NEW YORK UNIVERSITY, JULY 17-21, 2017

9th International Tunicate Meeting #9thITM

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9th INTERNATIONAL TUNICATE MEETING
PROGRAM AT A GLANCE

Sunday 7/16	Monday 7/17	Tuesday 7/18	Wednesday 7/19	Thursday 7/20	Friday 7/21
	8:15am-9:00am BREAKFAST	8:15am-9:15am BREAKFAST		8:15am-9:15am BREAKFAST	8:15am-9:15am BREAKFAST
	9:00-9:15am Opening Remarks				
	9:15am-10:55am Session 1. Beginning of Embryogenesis	9:15am-10:30am Session 5. Nervous system development and anatomy	9:30am-11:30am Career Session	9:15am-10:30am Session 9. Systematics and Evolution (1)	9:15am-10:30am Session 13. Late embryos and early larvae
	10:55am-11:15am COFFEE BREAK	10:30am-10:50am COFFEE BREAK		10:30am-10:50am COFFEE BREAK	10:30am-10:50am COFFEE BREAK
	11:15am-12:30pm Session 2. Developmental Cell Biology	10:50am-11:40am Session 6. Development and evolution of the nervous system		10:50am-11:40am Session 10. Systematics and Evolution (2)	11:15am-12:30pm Session 14. Post-embryonic development
		11:40am-12:40pm KEYNOTE Marianne Bronner		11:40am-12:40pm KEYNOTE Gretchen Lambert	
	12:30pm-2:00pm LUNCH BREAK	12:40pm-2:00pm LUNCH BREAK	EXCURSION	12:40pm-2:00pm LUNCH BREAK	12:40pm-2:00pm LUNCH BREAK
	2:00pm-3:15pm Session 3. From Development to Genomics	2:00pm-3:15pm Session 7. Gene regulation		2:00pm-3:15pm Session 11. Ecology (1)	2:00pm-3:40pm Session 15. Asexual reproduction and regeneration
	3:15pm-3:45pm GROUP PHOTO	3:15pm-3:35pm COFFEE BREAK		3:15pm-3:35pm COFFEE BREAK	
	3:45pm-4:05pm COFFEE BREAK	3:35pm-5:15pm Session 7. Imaging ascidian development		3:35pm-5:15pm Session 12. Ecology (2)	3:40pm-4:00pm COFFEE BREAK
					4:00pm-4:50pm Session 16. Anatomical Ontology
	4:05pm-5:20pm Session 4. Allorecognition and Immunity	5:15pm-7:15pm POSTER SESSION 1 Odd numbers		5:15pm-7:15pm POSTER SESSION 2 Even numbers	5:00-6:45pm ROUND TABLE DISCUSSIONS
6:00pm-8:00pm WELCOME RECEPTION	*Dinner on your own*				6:45pm-7:00pm Closing Remarks
		Dinner on your own		*Dinner on your own*	7:00pm-9:00pm FAREWELL BANQUET

9th INTERNATIONAL TUNICATE MEETING
DETAILED PROGRAM

Sunday, July 16th

Rosenthal Pavilion
6:00pm - 8:00pm WELCOME RECEPTION

Monday, July 17th
Rosenthal Pavilion
8:15am - 9am BREAKFAST

9:00am - 9:15am Opening remarks - Anna Di Gregorio and Lionel Christiaen

9:15am - 10:55am: **Session 1 - Beginning of Embryogenesis**
Chair: Hiroki Nishida

9:15am - 9:40am #1 **Silvia Caballero-Mancebo** (IST Austria). *Actin driven ooplasmic segregation in ascidian oocytes*

9:40am - 10:05am #2 **Benoit Godard** (IST Austria). *Cleavage plane positioning in early ascidian embryos*

10:05am - 10:30am #3 **Rémi Dumollard** (Sorbonne Universités, UPMC, CNRS). *Apical cell shape drives the invariant cleavage pattern of early ascidian embryos and relies on intrinsic and extrinsic cell deformations*

10:30am - 10:55am #4 **Ute Rothbächer** (University of Innsbruck). *Repression through Wnt signaling effectors*

10:55am - 11:15am COFFEE BREAK

11:15am - 12:30pm: **Session 2 - Developmental Cell Biology**
Chair: Michael Veeman

11:15am - 11:40am #5 **Takefumi Negishi** (Tohoku University). *Membrane invagination-mediated posterior ciliary positioning is involved in the neurula rotation in* Ciona intestinalis

11:40am - 12:05am #6 **Christina Cota** (Swarthmore College). *Mitotic progression choreographs FGF receptor*

redistribution during differential heart progenitor induction

12:05am - 12:30am #7 **Yelena Bernadskaya** (New York University). *Ddr coordinates cell polarity and matrix adhesion during cardiopharyngeal precursor migration*

12:30pm - 2:00pm LUNCH BREAK (*optional lunch box offered*).

2:00pm - 3:15pm: **Session 3 - From Development to Genomics**
Chair: Patrick Lemaire

2:00pm - 2:25pm #8 **Michael Veeman** (Kansas State University). *Shaping the ascidian notochord*

2:25pm - 2:50pm #9 **Robert Zeller** (San Diego State Univeristy). *Genome and transcriptome assemblies for two Ascidia species*

2:50pm - 3:15pm #10 **Megan Wilson** (University of Otago). *Tunicate evolution: Insights from recently sequenced genomes*

3:15pm - 3:45pm GROUP PHOTO (*Rosenthal Pavilion or Washington Square Park*)

3:45pm - 4:05pm COFFEE BREAK

4:05pm - 5:20pm: **Session 4 - Allorecognition and Immunity**
Chair: Tony De Tomaso

4:05pm - 4:30pm #11 **Marie Nydam** (Centre College). *Balancing selection on allorecognition genes in the colonial ascidian* Botryllus schlosseri

4:30pm - 4:55pm #12 **Loriano Ballarin** (University of Padova). *Amyloid and allorecognition in the colonial ascidian* Botryllus schlosseri

4:55pm - 5:20pm #13 **Ayelet Voskoboynik** (Stanford University). *BHF mediates induction and loss of tolerance in* Botryllus schlosseri *chimeras*

Dinner on your own

Tuesday, July 18th

Rosenthal Pavilion

8:15am - 9:15am BREAKFAST

9:15am - 10:30am: **Session 5 - Nervous System Development and Anatomy**
Chair: Ian Meinertzhagen

9:15am - 9:40am #14 **Kerrianne Ryan** (Dalhousie University). *Interpreting the connectome of a* Ciona intestinalis (L.) *larva*

9:40am - 10:05am #15 **Alberto Stolfi** (NYU/Georgia Institute of Technology). *A conserved RNA switch for Acetylcholine receptor clustering at the neuromuscular junction*

10:05am - 10:30am #16 **Kouhei Oonuma** (Konan University). *The cell lineage and the patterns of cell division for photoreceptor cells of the left-right asymmetric ocelli in the* Ciona intestinalis *larva*

10:30am - 10:50am COFFEE BREAK

10:50am - 11:40pm **Session 6 - Development and Evolution of the Nervous System**
Chair: Takehiro Kusakabe

10:50am - 11:15am #17 **Adeline Rausch** (Bioemergences - CNRS). *Putative neural crest-like cells in Tunicates, behavior, identity and fate of A10.64*

11:15am - 11:40pm #18 **Takeo Horie** (University of Tsukuba/Princeton University). *Neural crest and cranial placodes share a common evolutionary origin*

11:40am - 12:40pm **KEYNOTE: Marianne Bronner** (California Institute of Technology) *Evolutionary origin of vertebrate enteric neurons*
Chair: Lionel Christiaen

12:40pm - 2:00pm LUNCH BREAK (*optional lunch box offered*)

2:00pm - 3:15pm: **Session 7 - Gene regulation**
Chair: Kenneth Hastings

2:00pm - 2:25pm #19 **Wei Wang** (New York University). *A single cell transcriptional roadmap for cardiopharyngeal fate diversification.*

2:25pm - 2:50pm #20 **Claudia Racioppi** (New York University). *Chromatin accessibility underlying cardiac vs. pharyngeal muscle specification in* Ciona

2:50pm - 3:15pm #21 **Emma Farley** (UC San Diego). *Regulatory grammar governing enhancer specificity*

3:15pm - 3:35pm COFFEE BREAK

3:35pm - 5:15pm: **Session 8 - Imaging Ascidian Development**
Chair: Nadine Peyriéras

3:35pm - 4:00pm #22 **Ulla-Maj Fiuza** (EMBL). *A Nodal and Ephrin signalling relay drives the transition between apical constriction and apico-basal shortening during ascidian endoderm invagination*

4:00pm - 4:25pm #23 **Ed Munro** (University of Chicago). *Spatiotemporal control of actomyosin contractility during zippering and neural tube closure in a simple chordate*

4:25pm - 4:50pm #24 **Taichi Akahoshi** (Keio University). *Comprehensive characterization of Ca2+ transients in early Ascidian development*

4:50pm - 5:15pm #25 **Emmanuel Faure** (CNRS). *4D Clouds Embryos*

5:15pm - 7:15pm **POSTER SESSION 1** - all sessions, odd numbers

Dinner on your own

Wednesday, July 19th

Rosenthal Pavilion
9:30am - 11:30am **Session: career panel discussion**
contributors: *Yelena Bernadskaya, Emma Farley, Noa Shenkar, Cristian Cañestro, Federico Brown, Weiyang Shi, Steve Irvine, Shigeki Fujiwara*

Chair: Lionel Christiaen

EXCURSION

Thursday, July, 20th
Rosenthal Pavilion

8:15am - 9:15am BREAKFAST

9:15am - 10:30am: **Session 9: Systematics and Evolution (1)**
Chair: Billie Swalla

9:15am - 9:40am #26 **Billie Swalla** (University of Washington). *Molgulid tales*

9:40am - 10:05am #27 **Cristian Cañestro** (University of Barcelona). *Gains N' Losses during the evolution of RA and Wnt developmental pathways in* Oikopleura dioica *and ascidian species*

10:05am - 10:30am #28 **Dorothee Huchon** (Tel-Aviv University). *Insights from complete mitochondrial sequences on the phylogenetic relationships of* Aplousobranchia

10:30am - 10:50am COFFEE BREAK

10:50am - 12:30pm: **Session 10: Systematics and Evolution (2)**
Chair: Billie Swalla

10:50am - 11:15am #29 **Fabio Gasparini** (University of Padova). *Disentangling a species complex: the case of the colonial ascidian* Botryllus schlosseri

11:15am - 11:40am #30 **Rosana Rocha** (Universidade Federal do Paraná). *Is* Styela canopus *a complex of species?*

11:40am - 12:40pm **KEYNOTE: Gretchen Lambert** (University of Washington). *The continuing saga of a sea squirt detective: fouling ascidians in the Galapagos on artificial surfaces*
Chair: Anna Di Gregorio

12:40pm - 2:00pm LUNCH BREAK (*optional lunch box offered*)

2:00pm - 3:15pm: **Session 11: Ecology (1)**

Chair: Noa Shenkar

2:00pm - 2:25pm #31 **Shady Amin** (NYU Abu Dhabi). *Algal-bacterial interactions: Mutualism, antagonism and chemical signaling*

2:25pm - 2:50pm #32 **Mey-Tal Gewing** (Tel-Aviv University). *Med vs. Red: temperature sensitivity of the non-indigenous ascidian* Herdmania momus *in the Mediterranean Sea*

2:50pm - 3:15pm #33 **Steve Irvine** (University of Rhode Island). *Connecting water temperature to reproductive physiology and molecular signals in* C. intestinalis

3:15pm - 3:35pm COFFEE BREAK

3:35pm - 4:00pm: **Session 12: Ecology (2)**
Chair: Shigeki Fujiwara

3:35pm - 4:00pm #34 **Susanna Lopez-Legentil** (University of North Carolina Wilmington). *Introduced and native ascidian microbiomes from artificial versus natural habitats*

4:00pm - 4:25pm #35 **Lauren Stefaniak** (Georgia Southern University). *Moving to the reef: historical and present observations of the worldwide introduced species* Styela plicata *in the Northwest Atlantic continental shelf*

4:25pm - 4:50pm #36 **Khashayar Razghandi** (Humboldt-Universität zu Berlin). *Structure-Function Analysis of the Filter-House of the Appendicularian* Oikopleura dioica

4:50pm - 5:15pm #37 **Laurel Hiebert** (Universidade de São Paulo). *How ecological factors influence growth patterns of colonial and solitary ascidians*

5:15pm - 7:15pm **POSTER SESSION 2** - all sessions, even numbers.

