster near rearrangement breakpoints as predicted by theory. Finally, genetic-mapped based analyses of hybrid zones demonstrate that rearrangements have a large effect on interspecific gene flow near the center of hybrid zones, but that this effect does not extend to more distant populations as has been reported for some animal species.

681 Abstract Not Submitted

NOTES

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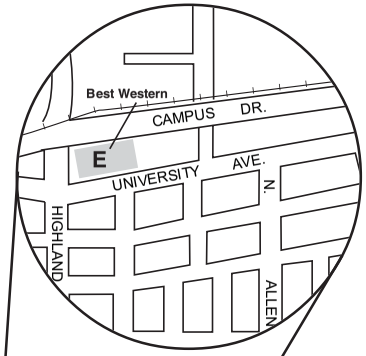
ACCOMODATION LOCATIONS

- A Double Tree (formerly Howard Johnsons)
- B Dahlmann Campus Inn
- C Fluno Center
- D University Inn
- E Best Western
- F Princeton House
- G Lowell Center
- H Union South Hotel
- I Chadbourne Hall



Direction to Best Western:

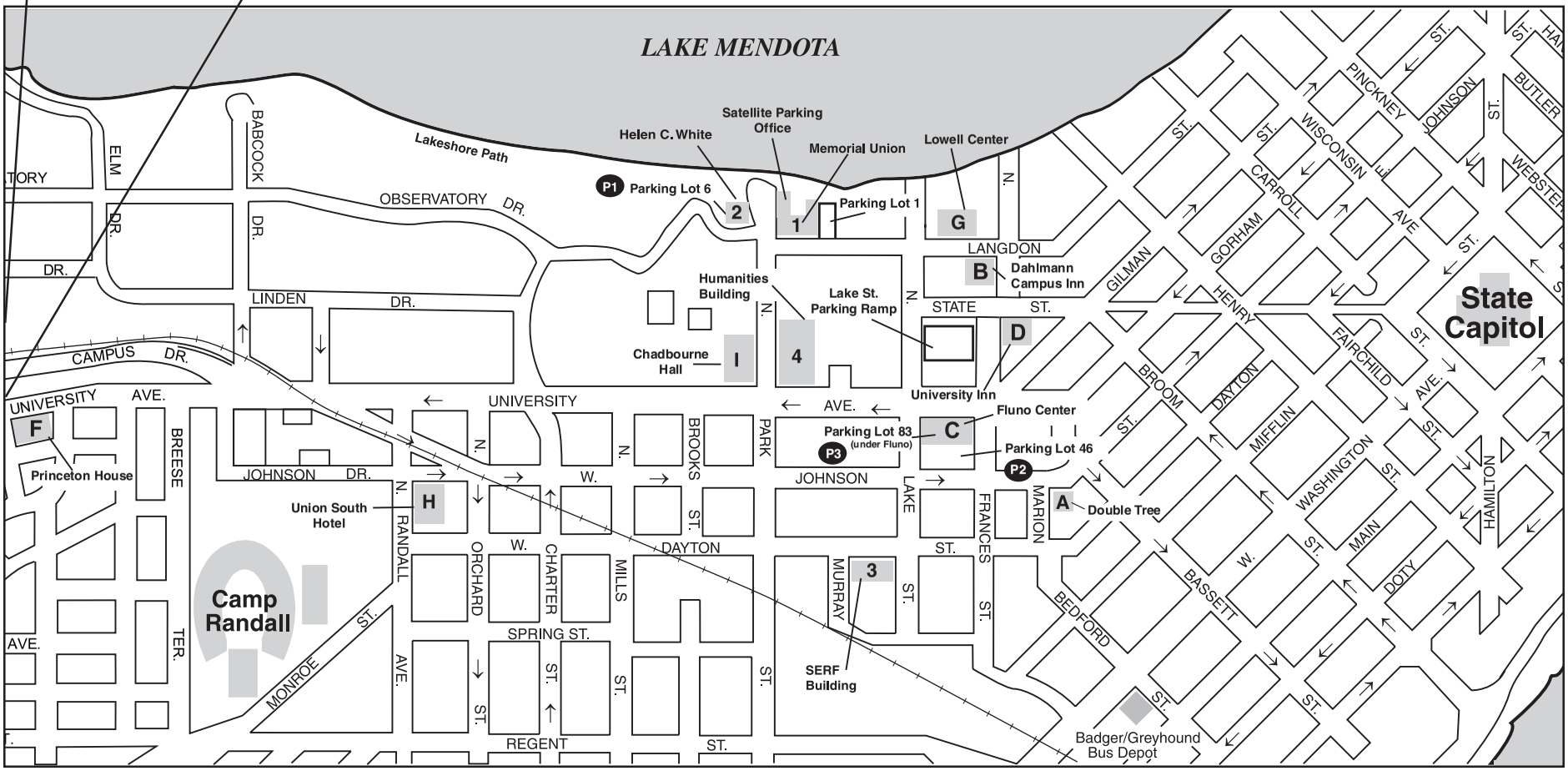
- Take University Ave. west.
- Take the 1500 - 2600 University Ave. Exit.
- (Do not take Campus Drive).
- Hotel is 8 blocks down on the right.