Dinner on your own

Friday, July 21st

Rosenthal Pavilion

8:15am - 9:15am BREAKFAST

9:15am - 10:30am: **Session 13: Late embryos and early larvae**
chair: Filomena Ristoratore

9:15am - 9:40am #38 **Antonio Palladino** (Stazione Zoologica Anton Dohrn). *Gsx in Ascidian Central Nervous System Development*

9:40am - 10:05am #39 **Ugo Coppola** (Stazione Zoologica Anton Dohrn). *Klhl21: a new player for pigmentation from* Ciona robusta

10:05am - 10:30am #40 **Brad Davidson** (Swarthmore College). *Laterally asymmetric morphogenesis of the heart and gut in* Ciona intestinalis

10:30am - 10:50am COFFEE BREAK

10:50am - 12:30am: **Session 14: Post-embryonic Development**
Chair: Thomas Stach

10:50am - 11:15am #41 **Gabriel Krasovec** (Sorbonne Universités, UPMC, IBPS, CNRS). *Morphogenetic function of apoptosis during tail regression at the metamorphosis stage of* Ciona intestinalis

11:15am - 11:40pm #42 **Hiroki Nishida** (Osaka University). *Patterning and morphogenesis of the intricate but stereotyped Oikoplastic epidermis of the appendicularian,* Oikopleura dioica

11:40pm - 12:05pm #43 **Jiankai Wei** (Ocean University of China). *Molecular composition and architecture of extracellular matrix in ascidian embryogenesis and larval metamorphosis*

12:05pm - 12:30pm #44 **Anthony De Tomaso** (UC Santa Barbara). In vivo *manipulation of the extracellular matrix induces vascular regression in a basal chordate*

12:30pm - 2:00pm LUNCH BREAK (*optional lunch box offered*)

2:00pm - 3:15pm: **Session 15 - asexual reproduction and regeneration**
Chair: Federico Brown

2:00pm - 2:25pm #45 **Maria Mandela Prünster** (Sorbonne Universités, UPMC, CNRS). *Detour to make muscles? Myogenesis in the budding process of a colonial ascidian,* Botryllus schlosseri

2:25pm - 2:50pm #46 **Federico Brown** (Universidade de São Paulo). *Asexual reproduction in Styelidae (Ascidiacea): and evo-devo approach*

2:50pm - 3:15pm #47 **Tal Gordon** (Tel-Aviv University). *When One becomes Two - unique regeneration abilities of the solitary ascidians* Polycarpa mytiligera

3:15pm - 3:40pm #48 **William Jeffery** (University of Maryland). *Partial Body Regeneration, Stem Cells, and Regenerative Aging in* Ciona

3:40pm - 4:00pm COFFEE BREAK

4:00pm - 4:50pm: **Session 16 - Anatomical Ontology**
Chair: Patrick Lemaire

4:00pm - 4:25pm #49 **Kohji Hotta** (Keio University). *The ontology of larval and metamorphosis stages in* Ciona. *I. Development*

4:25pm - 4:50pm #50 **Lucia Manni** (University of Padova). *The ontology of larval and metamorphosis stages in* Ciona. *II. Anatomy*

Room KC914
5:00pm - 6:45pm **Round table discussions**
Databases - Patrick Lemaire
#51 **Justine Dardaillon / Matija Brozovic**
#52 **Marc Rius**

Functional genomics methods & resources - Alberto Stolfi
#53 **Wei Deng**
Basile Gravez

6:45pm - 7:00pm	**Closing remarks** - Anna Di Gregorio and Lionel Christiaen

Rosenthal Pavilion

7:00pm - 9:00pm	**Farewell banquet**

9th Tunicate International Meeting

New York University, July 17-21, 2017

Abstracts

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TALKS

#Session 1 : Beginning of Embryogenesis

Chair: Hiroki Nishida

Talk 1, Poster 01
Actin driven ooplasmic segregation in ascidian oocytes

Silvia Caballero-Mancebo[1*] and Carl-Philipp Heisenberg[1]

1 Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria

* Presenting Author silvia.caballero@ist.ac.at

In ascidians the animal-vegetal axis is already defined in the mature oocyte due to asymmetric positioning of the meiotic spindle at the animal pole and the localization of two major cytoplasmic domains - a cortical endoplasmic reticulum-rich (cER) and a mitochondria-rich domain (myoplasm) – close to the vegetal pole. Fertilization triggers an actin-dependent cortical contraction wave that traverses the cell in an animal-to-vegetal direction and culminates in the formation of a transient structure at the vegetal pole called contraction pole (CP) [1,2]. Although CP formation has been extensively studied, the forcegenerating mechanisms remain unknown. Here we use the species Phallusia mammillata to decipher what are the mechanochemical forces involved in CP formation, and how CP formation triggers the asymmetric positioning of cER and myoplasm.

To address these questions, we analyzed the dynamic distribution of actin and myosin during CP formation. We found that sperm entry triggers the depletion of cortical actin at the animal pole and its accumulation at the vegetal pole cortex. Myosin is rapidly recruited to the cortex after sperm entry and follows then the same accumulation pattern as actin. We also found that the actin cortex displays calciumdependent changes in its organization from puncta in unfertilized oocytes to a filamentous network in fertilized oocytes. These changes coincide with the establishment of cortical actomyosin flows from the animal to the vegetal pole leading to CP formation.

To reveal the force-generating mechanism leading to CP formation, we analyzed cortical tension in unfertilized and fertilized oocytes. We found that tension is higher in vegetal compared to animal regions, and that this difference massively increases upon oocyte fertilization, suggesting that actomyosin network reorganization triggers CP formation by potentiating cortical tension anisotropy along the animal-vegetal axis of the oocyte.

Finally, to reveal how CP formation is linked to the reorganization of the cER and myoplasm domain within the oocyte, we ectopically increased cortical tension at the vegetal pole by micropipette aspiration and observed flows of cER and, to a lesser degree, myoplasm towards the aspiration site. Based on these preliminary observations, we propose a model in which the actin network at the cortex is linked to the cER so they both flow together towards the vegetal pole upon fertilization. The myoplasm, in contrast, is not directly linked to the cER and thus does not accumulate at a similar degree as the cER at the vegetal pole during CP. I will discuss these findings in the context of preexisting models of cortical actomyosin and cytoplasmic flows.

References
[1] Nishida. 2005, Developmental Dynamics 233: 1177-1193.
[2] Sardet et al. 2007, Developmental Dynamics 236:1716-1731.

Talk 2
Cleavage plane positioning in early ascidian embryos

Benoit G. Godard[1,*], Kohji Hotta[2], Nicolas Minc[3] and Carl-Philipp Heisenberg[1]

1 Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria
2 Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, Kouhoku-ku, Yokohama 223-8522, Japan
3 Institut Jacques Monod, UMR7592, 15, rue Hélène Brion, 75205 PARIS

* Presenting Author: benoit.godard@ist.ac.at

Embryonic development of ascidians is characterized by a small number of cells and an invariant cleavage pattern. The invariant cleavage pattern critically depends on a tight control of cleavage plane positioning. The third round of cleavages established the animal-vegetal axis of the embryo and relies on a precise orientation and centering of the mitotic spindle prior to this cleavage (4 cell stage). At this stage, the mitotic spindle in the posterior cells is oriented by the Centrosome-Attracting-Body, causing the vegetal-posterior blastomeres to protrude at the 8-cell stage (Negishi et al., 2007). In addition, the mitotic spindle is off-centered along the forming animal-vegetal axis, giving rise to smaller animal compared to vegetal cells after the third round of cleavages. This off-centering of the mitotic spindle has recently been speculated to be caused by the graded distribution of yolk along this axis (Pierre et al., 2016).

To address how off-centering of the mitotic spindle along the forming animal-vegetal axis at the 4 cell stage is achieved, we performed high-resolution time-lapse imaging of *Phallusia mammillata* embryos. We found that the astral microtubules project through the yolk and reach the cell cortex, pointing to the possibility that differences in cortex composition along the animal-vegetal axis rather than the subcellular distribution of yolk influences mitotic spindle positioning along this axis. Interestingly, by performing cortical ablations at the 1-cell stage, we found that the vegetal cell cortex at the 4-cell stage functions as a negative polarity domain for spindle positioning. I will discuss about the molecular nature of this new polarity domain and the origin of the pulling forces exerted on the microtubules.

References

T.Negishi, T.Takada, N.Kawai, H.Nishida, Current Biology 17, 1014-1025, June 19, 2007
A.Pierre, J.Sallé, M.Wühr, N.Minc, Developmental Cell 39, 667-682, December 19, 2016

Talk 3

Apical cell shape drives the invariant cleavage pattern of early ascidian embryos and relies on intrinsic and extrinsic cell deformations.

Rémi Dumollard, Lydia Besnardeau, Celine Hebras and Alex McDougall

Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV) UMR7009, Sorbonne Universités, Université Pierre-et-Marie-Curie, CNRS, Villefranche-sur-mer, France; *

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The ascidian embryo is an ideal system to investigate how cell position is determined during embryogenesis. Using 3D timelapse imaging and computational methods we analyzed the planar cell divisions in ascidian early embryos and found that spindles in every cell tend to align at metaphase in the long length of the apical surface except in cells undergoing unequal cleavage. Furthermore, the invariant and conserved cleavage pattern of ascidian embryos was found to consist in alternate planar cell divisions between ectoderm and endomesoderm. In order to test the importance of alternate cell divisions we abolished cell cycle asynchrony and found that it consistently disrupted the spindle orienting mechanism underpinning the invariant cleavage pattern.

Using dissociated embryos we now find that the apicobasal polarization of the blastomeres during the early development of ascidians is a cell autonomous process, as isolated blastomere become spontaneously polarized. Interestingly, these isolated blastomeres show a constriction perpendicular to the apicobasal axis where actomyosin accumulates in a ring-like structure. This local accumulation of actomyosin might correspond to the formation of the first contractile junctions at the 16 cell stage which are able to impact cell shape at the 16-32-44 cell stage.

Together our study suggests that intrinsic and extrinsic forces impact cell shape in the ascidian blastula to implement the invariant cleavage pattern specific to ascidian embryos.

Talk 4
Repression through Wnt signaling effectors

Willi Kari[1] , Izumi Oda-Ishii[3] , Fan Zeng[1] , Patrick Lemaire[2] , Lena Zitzelsberger[1] , Yutaka Satou[3] and Ute Rothbächer[1]*

1 Dpt. of Evolution and Development, Institute for Zoology, University Innsbruck, Austria
2 CRBM, CNRS, Montpellier, France;
3 Dpt. of Zoology, Kyoto University, Japan

* Presenting Author: ute.rothbaecher@uibk.ac.at

We have recently discovered a novel mode of direct transcriptional repression by the Wnt signaling effectors TCF/ß-catenin in Ciona that lacks canonical TCF DNA binding motifs (Rothbächer et al., 2007). The resulting opposite transcriptional target gene activation in daughters of asymmetrically dividing precursors assures mutual exclusiveness and robustness of binary cell fates in the segregating tissues. The repressive mechanism, we described in both, Ciona and C. elegans, is similar regarding the formation of a repressive complex between TCF/ß-catenin and another transcription factor to be repressed, i.e. Ref-2 in nematode neural precursors (Murgan, Kari et al., 2015) and Gata.a in ascidian early blastomeres (Oda-Ishii et al., 2016). Notably, we proposed a model in Ciona where Gata.a binding to its target sites is weakened in the presence of both, Tcf7 and ß-catenin, without direct competitive binding of the latter to DNA as suggested from EMSA or genomewide ChIP assays.

We could now refine the initial model in a qualitative and quantitative way concerning the signature around the target GATA binding sites and concerning the role of Tcf7 at certain types of GATA sites. By ex vivo DNA pulldown and reporter gene activity assays on representative sets of GATA site variants and within different target genes we monitored for previously observed repressive differences at different GATA sites. Indeed, we are now in the position to propose a repression signature that could more generally explain for how ubiquitous Gata.a protein is variably restricted to activate selected target genes required for regionalizing the early embryo.

References

1. Rothbächer, U, Bertrand, V, Lamy, C and Lemaire, P. A combinatorial code of maternal GATA, Ets and β-catenin-TCF transcription factors specifies and patterns the early ascidian ectoderm. (2007) Development; 134 (22): p. 4023-4032.
2. Murgan, S, Kari, W, Rothbächer, U, Iche-Torres, M, Melenec, P, Hobert, O and Bertrand, V. Atypical Transcriptional Activation by TCF via a Zic Transcription Factor in C. elegans Neuronal Precursors. (2015) Developmetal Cell; 33 (6): p. 737-745.
3. Oda-Ishii I, Kubo A, Kari W, Suzuki N, Rothbächer U, Satou Y. A Maternal System Initiating the Zygotic Developmental Program through Combinatorial Repression in the Ascidian Embryo. PLoS Genetics 2016 May 6;12(5):e1006045. doi: 10.1371/journal.pgen.1006045.

Notes: WK, FZ, LZ and UR are funded by the Austrian Academy of Sciences (ÖAW) and the University Innsbruck, Austria

#Session 2 : Developmental Cell Biology

Chair: Michael Veeman

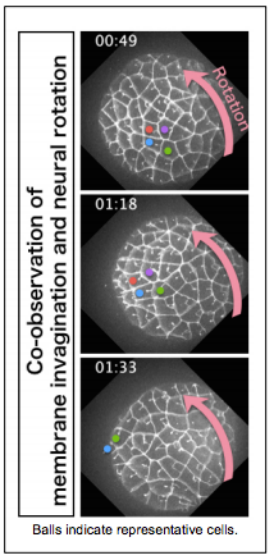
Talk 5
Membrane invagination-mediated posterior ciliary positioning is involved in the neurula rotation in Ciona intestinalis

Takefumi Negishi[1]* and Naoto Ueno[2]

1 Research Center for Marine Biology, Tohoku University, Aomori, Japan.
2 Division of Morphogenesis, National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan

* Presenting Author: negishi0525@gmail.com

Ciliary positioning has been proposed to establish left-right (L-R) asymmetry. The position of cilia is often determined by that of the basal body. The basal body is converted from centrosome during ciliogenesis.



In Ciona intestinalis embryo, we found a unique centriole-targeting of the membrane structure (membrane invagination) in epidermal cells, which divide stereotypically along the anterior-posterior axis. The membrane invagination pulls the centrosome to the posterior and orients mitotic spindle. Interestingly, we also found that cilia formed on the posterior side of the cell and its basal body remained associated with the invagination. We have revealed that disruption of directional membrane

invagination is strongly correlated with randomization of mitotic spindle orientation with the depletion of Dishevelled (Dsh). We also observed the epidermal cilia form in the center of the Dsh depleted-cells, instead of at a posterior position in the normal cells. These results imply us the membrane invagination is involved in the ciliary positioning, as well as spindle orientation (Negishi et al. 2016).

Based on the study of Halocynthia roretzi, it is proposed that counterclockwise rotation of the neurula embryo (neurula rotation), which is conserved in several ascidian species is involved in the establishment of L-R axis (Nishide et al. 2012). We found that membrane invaginations were formed during the neurula rotation (see left figure), and depletion of Dsh disturbs the axis of rotation in Ciona. These results support our hypothesis that posterior ciliary positioning mediated by membrane invagination is involved in the neurula rotation to establish left-right asymmetry in Ciona embryo. Balls indicate representative cells.

Talk 6, Poster 02
Mitotic progression choreographs FGF receptor redistribution during differential heart progenitor induction.

Christina D. Cota[1,*], Matthew S. Dreier[1] , William Colgan[1] , Anna Cha[2] , Chanel Ligon[3] and Brad Davidson[1]

1 Department of Biology, Swarthmore College, PA
2 Department of Systems Biology, Harvard Medical School, MA 3 Department of Environmental Health Sciences, Columbia University, NY

* Christina D. Cota: ccota1@swarthmore.edu

Receptor distribution profoundly impacts cellular signal transduction. Cell division requires extensive reorganization of cell membranes that alters the distribution of membrane-bound proteins. As such, mitotic trafficking of signaling components may play a profound, largely uncharacterized role in cell fate specification. We use the invertebrate chordate, Ciona intestinalis, to investigate in vivo signaling dynamics during FGF-dependent heart progenitor induction. The extreme genetic and cellular simplicity of Ciona embryos facilitates high-resolution analysis of in vivo signaling mechanisms. Previously we have shown that biased mitotic redistribution of FGF receptors is required to compartmentalize the response to FGF during differential heart progenitor induction. Here we show that mitotic progression driven by the mitotic kinases; Aurora kinase, Cyclin dependent kinase 1 (Cdk1) and Polo-like kinase (Plk1) directs FGFR trafficking during heart progenitor induction. Through selective perturbation of these mitotic kinases in precardiac founder cells we have found that Aurora Kinase is required for the initial internalization of FGF receptors at the onset of mitosis. During mitotic entry, CDK1 suppresses recycling of FGF receptor containing endosomes. Finally, PLK1 promotes directed recycling of FGF receptors to adherent membrane during mitotic exit. These results indicate that mitotic receptor redistribution may tune inductive signals during embryonic and stem cell divisions to promote a robust response during cell fate specification.

Talk 7
Ddr coordinates cell polarity and matrix adhesion during cardiopharyngeal precursor migration

Yelena Bernadskaya, and Lionel Christiaen

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Collective cell migration occurs during diverse morphogenetic processes such as gastrulation, angiogenesis and neural crest migration. Here we use the migration of bilateral pairs of cardiopharyngeal precursors of the chordate *Ciona robusta* as the simplest possible model to study the regulation of polarized collective cell movement. The cardiopharyngeal precursors (trunk ventral cells, TVCs) arise from asymmetric divisions of the B7.5 blastomeres and migrate with stereotypical leader/trailer cell identity from the embryo tail to the ventral trunk. We develop quantitative methods to describe the 4D migration, morphology, and polarity of the TVCs, allowing us to identify regulators of these processes. We find that two collagen receptors, Ddr and Intβ1, expressed in the TVCs maintain epidermal adhesion and directional movement of TVCs during migration, as disruption of these pathways produces uncoordinated TVC tumbling and loss of leader/trailer position maintenance. We also describe the localization of an endodermally secreted collagen, Col9-a1, and identify it as the extracellular cue required for Ddr activation and localization to the ventral TVC surface. In addition to promoting adhesion, Col9-a1 and Ddr, but not Intβ1, may induce leader/trailer cell identity prior to the onset of TVC migration through the asymmetric exposure of the leader TVC to Col9-a1. We therefore propose a dual role for Ddr as establishing leader cell identity early in development and modulating the differential adhesion of leader/trailer TVCs during collective migration.

#Session 3 : From Development to Genomics

Chair: Patrick Lemaire

Talk 8
Shaping the ascidian notochord

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My lab is using the *Ciona* notochord as a model to work towards a systems-level understanding of organogenesis. In one project we are seeking to understand the mechanisms controlling the notochord's graceful anterior-posterior taper, which in part involves AP differences in notochord cell volume. We have combined genetic fate mapping based on mosaic transgene expression with cell volume measurements by in toto imaging to map out the asymmetry of all the notochord cell divisions. We find that asymmetric cell division is the main driver of notochord cell volume differences, but that there is also a modest but statistically significant tendency for smaller cells to be 'sorted' towards the ends of the notochord during mediolateral intercalation. We have excellent new 4D timelapses of notochord morphogenesis in *Phallusia* that we are currently analyzing to test the hypothesis that many of the asymmetric notochord divisions involve a novel mechanism that is not driven by polarized displacement of the mitotic spindle.

In another project, we are using RNAseq to quantify notochord gene expression in a broad range of wildtype and experimentally perturbed conditions. Among other conclusions, I will present evidence that Brachyury is much less potent in its ability to transfate other cell types into notochord than previously believed, and is thus perhaps not a 'master regulatory gene' for notochord morphogenesis as formally defined.

Talk 9
Genome and transcriptome assemblies for two Ascidia species

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Comparative genomics approaches have proven useful for identifying conserved non-coding sequences that may serve as putative cis-regulatory regions. Although the ascidian community has started to sequence the genomes of several additional ascidian species, none of these were readily available locally to our lab. We identified a pair of species from the genus Ascidia that we can obtain year-round in San Diego. Their embryonic development proceeds on a timescale that is similar to Ciona. Importantly, both species can be made transgenic using a modified electroporation protocol suggesting that they can be readily manipulated using all of the transgene-based approaches developed in Ciona. We first sequenced the genomes of both species by obtaining 100 million short-insert paired end reads (Illumina) that we estimated would be equivalent to 100X coverage. These reads were assembled with three different programs that gave quite different assemblies. The best performing assembler was Discovar; for one species producing 73,000 contigs with an N50 of greater than 23kb (longest contig 450kb). A Busco analysis determined that this assembly contains 85% of expected metazoan genes. Scaffolding is currently underway using additional long-read sequences. Based on rVISTA analyses of several genetic loci, we hypothesize that the genomes of the two Ascidia species are about as diverged as the two Phallusia species currently being sequenced. We also generated transcriptomes for both species using RNA extracted from mixed embryonic stages. A Busco analysis of the transcriptomes suggests that they are both nearly compete containing about 85% of expected genes. We annotated both transcriptomes against the UniProtKB using Annocript and against the Ciona robusta gene annotation. Interestingly, both Ascidia species contain numerous genes that are absent in Ciona suggesting that they are less diverged than Ciona. Finally, we compared the two Ascidia transcriptomes against each other and identified a number of genes that are differentially expressed. Of particular interest is a set of genes that are involved in cell migration that are only expressed in one of the two Ascidia species. This species also has a population of cells that we have not previously observed in larvae from over a dozen other ascidian species. These cells are found at random locations throughout the embryo. The number of cells is quite variable from embryo to embryo with some embryos containing dozens of these cells while other embryos have very few. Their nuclear morphology is similar to the nuclei in cells of the central nervous system of the larva. Experiments are currently underway to determine if these novel cells express these migratory genes.

Talk 10
Histone deacetylase activity is required for Botrylloides leachii whole body regeneration.

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The colonial tunicate Botrylloides leachii is exceptional at regenerating from a piece of vascular tunic after loss of all adults from the colony. This regeneration process rapid, going from vascular tunic to new zooid in as little as 8 days. Transcriptome analysis of regenerating fragments revealed the range of gene expression changes that occur over the time course of regeneration. However, there is still little understanding of how rapid changes to gene expression required to drive regeneration are initiated and how the regeneration process is regulated. We investigated the expression and function of a group of key epigenetic modifiers, histone deacetylases (HDAC) that are known to play an important role in many biological processes such as development, healing and regeneration.

We identified several B. leachii HDAC genes, two of which were expressed during whole body regeneration (WBR). To determine if histone deacetylation was required for WBR, we inhibited its activity through valproic acid (VPA) and Trichostatin A (TSA). Inhibition of HDAC activity prevented regeneration, along with altering the expression of genes linked to regeneration in comparison to controls. , Here we show that HDAC activity, specifically HDAC I/IIa enzymes, is vital for B. leachii to successfully complete whole body regeneration.

#Session 4 : Allorecognition and Immunity

Chair: Tony De Tomaso

Talk 11

Balancing selection on allorecognition genes in the colonial ascidian Botryllus schlosseri

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Allorecognition is the capability of an organism to recognize its own or related tissues. The colonial ascidian Botryllus schlosseri, which comprises five genetically distinct and divergent species (Clades A-E), contains two adjacent genes that control allorecognition: fuhcsec and fuhctm. These genes have been characterized extensively in Clade A and are highly polymorphic. Using alleles from 10 populations across the range of Clade A, we investigated the type and strength of selection maintaining this variation. Both fuhc genes exhibit higher within-population variation and lower population differentiation measures (FST) than neutral loci. The fuhc genes contain a substantial number of codons with > 95% posterior probability of dN/dS > 1. fuhcsec and fuhctm also have polymorphisms shared between Clade A and Clade E that were present prior to speciation (trans-species polymorphisms). These results provide robust evidence that the fuhc genes are evolving under balancing selection.

Talk 12
Amyloid and allorecognition in the colonial ascidian Botryllus schlosseri

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Allorecognition, i.e., the ability of intraspecific nonself recognition is widely distributed among colonial, sessile marine organisms in the form of colony specificity. In the cosmopolitan compound ascidian Botryllus schlosseri, colony specificity is controlled by a highly polymorphic Fu/HC locus: two colonies sharing at least one allele at the Fu/HC locus can fuse into a chimeric colony; if no alleles are shared, a typical inflammatory reaction occurs, with the recruitment of a specific hemocyte type, the cytotoxic morula cells (MCs), inside the tips of the ampullae (the blind termini of the tunic vasculature) extending towards the alien colony, their extravasation in the tunic and their final degranulation. As a consequence of allorecognition, necrotic, melanic spots (points of rejection; PORs) form along the contact border, due to the release, by MCs, of their granular content, mainly represented by quinones, polyphenols and the enzyme phenoloxidase (PO), upon the perception of the allogeneic humoral factors diffusing from the alien colony through the partially fused tunics. It is remarkable that the deposition of melanin and the cell death is confined to the immediate outside of the ampullar tips, suggesting that the diffusion of PO and the products of its activity are, in some way, prevented in order to limit cytotoxicity to the immediate neighbourhood of the contact region. In this context, we looked for factors released by MCs that could limit the spreading of cytotoxicity and melanisation.

We found that MCs share with vertebrate melanocytes similar packaging of melanin precursors, entrapped in a 3Dscaffold of amyloid fibrils. They contribute to form the electron dense content of MC granules that, after stimulation, flake off and is released in the surrounding medium. Released amyloid fibrils limit the diffusion of the produced melanin.

The search for genes and factor controlling both melanogenesis and amyloidogenesis, revealed an evolutionary conserved machinery involved in the processes and an unexpected cross talk between the two Botryllus immunocyte types, i.e., phagocytes and MCs. Furthermore, this work confirms the physiological role of amyloid in tunicate immunity.

a)

Talk 13

BHF mediates induction and loss of tolerance in Botryllus schlosseri chimeras

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Histocompatibility is the basis by which multicellular organisms of the same species distinguish self from nonself. To gain insights into the evolution and molecular characteristics of allorecognition, we are studying Botryllus schlosseri, a member of the tunicates. B. schlosseri engages in a natural transplantation reaction, whereby self-recognition between colonies leads to the formation of chimeras with a common vasculature, termed fusion, and non-self recognition results in rejection. Progeny from crosses between histocompatible B. schlosseri colonies are known to segregate as expected from a monogenic trait. We have found a polymorphic gene, called BHF, or Botryllus histocompatibility factor, that governs fusion or rejection outcomes between Botryllus colonies. Colonies fuse if they share at least one BHF allele AB=AB or AB=BC and reject if there are no alleles in common, AB=CD (Voskoboynik and Newman et al. 2013). Following fusion, one chimeric partner is often eliminated in a process of allogeneic resorption (Corey and Rosental et al. 2016). But stable chimerism where both partners thrive can also occur. Here, based on long term studies aimed to characterize molecular and morphological phenotypes of chimeras we provide evidence that BHF, and genes that promote immune response and death, are highly expressed in the eliminated chimeric partner. Furthermore, the degree of allelic differences between the non-shared BHF allele (e.g. A vs. C in AB=BC chimera) determines shortterm/stable chimerism phenotypes. Just as HLA haplotyping predicts the success of organ transplantation in humans, BHF haplotyping can predict both fusion/rejection outcomes and, short term/stable chimerism phenotypes in the Botryllus chimera.