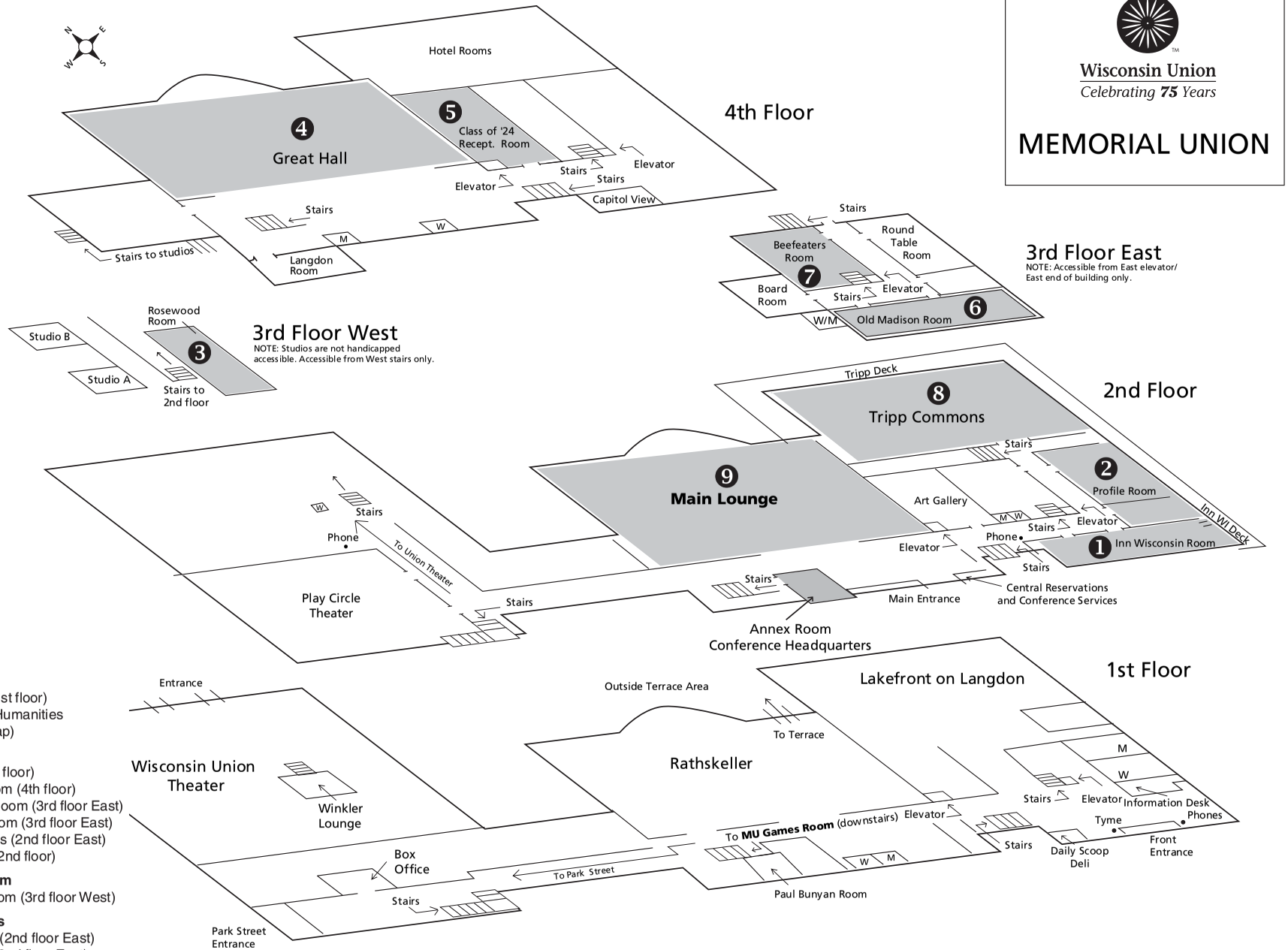
PARKING LOTS

- P1 Parking Lot 6
- P2 Parking Lot 46
- P3 Parking Lot 83

UNIVERSITY BUILDINGS

- 1 Memorial Union
- 2 Helen C. White
- 3 SERF Building
- 4 Humanities Building





Arabidopsis

Oral Sessions
- Union Theater (1st floor)
- 3650 and 1111 Humanities (see Campus Map)

Poster Sessions
- ❹ Great Hall (4th floor)
- ❺ Reception Room (4th floor)
- ❻ Old Madison Room (3rd floor East)
- ❼ Beefeaters Room (3rd floor East)
- ❽ Tripp Commons (2nd floor East)
- ❾ Main Lounge (2nd floor)

Slide Preview Room
- ❸ Rosewood Room (3rd floor West)

Meal/Buffet Rooms
- ❶ Inn Wisconsin (2nd floor East)
- ❷ Profile Room (2nd floor East)

Open Meeting Rooms
- Langdon Room (4th floor)
- Capitol View Room (4th floor)