#Session 5 : Nervous System Development and Anatomy

Chair: Ian Meinertzhagen

Talk 14
Interpreting the connectome of a Ciona intestinalis (L.) larva

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The nervous systems that drive animal behaviour are founded on synaptic connections between cells therein. Small nervous systems allow us to examine the entirety of a nervous system in great detail, revealing both ultrastructural and network details. We have used anatomical connectomic approaches by means of serial-section EM (ssEM) to analyze the number and distribution of synapses within the networks of the larval brain of Ciona intestinalis (Ryan et al., 2016; Ryan et al., 2017). The anatomical connectome reveals the components and connections of pathways for sensory integration of visual, gravity, and peripheral information both to and from pathways of other sensory systems. These sensory pathways feed into motor networks described through the current analysis that are involved in central pattern generation and a putative escape response (Ryan et al., 2017). Cell by cell analysis of morphology and all synaptic connections enables the identification of new cell types and the classification of all neurons in this small model chordate nervous system. The network complexity allowing integration at multiple levels provides a substrate for more complex larval behaviour based on interactions between pathways that converge on the motor generating network of the Ciona intestinalis larva. Thus, this hypothesis-generating analysis can help to propel the field of tunicate neurobiology and physiology into the era of connectomics.

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Talk 15
A conserved RNA switch for Acetylcholine receptor clustering at the neuromuscular junction

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The Ciona larva has emerged as a model for studying chordate-specific mechanisms of neural specification, patterning, and morphogenesis. Its nervous system comprises of a mere 177 neurons, and its connectome is only the second to be completely mapped, after the nematode C. elegans. Here we show that the larval motor neuron-specific expression of the RNA-binding protein Nova promotes neuromuscular synapse maturation through alternative splicing of mRNA transcripts of the Agrin gene, which encodes a secreted proteoglycan. Motor neuron-specific isoforms of Agrin promote the clustering of post-synaptic Acetylcholine receptors by interacting with the transmembrane receptor Lrp4/8 in tail muscle cells. This Nova-Agrin-Lrp pathway for Acetylcholine receptor clustering is shared between Ciona and mammals. However, we present evidence for coevolution between Nova proteins and the cisregulatory sequences embedded in Agrin introns that promote Nova-dependent alternative splicing, revealing "developmental system drift" of an otherwise highly conserved RNA splicingdependent molecular switch.

Talk 16

The cell lineage and the patterns of cell division for photoreceptor cells of the left-right asymmetric ocelli in the Ciona intestinalis larva

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The brain of the ascidian Ciona intestinalis larva contains two distinct photoreceptor organs, a conventional pigmented ocellus and a nonpigmented ocellus which are located at the right and left side of the larva, respectively. The photoreceptor cells of these ocelli are ciliary photoreceptor cells resembling visual cells of the vertebrate retina. Precise elucidation of cell lineage of the photoreceptor cells in the ascidian larva is a key to understand developmental mechanisms of these cells as well as evolutionary relationships between photoreceptor organs of ascidians and vertebrates. Previous studies inferred that the photoreceptor cells of the pigmented ocellus are derived from the right a9.33 and a9.37 cells, descendants of a blastomere of the anterior animal hemisphere. The photoreceptor cell lineage is, however, not conclusive because it was only a speculation based on observation of unlabeled embryos without using any photoreceptor markers. Here, we traced the fate of neural plate cells from the late gastrula to larval stages by labeling particular cells of intact (non-dechorionated) embryos at single-cell resolution using the photo-convertible fluorescent protein Kaede. We investigated which cells give rise to the photoreceptor cells by using photoreceptor-specific markers. Our results conclusively indicate that two right medial cells (A9.14 and A9.16) of the neural plate give rise to the photoreceptor cells of pigmented and nonpigmented ocelli, respectively. We further elucidated the temporal and spatial patterns of cell divisions up to the terminal differentiation of the photoreceptor cells. Our results reveal that dynamic yet stereotyped cell divisions shape the left-right asymmetric ocelli.

#Session 6 : Development and Evolution of the Nervous System

Chair: Takehiro Kusakabe

Talk 17, Poster 04
Putative neural crest-like cells in Tunicates, behavior, identity and fate of A10.64

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The evolutionary origin of the neural crest (NC), characteristic of vertebrates, is a matter of debate. Possible scenarios have been built from investigations in basal chordates including Cephalochordates and Tunicates, with a special interest for the latter since genome studies identified them as the closest emergence to vertebrates. Investigating the presence in Tunicates of cell types with NC typical characteristics proved being heuristic. Expected phenotypic features include epithelium to mesenchyme transition (EMT) at the border of the neural plate, migration, mesenchyme to epithelium transition and differentiation in NC derived specific cell types. Together with a major revision of the neural plate lineage, the recent observation of A10.64 migratory behaviour (Navarrete & Levine, 2016) suggests that it might be another NC-like candidate that has been overlooked so far. Hypotheses regarding the status of A10.64 include: i) the possibility that these cells respond to the same migratory cues as the bipolar tail neurons (BTN) previously identified as possessing some of the properties of neural crest derivatives (Stolfi et al., 2015) and ii) the possibility that A10.64 cells correspond to Islet-positive neurons of the visceral ganglion, i.e., an identity previously proposed for A10.57 in the context of erroneous lineage assignation. We further investigated these issues through 3D+time imaging and lineage reconstruction and analysis in Phallusia mammillata. Plasmid reporter construct injection was used to drive the expression of GFP under the control of Ci-coe regulatory sequences and follow these cells' delamination and migration with high spatiotemporal resolution. We provide insights into A10.64 behaviour and identity as well as conclusions about similarities in Ciona and Phallusia.

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b)

Talk 18, Poster 70
Neural crest and cranial placodes share a common evolutionary origin

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Neural crest and cranial placodes are the source of the most striking innovations of the Vertebrata, including a "new head" with centralized organs of special sense.The origins and relationship of neural crest and cranial placodes are long-standing mysteries. Experimental and molecular manipulations in Xenopus and chick embryos reveal a precocious demarcation in the developmental potential of lateral neural plate derivatives in cranial and trunk regions, raising the possibility that cranial placodes and neural crest arose independently of one another during the advent of the vertebrates. To address this issue, we examined the gene networks responsible for delineating the proto-placode and proto-neural crest territories in the Ciona embryos. The lateral plate ectoderm abutting the anterior-most regions of the neural tube was shown to be the source of the aATEN neurons, which share a number of common features with vomeronasal neurons derived from the olfactory placodes of vertebrates. In contrast, the lateral plate ectoderm adjacent to the posterior-most regions of the neural tube give rise to pATEN neurons and BTN neurons, which are thought to be homologous to dorsal root ganglia, a key derivative of the neural crest of vertebrates.

We present evidence that the lateral ectoderm is subdivided into rostral and caudal territories by the regulatory genes Dmrt.a and Msxb, respectively. Msxb maintains this boundary through the direct repression of the placodal determinants Six1/2 and Eyes absent (Eya). Lateral plate derivatives are transformed in Dmrt.a and Msxb morphants, including the conversion of aATEN neurons (proto-placode) into pATENs (proto-crest) and vice versa. Dmrt.a;Msxb double morphants exhibit a proto-placodal phenotype, whereby proto-neural crest sensory neurons are transformed into supernumerary aATENs. These studies suggest a common evolutionary origin for placodes and neural crest, and also raise the possibility that neural crest arose through the modification of a proto-placodal ground state.

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c)

d) KEYNOTE LECTURE

Expansion of the neural crest in the vertebrate lineage: evolutionary origin of vertebrate enteric neurons.

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Evolution of vertebrates is intimately linked to the advent of the neural crest, a migratory and multipotent cell population that gives rise to many defining vertebrate characteristics, including a well-defined head and peripheral ganglia. In all vertebrates, neural crest cells originate at the border of neural and non-neural ectoderm, migrate from the neural tube along defined pathways, populate numerous sites and differentiate into diverse cell types including melanocytes, sensory ganglia, and craniofacial cartilage. One important and early-branching group of vertebrates, the jawless fish (including the lamprey *Petromyzon marinus)* has bona fide neural crest cells, but lacks the full complement of neural crest derivatives, including jaws and sympathetic ganglia. Here we explore the origin of the lamprey enteric nervous system (ENS). The ENS is composed of an autonomous network of thousands of interconnected ganglia embedded within the walls of the gastrointestinal tract that regulate muscle contraction, secretion and water balance. Our data suggest that lampreys may be missing a discrete "vagal" neural crest population akin to those forming the ENS of jawed vertebrates. Rather, we find that late-migrating trunk neural crest cells, originating from the DiI-labeled trunk neural tube and closely associated with nerve fibers, can differentiate into serotonergic neurons within the gut wall. These trunk neural crest cells appear to be homologous to Schwann cell precursors of mammals, recently shown to populate post-embryonic parasympathetic including enteric ganglia of the colon. Thus, we propose that ENS ganglia may represent a novel neural crest-derived cell type that arose in the vertebrate lineage with the advent of jawed vertebrates.

#Session 7 : Gene regulation

Chair: Kenneth Hastings

Talk 19, Poster 03
A single cell transcriptional roadmap for cardiopharyngeal fate diversification

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Heart and pharyngeal muscles share developmental origins from a common pool of multipotent mesoderm progenitor cells - the cardiopharyngeal progenitors (CPPs). The failure of proper CPP specification is thought to cause severe cardiocraniofacial defects, including the DiGeorge/Velocardiofacial syndrome. However, the regulatory mechanisms governing cardiopharyngeal multipotency and early fate specification remain elusive. In particular, the complexity of mammalian organogenesis limits our understanding of the genome-wide spatialtemporal dynamics of early cardiopharyngeal development. Here, we took the advantage of a simple chordate model, the ascidian Ciona, to address fundamental questions regarding cardiopharyngeal specification. To obtain comprehensive transcription profiles encompassing the transition from multipotent cardiopharyngeal progenitors to distinct fate-restricted precursors, we performed single cell RNA-seq on FACS-purified cardiopharyngeal progenitor cells. We obtained a total of 848 high-quality single-cell transcriptomes from 5 developmental stages covering early cardiopharyngeal specification. Using the Seurat pipeline (http://www.satijalab.org/seurat.html), we identified each cell type based on their unique transcriptional signatures, including the first and second heart precursors (FHPs and SHPs), and the inner/outer atrial siphon muscle precursors (iASMs/oASMs). For each lineage, we reconstructed the developmental trajectory by mapping the cells onto their developmental 'pseudotime'. By focusing on the gene expression dynamics along each trajectory, we identified distinct regulatory states and developmental transitions, including those marked by cell divisions.

We observed that a fraction of the pan-cardiac and ASM genes were both primed in the multipotent progenitors, and then they were progressively excluded from the ASM and cardiac lineages respectively due to the asymmetric activation of FGF-MAPK signaling pathway. A total of 192 Ebf downstream genes were activated in two temporal waves during ASM maturation, contributing to the two-step transition from secondary TVCs to early ASMFs and early ASMFs to more specified ASMs. In parallel, the de novo pan-cardiac genes were activated lately in both FHPs and SHPs before the newly identified FHP and SHP specific genes, indicating the preferential organ (heart) fate determination prior to cell type determination during cardiac specification. We also found that the FHPs produced the beating MHC2+ cardiomyocytes, whereas SHPs were specified into MHC2- cells, which remained as a largely separated domain, adding to the FHPderived cells to form the more distal base of the conical heart in metamorphosed juveniles. With our single cell RNA-seq data, we identified a novel SHP-specific regulator Dach, a Dachshund-family transcription factor, as an SHP-specific target of Tbx1/10 and FGF-MAPK, that was required to oppose the FHP-specific program in newborn SHPs.

By deciphering the spatial-temporal dynamics underlying early cardiopharyngeal specification in Ciona TVC progeny using single cell RNA-seq, we are able to provide a blueprint of the early cardiogenesis, which will contribute to understand the cause of congenital cardio-craniofacial defects and find the potential genetic therapeutic targets.

Talk 20, Poster 05
Chromatin accessibility underlying cardiac vs pharyngeal muscle specification in Ciona

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The vertebrate heart and pharyngeal head muscles share common developmental origins and genetic underpinnings, but the relative complexity of vertebrate embryos has hindered the identification of common progenitor cells and detailed analyses of the cellular and molecular mechanisms underlying cardiopharyngeal development. Here, we harness the simplicity of the basal chordate Ciona to characterize the dynamics underlying the regulation of multipotency and progressive fate restriction during heart vs pharyngeal muscles (ASM) specification. In Ciona, founder cells produce anterior tail muscle (ATM) and bipotent cardiopharyngeal (TVC) cells that are multilineage primed and regulatory cross-antagonisms between the heart and ASM programs ensure fate segregation. To document open chromatin profiling of cardiopharyngeal precursors, we combined chromatin accessibility profiling to FACS-purified cells isolated following targeted molecular perturbations that alter heart vs ASM fate specification. Colleting ATACseq from a window encompassing developmental transitions from progenitor cells to heart- vs ASM-specific precursor, we built an atlas of accessible regions from all the replicates, and cells outside the cardiopharyngeal lineage, used to analyze differential accessibility.

We revealed that chromatin accessibility in founder cells is vastly different from that in TVCs and ATMs suggesting that chromatin remodeling cooperates with TVC/ATM induction. Moreover, the cis-regulatory landscape of active enhancers changes drastically between the TVCs and ATMs while more moderate changes are observed at late developmental stages where the majority of accessible regions are cell-lineage-specific, with few differences in accessibility between heart and ASM precursors. By motif enrichment analyses, we found the well-known pioneer factors GATA and Forkhead families motifs are enriched motifs in TVC-specific peaks while muscle regulators and pioneer factor MyoD and Myf5 are the top two most enriched motifs in ATMs. These results suggest that chromatin accessibility patterns are established early by pioneer transcription factors to define the competence of the cardiopharyngeal lineage by orchestrating the physical conformation of chromatin changes to form both heart and pharyngeal muscles.

Talk 21
Regulatory grammar governing enhancer specificity

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Enhancers are segments of DNA that act as switches to regulate when and where genes are expressed (for example turning on heart genes in the heart and neuronal genes in the nervous system). As such, enhancers provide the instructions for tissue specific gene expression, ensuring successful development and cellular integrity. An array of studies have demonstrated that mutations in enhancers can alter tissue specific expression causing phenotypic variation and disease; while, computational analysis suggests that the majority of mutations associated with disease are located within enhancers. Despite the fundamental importance of enhancers for successful development and organismal integrity, we lack a broad understanding of how enhancer sequence encodes tissue specific expression.

In particular the significance of enhancer grammar, the relationship between binding sites within an enhancer and their syntax (the order, orientation, and spacing of binding sites) is poorly understood. To define how enhancer grammar encodes tissue specific gene expression many grammatical variants need to be tested for function in all cells of a developing embryo. Ciona is the ideal model organism for such indepth functional approaches because millions of fertilized eggs can be electroporated with millions of enhancer variants in a single experiment. We have made half a million grammatical variants of a neural enhancer and have tested these for function in developing Ciona embryos. We have found grammatical variants that are non-functional, grammatical variants that results in ectopic expression and grammars that results in the endogenous neural expression pattern. I will present our current progress defining a grammatical code for neural specific enhancer activity.

#Session 8 : Imaging Ascidian Development

Chair: Nadine Peyriéras

Talk 22

A Nodal and Ephrin signalling relay drives the transition between apical constriction and apico-basal shortening during ascidian endoderm invagination

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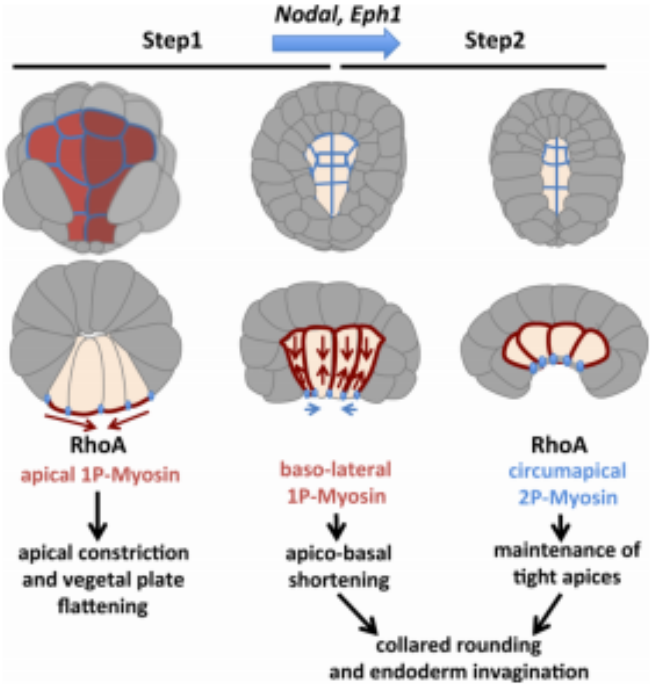
Gastrulation is a key early embryonic morphogenetic event. In ascidians the onset of gastrulation, endoderm invagination, takes place between 64-cell and late 112-cell stages when the embryo consists of a simple epithelial monolayer. Exactly 10 endoderm precursor cells undergo individual cell shape changes that ultimately drive tissue invagination of prospective endoderm and mesoderm cells. Endoderm cell shape changes comprehend a two-step process consisting of apical constriction (step1) followed by apico-basal shortening with maintenance of tight apices (step2) that induces endoderm invagination. This is driven by localized contractile activity of myosin. What causes the transition between steps 1 to 2 has remained elusive. Here we show in two distantly related ascidian species (Phallusia mammillata and Ciona intestinalis) that Nodal signalling activity at 32-cell stage relayed by Eph signalling controls the transition between steps 1 to 2 of endoderm invagination. This signalling activity does not affect endoderm cell fate specification providing a system where the invagination gene regulatory network can be at least partially uncoupled from the cell fate specification control.


Nodal, Eph1
Step1
Step2
RhoA
apical 1P-Myosin
apical constriction
and vegetal plate
flattening
baso-lateral
1P-Myosin
apico-basal
shortening
RhoA
circumapical
2P-Myosin
maintenance of
tight apices
collared rounding
and endoderm invagination

Talk 23
Spatiotemporal control of actomyosin contractility during zippering and neural tube closure in a simple chordate

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Tissue morphogenesis requires the dynamic coordination of cell shape changes in space and time. We are using ascidian embryos as a simple model system to study the spatiotemporal control of force generation during zippering in neural tube closure. We previously showed that zippering is driven by tissue-level asymmetries in actomyosin contractility: Local activation of Myosin II at apical junctions along the Neural/Epidermal (Ne/Epi) boundary ahead of the zipper produces forces that pull the neural folds together and drive the zipper forward. Behind the zipper, junctional exchange (Ne/Epi+Ne/Epi -> Ne/Ne+Epi/Epi), cell detachment, and local inhibition of Myosin II on new junctions, reduce posterior resistance to zipper progression. Here, we show that differential expression of a classical cadherin, Cadherin-2 (Cad-2), controls Myosin II activation at Ne/Epi junctions and zipper progression. Cad-2 is normally expressed only on the neural side of the Ne/Epi boundary; GFP-tagged Cad-2 protein accumulates at homotypic Ne/Ne junctions and is absent from heterotypic Ne/Epi junctions. Equalizing Cad-2 expression across the Ne/Epi boundary causes local accumulation of Cad-2 at apical Ne/Epi junctions, local inhibition of myosin activation at apical junctions along that boundary and prevents zippering. In contrast, creating ectopic Cad-2 expression boundaries directs ectopic Myosin II activation to apical junctions boundaries between Cad-2-expressing and non-expressing cells. We show further that differential expression of Cad-2 controls local activation Myosin II by locally activating the small GTPase RhoA. We identify a putative RhoA GAP (Gap-21), which is required for zippering/neural tube closure, and we present evidence that Cad-2 directs local activation of RhoA to Ne/Epi junctions by recruiting Gap-21 to Ne/Ne contacts and away from Ne/Epi contacts. Together, these results suggest a mechanism by which homophilic interactions between Cad-2 can inhibit local actomyosin accumulation along homotypic Ne/Ne boundaries, and direct actomyosin accumulation to heterotypic Ne/Epi boundaries ahead of the zipper. Combined with local inhibition of Myosin II at homotypic Ne/Ne contacts behind the zipper, Cad-2 creates a global contractile asymmetry that is required for unidirectional zipper progression.

Talk 24, Poster 06
Comprehensive characterization of Ca2+ transients in early Ascidian development

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Calcium ion (Ca2+) is one of the major second messengers, and the rapid and local Ca2+ increase (Ca2+ transient) is known to be involved in many biological processes. Although Ca2+ transients may be observed among wide-range of developmental stages and involved in important process of early development, it has been difficult to specify when and where Ca2+ transients would be observed in a developing whole-animal embryo. To characterize Ca2+ transients comprehensively during early embryogenesis of Ciona intestinalis, we used a fluorescent protein-based Ca2+ indicator, GCaMP6, combining with finely tuned fluorescence microscope systems.

We observed the GCaMP6-expressing embryo continuously for 6 to 8 hours from gastrula to tailbud stages. As a result, distinct Ca2+ transients were newly discovered in different cell lineages in addition to previously reported ones (Abdul-Wajid et al., 2015; Hackley et al., 2013). Interestingly, at the late tailbud stages, the frequency of Ca2+ transients in epidermal cells were drastically increased, and the timing of the increase was consistent with that of the relaxation of the tail curve.

The wave form of Ca2+ transients observed in this study are also characterized and categorized depending on the features like their duration and frequency. These comprehensive characterization of Ca2+ transients during early ascidian embryogenesis will provide a first step for understanding a wide variety of developmental roles of Ca2+ signaling in chordate embryogenesis.

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Talk 25
4D Clouds Embryos

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Ascidian embryos are particularly well adapted to high-resolution live imaging using multi-angle light sheet microscopy (MuViSPIM). The size of high-throughput light-sheet datasets however makes them difficult to interactively visualize. In order to characterize cellular behaviors during ascidians embryogenesis, we have developed a web interface to visualize and interact with whole embryo imaging datasets.

This tool, the "4D Clouds Embryos", is based on a video games framework and allows the manipulation of digitalised embryos at the single-cell level with very high spatio-temporal resolution. Running on standard web browsers (such as Firefox), 4D Cloud Embryos does not require any local software installation and offers a user-friendly visual interface.

Biologist users can perform a panoply of classical actions with simple clicks: the embryo can be cut, cells can be selected and tracked, specification events and cell lineage trees can be precisely displayed, colors maps can be applied to reveal quantitative cellular features including cell volumes, cell lifetime, geometric anisotropy, etc. Gene expression data from the ANISEED database can also be projected onto individual cells. Finally, 4D Cloud Embryos can be used for more complex quantitative analyses through its python API. 4D cloud embryos can also be used to visualize the results of mechanical simulations of embryogenesis.

The power of 4D Cloud Embryos will be exemplified by its application to the study of embryonic bilateral symmetry and to the analysis of cell division geometry - and its relationship to the classical Hertwig rule - at the single-cell level, allowing for an unprecedented depth in the analysis of morphogenetic events.

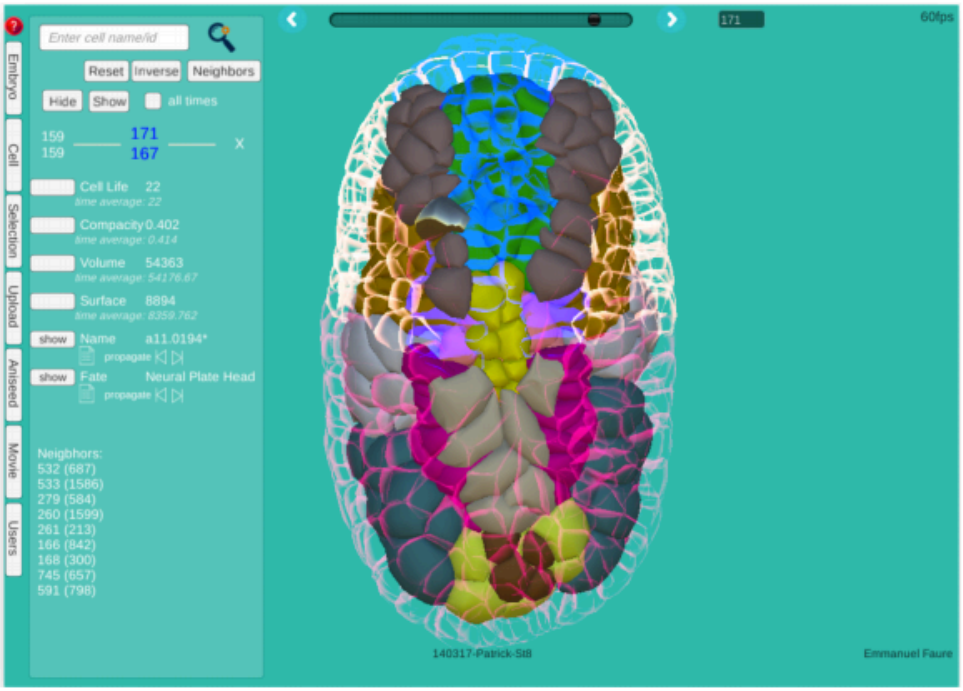

Figure : Screenshot of "4D Clouds Embryos"

#Session 9 : Systematics and Evolution (1)

Chair: Billie Swalla

Talk 26
Molgulid Tales

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Transcriptome and genome data offer an exciting new approach to examine the origin and evolution of the chordate body plan. Chordate body plan evolution can be studied with two tunicate species with radically different larval body plans that are found sympatrically off the coast of Roscoff, France - the tailed ascidian Molgula oculata and the tailless M. occulta. Tailed M. oculata embryos have forty notochord cells that are converged and extended in the center of the tadpole tail, as most ascidian larvae. The larvae also have tail muscle cells flanking the notochord in the tail, and in the head is the otolith, a gravity sensory organ. The tailless M. occulta does not form a tail in their larval stage, and have only twenty notochord cells that do not converge and extend during larval development. We have sequenced the genomes of these two species and a third species, M. occidentalis in collaboration with the Christiaen lab, and they are available on Aniseed (Stolfi et al. 2014). We show by transcriptome and in situ hybridization analysis that the notochord gene network is expressed at the right time and place in the tailless M. occulta embryos and larvae, although the notochord collapses into a “notoball” near the posterior. We show by transcriptome analyses that the ascidian metamorphosis program begins much earlier in molgulid ascidians, during early development. This radical heterochronic shift has been documented in another tailless ascidian, Molgula tectiformis, and is now reported for three additional species: the tailed molgulid species, Molgula oculata, Molgula occidentalis, and the tailless Molgula occulta. Further functional data is necessary to determine if this pronounced heterochrony is the necessary preadaptation for tailless tadpole to evolve in molgulid ascidians. This is an excellent model system to study the evolution of gene networks underlying morphology.

References

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Talk 27

Gains N' Losses during the evolution of RA and Wnt developmental pathways in *Oikopleura dioica* and ascidian species

Josep Martí-Solans[1], Alfonso Ferrández-Roldán[1], Miriam Diaz-Gracia[1], Ildiko Somorjai[2], Alba Almazán-Almazán[1], Marcos Plana[1], Paula Bujosa[1], Enya Duran-Bello[1], Kaoru S Imai[3], Hiroki Nishida[3], Ricard Albalat[1], and Cristian Cañestro[1,*]

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The bloom of Genomics is revealing a new perspective of gene loss as a pervasive evolutionary source of genetic variation that can influence the evolution of the mechanism of development of animal species. Our group, using the dismantling of developmental gene networks in the larvacean *Oikopleura dioica* and several ascidian species, investigates how losses (and duplications) affecting gene signaling pathways (e.g. Retinoic Acid and Wnt) have impacted on the evolution of urochordates, such as early AP axis specification. Our work also illustrates how the identification of patterns of gene co-elimination can be a useful strategy to recognize developmental gene network modules associated to distinct embryonic functions, and how the identification of survival genes can help to recognize neofunctionalization events and ancestral functions (Martí-Solans et al., 2016). Finally, our recent work on the mechanisms of heart development on *O. dioica* reveals another example of the inverse paradox of Evo-Devo, this is how similar structures at the morphological level are build despite important differences in their developmental genetic toolkits.

References

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Talk 28
Insights from complete mitochondrial sequences on the phylogenetic relationships of Aplousobranchia

Dorothée Huchon1,2*, Michal Hadass Sasson1 , Liran Dray1 , Emmanuel JP Douzery3 , Xavier Turon4 , Merrick Ekins5 , Gustav Paulay6 , Carmela Gissi7 , Noa Shenkar1,2

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2 The Steinhardt Museum of Natural History, National Research Center, Tel Aviv Israel
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The order Aplousobranchia contains approximately 1,500 species, both colonial and solitary. Although aplousobranchians include several harmful invasive-species, very little is known about their phylogeny. Our lack of knowledge regarding Aplousobranchia phylogenetic relationships can be explained by the fact that tiny distinguishing morphological characteristics are used for species identification. Complete mitochondrial (mt) sequences have been shown to be highly efficient for solving difficult phylogenetic relationships within tunicates. However, large-scale mt phylogenomic analyses of Aplousobranchia relationships are lacking. We here present an analysis, based on the 13 proteins encoded by complete mt sequences, which encompass representatives of 11 or the 12 Aplousobranchia families.

Our analysis includes 15 new mt sequences and provides some unexpected insights regarding Aplousobranchia relationships. First, three of the five Aplousobranchia families for which more than one species was sampled appear to be polyphyletic (i.e., Holozoidae, Polyclinidae, and Polycitoridae). Only Didemnidae and Clavelinidae are monophyletic. Furthermore, we found three well-supported novel, unexpected clades cutting across traditional families. The first new grouping clusters one Holozoidae species (Sigillina), Pseudodistomidae (Pseudodistoma), Protopolyclinidae (Condominium), two Polycitoridae species (both Eudistoma), and Stomozoidae (Stomozoa). The second unexpected grouping clusters the members of the genus Aplidium (Polyclinidae) with Ritterellidae and the second Holozoidae species considered (Distaplia). The last unexpected clade clusters another analyzed Polycitoridae species (Polycitor) and Euherdmaniidae (Euherdmania). Finally, Cystodytes (the remaining analyzed Polycitoridae) clusters with Didemnidae, albeit with moderate support. Other findings confirm the sister-clade relationship of Cionidae and Aplousobranchia, the early divergence of Clavelinidae among aplousobranchians, and the nested position of Diazonidae within Aplousobranchia.

These results suggest that a larger taxonomic sampling within each family is required to achieve a comprehensive understanding of the relationships among Aplousobranchia and of the mono/poly-phyly of each family.

#Session 10 : Systematics and Evolution (2)

Chair: Billie Swalla

Talk 29
Disentangling a species complex: the case of the colonial ascidian Botryllus schlosseri

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2 Department of Biosciences, Biotechnology and Biopharmaceutics, Università degli Studi di Bari "A. Moro", Italy
3 Natural History Museum of Venice, Italy
4 Department of Biology, Università degli Studi di Bari "A. Moro", Italy

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Botryllus schlosseri is a colonial ascidian commonly considered cosmopolitan and amply used as model for the study of asexual reproduction, developmental biology and immunobiology.[1,2] Recently, molecular data lead to hypothesize that the species named B. schlosseri is a species complex that consists of several cryptic species (namely, clades A-E).[3] In this context, and lacking both a type and a detailed morphological description, aim of the work was to designate a neotype and re-describe the species, as taxonomic reference point. Therefore, we provided a morphological description of samples from the Lagoon of Venice (Adriatic Sea, Italy). The description takes into account several developmental stages (oozooid, zooid of first asexual generations, and mature zooid), and was carefully compared with the previous morphological descriptions of samples coming from distant geographic areas (e.g., [4]). Both formalin- and ethanol-preserved specimens were deposited at the Natural History Museum of Venice, Italy, as neotype and topotypes. We associated our morphological description to a "DNA barcode", consisting of a longest sequence of the mitochondrial COI gene determined through new PCR primers: the designed neotype resulted to belong to the cryptic species previously named clade A. We think that the present study represents a prerequisite to investigate the existence of morphological and developmental differences between the other "cryptic species" hidden under the current name B. schlosseri. This will clarify if the research community is running into a second case of challenging nomenclature division referred to a model organism, similar to the recently one occurred in the solitary ascidian Ciona intestinalis[5].

References

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Talk 30
Is Styela canopus a complex of species?

Rodolfo Corrêa de Barros1,2 , Rosana Moreira da Rocha 2,* 1 Universidade Positivo, Curitiba, Paraná, Brazil 2 Universidade Federal do Paraná, Curitiba, Paraná, Brazil *

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With the advent of DNA barcoding it is now possible to recognize a growing number of species that are not clearly identified by morphological characters and which form complexes of cryptic species. Styela canopus (Savigny, 1816) is a solitary ascidian, rarely > 2 cm in length, and usually identified by a pair of elongated gonads on each side of the body. Its variable external morphology (Abbott et al., 1997) and wide geographic distribution suggest that cryptic species are being united under a single name. To investigate this possibility, we are using the CO1 marker to estimate genetic relationships among S. canopus populations of the world. Phylogenetic relationships were inferred using the Maximum Likelihood method in MEGA 6.0, using GTR+G+I model, with Styela plicata (GenBank: AM292601.1) and Styela clava (GenBank: HG931920.1) as outgroups and bootstrap support (100 replicates). Gene flow was estimated using Fst statistic with help of DNA SP 5.1 software.

Eleven populations were sampled from Brazil (4) and elsewhere around the world (7). A total of 147 S. canopus sequences (633 bp) were obtained with 75 distinct haplotypes (Hd: 0.98209). All haplotypes from Japan (blue) were included in a monophyletic clade, with S. clava as the sister group (Fig. 1). Also, all haplotypes from Panama (yellow) and from Massachusetts/Georgia (pink and green, respectively) formed monophyletic clades. The Fst value between Massachusetts and Georgia was zero, indicating that both regions comprise the same population. Additionally, Fst values from Japan, Panama and Massachusetts/Georgia and the remaining populations were > 0.5, suggesting very low gene flow between these populations. All other haplotypes formed another monophyletic clade, comprising different local populations with low Fst values, suggesting that they all belong to a single, connected, population. These partial results indicate that the heretofore S. canopus is rather a complex of different species. We are now searching for morphological differences that will distinguish them. Next, we will include five more populations (four from Brazil, and one from the Galapagos) in the analysis and compare topologies of CO1 and ANT (nuclear gene) phylogenetic trees.

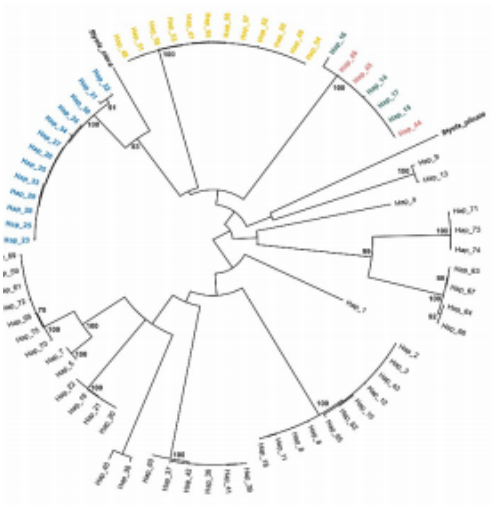

Figure 1. Phylogenetic relationships among haplotypes of *Styela canopus* based on Maximum Likelihood. The number above a node indicates its bootstrap support. Only support values > 75% are shown.

References

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Notes: partial results of PhD thesis of RCB financed by CNPq (445783/2014-1).

KEYNOTE LECTURE

The continuing saga of a sea squirt detective: fouling ascidians in the Galapagos on artificial surfaces

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From April 24-May 4, 2016 a group from the Smithsonian Environmental Research Center (SERC) Invasions Lab surveyed a number of floating docks and pilings and numerous settlement panels both uncaged and caged, on two of the Galapagos Islands: Santa Cruz and Baltra. The panels had been submerged for either 3 months or 14 months. Studies were concentrated on several sites on Santa Cruz Island because this is the location of the main town, Puerto Ayora, and has the most boat and ship traffic. The airport is located on the small adjacent Baltra Island and there is frequent boat traffic there, as well as a large Navy base and dock. Ascidians were the dominant foulers on most panels, especially the caged ones, with a dramatic difference in coverage between caged and uncaged which is presumed to result from differential predation pressure. Since the panels were all suspended with none touching the benthos, the predators are hypothesized to be primarily fish. There were schools of small fish around all the docks, and though they were not identified they were observed to be constantly nipping at the dock surfaces. While this is not the first fouling study to compare caged and uncaged surfaces, it does show that similar studies should include both treatments of settlement panels.

Nineteen species of ascidians were collected. The most common were Didemnum perlucidum, Ascidia sydneiensis, an Ascidia sp. with long tunic spines, 2 small pink Pyura spp. one of which has long siphonal spines, two unidentified didemnids, Diplosoma cf. listerianum, Polyclinum constellatum, Polyandrocarpa zorritensis, and Styela canopus. Several colonies of Botrylloides giganteum were retrieved from plates on the Baltra Navy dock. Other common foulers included Botrylloides nigrum and a few small colonies of Symplegma sp. Two large specimens of Halocynthia dumosa (~6 cm) (usually incorrectly referred to as H. hispida) were collected from the Baltra Is. Navy floating dock and one by SCUBA at 40 ft off Bartolome Is. by Greg Ruiz. A complete species list will be presented, including new records for the Galapagos, and a comparison with previous published and unpublished records.

I thank SERC for their support for this study.

#Session 11 : Ecology (1)

Chair: Noa Shenkar

Talk 31
Algal-bacterial interactions: Mutualism, antagonism and chemical signaling

Shady Amin

NYU Abu Dhabi

Interactions between phytoplankton and bacteria arguably represent the most important interorganism association in aquatic environments. These relationships influence fundamental processes that include nutrient provision and regeneration, primary production, harmful blooms and biogeochemical cycling. Although typically studied over large spatiotemporal scales, emerging evidence indicates that this relationship is often governed by microscale interactions played out within the region immediately surrounding individual phytoplankton cells known as 'the phycosphere.' The exchange of metabolites and infochemicals at this interface governs phytoplankton-bacteria relationships, which span mutualism, commensalism antagonism, parasitism and competition. My talk will highlight how the exchange of chemical currencies between algae and bacteria leads to different modes of interactions in several algal-bacterial model systems and evidence that such interactions are important in the marine environment. I will also discuss the population and chemical dynamics of the algal microbiome and potential importance to biogeochemistry.

Talk 32

Med vs. Red: temperature sensitivity of the non-indigenous ascidian Herdmania momus in the Mediterranean Sea

Mey-Tal Gewing1,* , Noa Shenkar1, 2

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The non-indigenous ascidian Herdmania momus (Savigny, 1816) is rapidly expanding its distribution range across the Mediterranean1. The change in its distribution and the fact it is becoming well established along the western edge of the Levantine Basin and central Mediterranean2, indicates its transition from "non-indigenous" status to "invasive". Better understanding of dispersal and establishment patterns in new environments may assist us in evaluating its possible impact on native communities and future dispersal to new regions, in addition to understanding the factors contributing to successful invasions of tropical-origin species. In order to determine the environmental tolerance range of H. momus, we examined the effect of varying temperature on fertilization and larval development, as well as the survival of adult individuals from native (from Red-Sea) and non-indigenous (Mediterranean) populations in several laboratory settings. Our results revealed that fertilization and larval development rate were significantly higher in 24oC and 31oC, compared to 16oC. When we compared adult individual survivability of Mediterranean vs. Red-Sea populations, a significantly higher survivability was observed in the Mediterranean population. Adult individuals from native fauna demonstrated significantly lower survivability in 16oC and 31oC treatments, while the survival of adult specimens from Mediterranean population was lower only in 16oC, compared to 24oC and 31oC. We concluded that despite its sensitivity to cold water, it seems that the Mediterranean population has adapted to a wider range of temperature. We expect this ability will enable this species to further spread into new locations along the Mediterranean coast.

References

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Talk 33
Connecting water temperature to reproductive physiology and molecular signals in C. intestinalis

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Ciona intestinalis exhibits lower reproductive success at the upper extreme of water temperatures it experiences in coastal New England. In order to understand the changes in protein expression associated with elevated temperatures, and possible response to global temperature change, we reared C. intestinalis from embryos to adults at 18°C (a temperature at which they reproduce normally at our collection site in Rhode Island) and 22°C (the upper end of the local temperature range). We then dissected ovaries from animals at each temperature, extracted protein, and measured proteomic levels using shotgun mass spectrometry (LC-MS/MS). 1532 proteins were detected at a 1% false discovery rate present in both temperature groups by our LCMS/MS method. 62 of those proteins are considered up or downregulated according to our statistical criteria. Principal component analysis shows a clear distinction in protein expression pattern between the control (18°C) group and high temperature (22°C) group. Apart from findings consistent with previous studies, we observed extreme upregulation of certain signal transduction and lipid transport proteins, including PTPN11, CrkL, Apolipoprotein B, and Sec1, in the higher temperature group. We propose a working model for the high temperature response in C. intestinalis ovaries whereby increased temperature induces upregulation of signal transduction pathways involving PTPN11 and CrkL and activating coordinated changes in the proteome, especially in large lipid transport proteins, cellular stress responses, cytoskeleton, and downregulation of energy metabolism. We present plans to test these hypotheses using CRISPR/Cas9 gene editing and assays of reproductive success.

#Session 12 : Ecology (2)

Chair: Shigeki Fujiwara

Talk 34
Introduced and native ascidian microbiomes from artificial versus natural habitats

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Harbor systems act as passive gateways for the introduction and long-term establishment of ascidian species worldwide. Once established, introduced ascidians can spread from artificial harbor habitats and colonize the adjacent natural environment by larval dispersal. To investigate the potential role of microbial symbionts in the success of ascidian introductions and spread, we examined the microbial communities of three colonial ascidian species introduced in North Carolina (NC) harbors (Distaplia bermudensis, Polyandrocarpa anguinea, and P. zorritensisi) and of two colonial species found both in harbors and adjacent natural reef systems (the native ascidian Eudistoma capsulatum and the introduced D. bermudensis). Microbial communities were characterized by next-generation (Illumina) sequencing of partial (V4) 16S rRNA gene sequences. Introduced ascidians in NC hosted diverse symbiont communities, consisting of 5,696 unique microbial operational taxonomic units (OTUs; at 97% sequenced identity) from 47 bacterial and 3 archaeal phyla. Permutational multivariate analyses of variance revealed clear differentiation of ascidian symbionts compared to seawater bacterioplankton, and distinct microbial communities inhabiting each ascidian species. 103 universal core OTUs (present in all ascidian replicates) were identified, including taxa previously described in marine invertebrate microbiomes that have been linked to ammonia-oxidization, denitrification, pathogenesis, and heavy-metal processing. Further, native ascidians collected from artificial and natural habitats hosted significantly different symbiont communities, though this was not the case for the introduced species. These results suggest that ascidian microbial symbionts exhibit a high degree of host-specificity, forming intimate associations that may contribute to host adaptation to new environments via increased tolerance thresholds and enhanced holobiont functioning. Moreover, these associations were stronger for introduced species than native ones, potentially enhancing the survival and spread of introduced ascidians across a wide range of environmental conditions.

Talk 35

Moving to the reef: historical and present observations of the worldwide introduced species Styela plicata in the Northwest Atlantic continental shelf

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Despite reports of the cosmopolitan range of the solitary ascidian Styela plicata (Lesueur, 1823) in warm temperate and tropical waters dating back to 1882, S. plicata was not described as non-native to any particular locality until 1983. The wide distribution of the species, coupled with the fact that the type specimen was collected from a transient vessel in a locality where resident populations were not known to occur (Van Name 1945), has effectively obscured the historical records with regard to the native range of S.plicata. Previous attempts to determine the native range of S. plicata via global phylogeography using both nuclear and mitochondrial genes have been unsuccessful, possibly due to a loss of the molecular signal by centuries of S. plicata constantly being transported by global ship movements (Pineda et al. 2011). In most cases, S. plicata distribution is restricted to artificial substrates (e.g. Lambert & Lambert 2003, Rocha & Kremer 2005; Barros et al. 2009; Pineda et al. 2011, 2016; López-Legentil et al. 2015), with occasional citations of individuals off the coast of Spain, Brazil, and Japan. An extensive literature review found one major exception to this trend, with well-established and abundant populations living off the Northwest Atlantic continental shelf along the southern United States. Known from coastal North Carolina (NC) as early as 1871, S. plicata was reported as abundant on natural substrates as early as 1900 and is currently a common member of live bottom temperate reef communities off the coast of Georgia (GA) and NC. Here, we investigated whether the Northwest Atlantic continental shelf could be the native region of S. plicata by sequencing a fragment of the mitochondrial cytochrome oxidase I gene (COI) from individuals collected from NC and GA reefs (natural substrates) as well as a NC harbor (artificial substrate). We then compared the resulting sequences (n=51) with all representative haplotype sequences for this species available in GenBank. Results showed a high number of private haplotypes for this region; however, all haplotypes clustered exclusively within Group 2 described in Pineda et al. (2011). These results suggest that, despite the extended and well-documented presence of S. plicata on natural substrates for this region, S. plicata is not native to the northwest Atlantic continental shelf. Rather, this species was able to colonize and establish sustainable populations in this area a long time ago. Further research should establish whether natural ecosystem functioning is disturbed by the presence of this species and whether it is necessary to elevate the current status of the species in the area from 'introduced' to 'invasive.'

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Talk 36, Poster 07
Structure-Function Analysis of the Filter-House of the Appendicularian Oikopleura diocia

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The gelatinous cellulosic house of the appendicularian Oikopleura dioica is a sophisticated filter machine. The solitary free-swimming larvacean secret an elaborate multilayered rudimentary tunica around its trunk, which later can be inflated and serve as filtering-house for the animal. When inside the house, through an undulatory movement of the tail, the animal sucks surrounding water into the house and circulates it through various compartments of the multi-chambered structure, within which the food particles get trapped and accumulate inside a complex meshed membrane system to nourish the animal. The 3D architecture of the so called filter-house in the inflated functioning, and in the folded rudimentary states are of crucial importance for better understanding of the filter operation as well as the unfolding mechanism of the complex structure.

A complementary range of approaches such as 3D reconstruction of histological sections, confocal laser scanning microscopy, electron microscopy studies with SEM, TEM and FIB SEM etc. were employed to address the structure of the house rudiment in the folded state. While a wide range of light microscopy images and films combined with light-sheet microscopy imaging provided the basis for a 3-dimensional understanding and visualization of the house in the inflated state, followed by the finite element modeling of the flow dynamics to shed light on the inflation mechanism of the house.

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Talk 37
How ecological factors influence growth patterns of colonial and solitary ascidians

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Colonial animals, those made up of connected asexually-derived modules, occur in a number of metazoan lineages. While solitary animals grow larger as a single unit, colonial animals grow by adding modules. The modules of colonial animals are typically smaller than solitary animals, however, whole colonies can attain larger biomasses than solitary species, and have great regenerative and propagative abilities. These two distinct morphogenetic life history strategies are linked to ecological differences that may affect their ability to compete for space or withstand disturbance. To what extent colonial and solitary life histories are affected by ecological factors remains poorly understood. The tunicates are one of only several animal taxa that contain both solitary and colonial species, making them a useful model for the study of the significance of these two life history strategies. In order to understand the effects of ecological factors on solitary and colonial ascidians, we conducted a manipulative experiment to measure the effects of competition and predation on growth. Eight species from southeastern Brazil – two solitary and six colonial – were subjected to a predation treatment (caged vs. uncaged) and a competition treatment (removing competitors by hand or leaving them as-is) to assess main effects and interaction effects. The area of individual or colonial forms was measured over the course of three months. We found that predation had a greater effect on the growth of colonial forms compared to solitary species. Competition did not show consistent patterns between life forms. These results suggest the importance of predation on growth patterns of ascidian life forms, with colonial species showing more susceptibility to predation. We hypothesize that colonial animals may put less energy into defenses since they have the ability to regrow lost members when partially consumed and they exhibit plasticity in shape and directional growth that may allow them to grow into spatial refuges free of predators.

#Session 13 : Late embryos and early larvae

Chair: Filomena Ristoratore

Talk 38
Gsx in Ascidian Central Nervous System Development

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The neural plate of *Ciona* consists of a grid-like array of cells arranged into columns and rows. Recently, we have shown that a unique combination of three signaling pathways, Nodal, Delta2/Notch and FGF/MEK/ERK, defines distinct cell identities in the a-lineage row III of the neural plate, with Nodal/Delta-Notch involved in mediolateral patterning and FGF/MEK/ERK signalling required for posterior identities. We have investigated the links between the identified patterning mechanisms and the cis-regulatory modules of *Gsx*, a gene that is expressed specifically in the intermediate column of row III at neural plate stage. In addition, we have revealed an intriguing expression pattern of *Gsx* from neural plate to late tailbud stages, which prompted us to explore the involvement of this gene in photoreceptor cell differentiation.

Talk 39

Klhl21: a new player for pigmentation from *Ciona robusta*

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1- Background

Pigmentation is one of the most fascinating topics in animal kingdom, central in diverse evolutionary challenges. The ascidian *C. robusta* larva has two pigmented organs, the otolith and the ocellus, inside sensory vesicle (1). The first is a single melanized cell used for geotactic response (2) while ocellus a photosensitive multicellular organ with three lens cells, 30 photoreceptors and one melanophore. These arise from a8.25 blastomeres that during gastrulation, divide one time in a9.50 and a9.49 cell pairs: the latters are pigment cell precursors (PCPs). Ascidians are the closest living relatives of vertebrates (3) and it is possible to combine developmental and genomic studies to precise cellular resolution analyses, rendering *C. robusta* a powerful model to study pigmentation. Recently, it has shown a decisive role for *Ciona* FGF/MAPK/Ets pathway in pigment cell specification (4).

2- Results and Conclusions

Using a combination of developmental biology and genomics we discovered a new FGF-dependent player in ascidian pigmentation, the Klhl21, belonging to big Kelch-like family. An evolutionary survey clarifies its orthology with vertebrate Klhl21s, involved in cytokinesis (5). During *Ciona robusta* embryogenesis, this gene is expressed solely in the two PCPs at early tailbud stage and only otolith from mid-tailbud stage (Fig. 1A). To study its transcriptional regulation, we analyzed 1kb region (klA) ability to recapitulate endogenous expression by GFP reporter assay: it drives larvae otolith expression (Fig. 1B). Several delections demonstrated that a 382 bp fragment (klB) is the smallest able to restore GFP activity. A bioinformatic and mutational survey on KLB indicates Mitf as the principal activator of Klhl21 with Dmrt and Msx as crucial co-activators. Given the zebrafish ortholog is present solely in non-pigmented nervous system territories, we suppose a functional diversification across different chordates. Next steps will be: to deepen Dmrt and Msx role in Klhl21 regulation and the possible function of this gene, through CRISPR/Cas9 system (6)

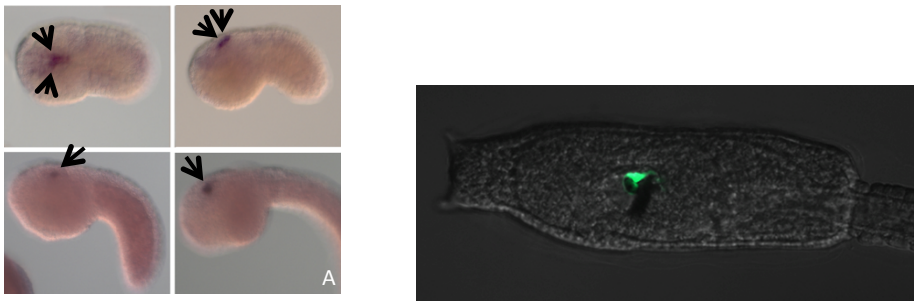

Figure 1. Klhl21 expression during *C. robusta* embryogenesis and klA>GFP activity.

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Talk 40
Laterally asymmetric morphogenesis of the heart and gut in Ciona intestinalis

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The evolutionary emergence and diversification of the Chordates involved dramatic changes in organ morphogenesis along the left-right axis. However, the ancestral chordate mechanism for establishing lateral asymmetry remains ambiguous. Additionally, links between initial establishment of lateral asymmetry and subsequent asymmetries in organ morphogenesis are poorly characterized. To explore asymmetric organ morphogenesis during chordate evolution, we have begun to characterize left/right patterning of the Ciona heart and endodermal organs. Here we show that Ciona has a laterally asymmetric, right-sided heart. Our data indicate that cardiac lateral asymmetry requires H+ /K+ ion flux but is independent of Nodal signaling. Our pharmacological inhibitor studies show that ion flux is required for polarization of epidermal cilia and neurula rotation and suggest that ion flux functions synergistically with chorion contact to drive cardiac laterality. Live imaging analysis revealed that larval heart progenitor cells undergo a lateral shift without displaying any migratory behaviors. Instead, out data suggests that ion flux promotes laterally asymmetric morphogenesis of the larval endoderm rudiment leading to a passive shift in the position of associated heart progenitor cells. To better understand the gene regulatory network driving lateral organ morphogenesis, we have also begun characterizing the function of genes displayed laterally asymmetric expression in late tailbud stage embryos, when initial asymmetries in cardiac and endodermal rudiments are first detected.

#Session 14 : Post-embryonic Development

Chair: Thomas Stach

Talk 41, Poster 08

Morphogenetic function of apoptosis during tail regression at the metamorphosis stage of Ciona intestinalis

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Animal metamorphosis refers to the profound morphological changes that affect many organisms during their life cycle. This developmental transition is characterized by various cellular mechanisms, such as migration, proliferation, differentiation and programmed cell death (PCD). Apoptosis (PCD type I) is a genetically controlled process defined by a characteristic and conserved set of morphological features which depend on caspase activation. The caspase protein family are present in all metazoans but essentially studied in vertebrate. This family is divided in two functional groups, the inflammatory caspases and the apoptotic ones. Among the apoptotic group there are two sub-groups, the executioner (caspase 3, 6 and 7) and the initiator (caspase 2, 8, 9 and 10), which are both implicated in the two main apoptotic pathways: the extrinsic pathway implies death receptor on the cellular membrane, whereas the intrinsic pathway is based on the mitochondria and the non-apoptotic caspases (1, 4 and 5). Apoptosis has long been described in metamorphosis as merely required for the removal of larval structures. However, studies in hydra, drosophila and chicken gave evidences of caspase-dependent signals released by apoptotic cells that promote cell proliferation, migration and survival (1, 2 and 3). This study focus on apoptosis during the metamorphosis of Ciona intestinalis in which our previous results revealed this potential new morphogenetic function. We also address the status of the caspase family by phylogenetic analysis to understand the evolution of this family at urochordates but also metazoans scale. At the onset of the metamorphosis of the Ciona intestinalis tadpole-swimming larva (urochordates), the driving force of the tail regression is an apoptotic polarized wave from the posterior to its anterior part (4). All the tail' tissues are eliminated except (i) the endodermal strand and (ii) the primordial germ cells (PGCs). Endoderm and PGCs migrate at the onset and during the apoptotic polarized wave progression respectively (5, 6 and 7). Our results shown that the PGCs movement and the endodermal strand migration speed is correlated with the apoptotic wave propagation. Moreover, inhibition of the caspase activities (Z-VAD-Fmk) block tail regression but also PGCs movement that strongly comfort a caspase-dependent process. A phylogeny with vertebrates sequences allow us to show a monophyly of the executioner and two distinct groups of initiators. The high number of caspases in urochordates requires phylogenetic analysis to understand the homologies relationship between caspases. The absence of some fundamental initiator caspases in Ciona intestinalis and multiple duplications which took place in urochordates argue of an evolutionary scenario different than vertebrates and more complex than currently accepted.

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Talk 42
Patterning and morphogenesis of the intricate but stereotyped Oikoplastic epidermis of the appendicularian, *Oikopleura dioica*

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Mechanisms for morphogenetic processes that generate complex patterns in a reproducible manner remain elusive. Live imaging provides a powerful tool to record cell behaviors. The appendicularian, *Oikopleura dioica*, has a rapid developmental speed, small number of cells (less than 3500 cells in a juvenile), and a transparent body. The trunk epidermis, called the oikoplastic epithelium (OE), has elaborate cellular arrangements showing a complex pattern to secrete extracellular "house". The OE is characterized by invariant number, size, and shape of the monolayer epithelial cells. Pattern formation is achieved during 5 hours of larval development without growth of the body, making this a suitable system for live imaging of 2D sheet. First, we subdivided the OE and defined several domains by cellular resolution, and systematically gave names to the constituent cells, since there is no variation among individuals.

Time-lapse imaging of the epidermal cells revealed intriguing and region-specific pattern formation processes. Each identified domain served as a compartment into which distribution of descendant cells of founder cells is restricted. Regulation of orientation, timing, and the number of rounds of cell divisions, but not cell death and migration, was a critical mechanism for determination of final cell arrangement and size. In addition, displacement of epithelial sheet plates was observed in the Eisen region. Stem-cell-like cell divisions, whereby large mother stem cells generate a chain of small daughter cells, were involved in formation of the Nasse region and ventral sensory organ. These are the first examples of this kind of stem-cell-like cell division in deuterostomes.

Furthermore, labeling of the left or right blastomere of the two-cell-stage embryo, which roughly gives rise to the left or right side of the body, respectively, revealed that the boundary of the descendant cells does not match with the midline of the trunk epidermis. Left and right descendants largely invade into the opposite side in an invariant way, suggesting the possibility that specification of the OE cell identities may occur later in development, most probably around hatching, depending on cell position in the OE epithelial sheet. These detailed descriptions of OE patterning processes provide basic and essential information to analyze further cell behaviors in the generation of elaborate and intricate but stereotyped 2D cellular patterns in this advantageous model system.

Talk 43, Poster 09
Molecular composition and architecture of extracellular matrix in ascidian embryogenesis and larval metamorphosis

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The extracellular matrix (ECM) not only provides essential physical scaffolding for cellular constituents but also initiates crucial biochemical and biomechanical cues that are required for tissue morphogenesis. In this study, we used the RNA-seq data from embryos and larvae of Ciona savignyi to identify ECM genes and acquire their expression patterns. 114 unigenes were identified as 66 ECM genes. 77 unigenes showed dynamic expression changes between different stages. Then we utilized wheat germ agglutinin (WGA) staining to characterize the ECM architecture. The results showed three distinct populations of ECM presenting in Ciona embryogenesis: the outer layer localized at the surface of embryo, while the inner one was notochord sheath and the third one was apical ECM secreted by notochord. To further elucidate the precise structure of Ciona embryonic ECM, we employed scanning and transmission electron microscope and found that the outer membrane was relatively thicker with short fibers, whereas the notochord sheath was orientated along the body axis with fibril architecture; the lumen between notochord cells was hydrostatic and sticky. Thus, our results present the profile of molecular composition, dynamic expression and architecture of ECM in ascidian embryogenesis, providing intriguing cues for understanding function of ECM in chordate development.

Talk 44
In vivo manipulation of the extracellular matrix induces vascular regression in a basal chordate

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We investigated the physical role of the extracellular matrix in vascular homeostasis in the basal chordate Botryllus schlosseri, which has a large, transparent extracorporeal vascular network encompassing areas >100 cm2. We determined that collagen crosslinking enzyme lysyl oxidase 1 (LOX1) is expressed in all vascular cells. In vivo inhibition of LOX1 by a specific inhibitor, ß-aminopropionitrile (BAPN), caused a rapid regression of the entire vascular bed, with some vessels regressing >10 mm within 16 hours. BAPN treatment disrupted the collagen ultrastructure in the basement membrane and was detected by integrin signaling. Regression is driven by apoptosis and extrusion of cells through the basal lamina and into the blood stream, which are then engulfed by blood-borne phagocytes. Extrusion and regression occurred in a coordinated manner that maintained vessel integrity, with no loss of barrier function. This suggests the presence of regulatory mechanisms linking physical changes to a homeostatic, tissue level response.

#Session 15 : asexual reproduction and regeneration

Chair: Federico Brown

Talk 45
Detour to make muscles? Myogenesis in the budding process of a colonial ascidian, Botryllus schlosseri

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Colonial ascidians such as Botryllus schlosseri develop adult zooids not only through embryogenesis and larval metamorphosis, but also asexually by budding of the atrial epithelium and epidermis (blastogenesis). The process of generating muscle, myogenesis, has been studied during embryogenesis and through metamorphosis, but it is still unclear how muscles develop during blastogenesis.

Similarly to solitary species, the musculature of B. schlosseri blastozooids is formed by circular and longitudinal non-striated body muscles and cardiac muscles. In Ciona intestinalis and Halocynthia roretzi myogenesis a few conserved effectors are crucial to achieve muscle fate: 1) the maternally expressed Zic.r.a (Macho-1) drives activation of the transcription factor Tbx6 during early embryogenesis; 2) in tadpoles, the Mesp+ descendent of the blastomeres B7.5 lead to Mrf expressing tail muscles and to Tbx1/10 expressing ventral trunk cells (TVCs), i.e. the heart and adult body muscle precursors; (3) the fate of TVCs is regulated by Nk-4 for heart and Ebf for body muscle development.

To investigate the myoblast origin and the mechanisms that lead to blastogenetic muscle formation and in order to test the potential co-option of a myogenic motif during asexual development, we isolated the molecular components of embryonic and metamorphic myogenesis, followed their expression during blastogenesis of B.schlosseri and reconstructed the dynamics of muscle formation.

In our study: 1) we confirm that the same molecular players as in solitary species are employed in embryonic and metamorphic myogenesis of Botryllus ; 2) we reveal that myogenesis is triggered de novo during morphogenesis, in every new blastogenetic cycle; 3) we observe the absence of maternal determinants as well as of the molecular modules involved in larval muscle formation and 4) we see that co-option of the molecular motifs leading to heart and adult body muscle formation occurs only in late morphogenesis and its linked to cell and tissues behavior different from the ones described during embryogenesis/metamorphosis.

In summary, regardless of the developmental pathways followed, i.e. embryonic or blastogenic adult muscles have the same molecular and anatomical setup (Myosin Heavy Chain III,II) but their regulations are characterised by an orchestration of co-option and loss of molecular modules.

Talk 46
Asexual reproduction in Styelidae (Ascidiacea): and evo-devo approach

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Styelidae (Stolidobranchia) groups more than 550 species and comprises both solitary and colonial forms. Whereas solitary species can reproduce only sexually, colonial ones have the ability to also propagate asexually by different modes of budding and often can regenerate the body completely after injury. Depending on the species, budding and whole body regeneration can be triggered either by stem cells and/or by trans-differentiation of specific epithelia. Despite low support for several branches, the most recent Styelidae phylogeny suggests independent acquisition of asexual reproduction at least twice: in Symplegma+Botryllidae and in Polyandrocarpa. Here, we present two approaches to elucidate the molecular mechanisms underlying the evolution of coloniality and regeneration in ascidians. First we generated a robust phylogeny of Styelidae based on 20 transcriptomes. Second, we established a laboratory culture of the species *Polyandrocarpa zorritensis* and described its life cycle and mode of budding to better comprehend the diversity of reproductive strategies found in Styelidae, These studies complement previous transcriptomic and developmental studies in the well-studied *Botryllus schlosseri*. Here, we will present ongoing work in our laboratories that combines anatomical, transcriptomic and phylogenomic data.

Talk 47

When One becomes Two: unique regeneration abilities of the solitary ascidians Polycarpa mytiligera

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Ascidians are well known for their powerful capacities for regeneration, and Ciona intestinalis has long been used as a model for studying regenerative mechanisms1. Our recent study showed that Polycarpa mytiligera, a solitary ascidian of the order Stolidobranchia has impressive capability of regenerating eviscerated organs, siphonal structure and neural complex within solitary Stolidobranchia species2,3 . The aims of the present study were: a) studying additional aspects of P. mytiligera regeneration abilities of internal and peripheral organs b) investigating the relationship between age and evisceration ability c) initiating reproduction under controlled conditions in order to test the applicability of using this species as a model organism. P. mytiligera extreme amputation treatment of about 50% of its body, revealed the ability of this species to fully survive and regenerate into two separated individuals. This ability of P. mytiligera to regenerate a new posterior part of its body, containing a new digestive system and heart, from a residual anterior part is a unique and previously unknown ability among solitary ascidians. In repeated evisceration experiments, regeneration of the digestive system was significantly related to age, as only young individuals were able to regenerate internal organs following three evisceration events. Our work suggests that P. mytiligera with its impressive regeneration abilities has the potential to become a new chordate model system for understanding the mechanisms of soft-tissue regeneration following evisceration, and for studying the regeneration mechanisms of the neural complex and siphon-associated structures and nerves.

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Talk 48
Partial Body Regeneration, Stem Cells, and Regenerative Aging in *Ciona*

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Partial body regeneration (PBR) refers to the phenomenon in which adult *Ciona intestinalis* and other solitary ascidians replace distal organs, such as the siphons and neural complex, from the remaining proximal part of the body, but are unable to regenerate proximal organs, such as the heart and viscera, from the excised distal parts. The term PBR was coined to distinguish regeneration in solitary ascidians from whole body regeneration in colonial ascidians. *Ciona* PBR is robust in young adults, diminishes continuously during aging, and is lost precipitously at senility. Stem cell markers, the cell proliferation indicator EdU, and transplantation methods have been used to identify the location and behavior of the stem cells responsible for PBR during the *Ciona* life cycle. Stem cells were detected in the gonads, gut, branchial sac, and the basal holdfast organ. Only the branchial sac stem cells responded to extirpation of the siphons or neural complex by dividing and dispatching migratory progenitor cells to the regenerating areas. Wounding rather than extirpation did not induce stem cell division, indicating that stem cell activation is specific to regeneration. Activation of branchial sac stem cells to divide and produce migratory progenitor cells occurred after either the oral siphon or the neural complex were removed, suggesting that the same progenitor cells may be involved in the replacement of these very different organs. When part of a branchial sac was transplanted from an EdU labeled donor into an unlabeled host, and the host oral siphon was removed, proliferating cells migrating from the graft participated in regeneration. The number of branchial sac stem cells decreased continuously during the life cycle, and proliferating stem cells were undetectable after regenerative ability was lost. Transplantation of part of a branchial sac from a young animal into an old animal provided a new source of dividing progenitor cells. After oral siphon amputation, these progenitor cells migrated into the stump and appeared to support limited reactivation of regeneration. The results show that the *Ciona* branchial sac contains a stem cell niche that supplies progenitor cells to distal organs during PBR and that a decline in stem cell potency and/or number may be responsible for progressive loss of PBR during aging. This “empty stem cell niche” hypothesis for regenerative aging is currently under further investigation.

#Session 16 : Anatomical Ontology

Chair: Patrick Lemaire

Talk 49
The ontology of larval and metamorphosis stages in Ciona. I. Development

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Although the solitary ascidian Ciona sp. is an established model organism for the scientific research communities and its embryonic development has been described in high detail, there is no standardized ontology of anatomy and development of post hatching larva, including larval and metamorphosis stages. In this study, combining time-lapse movies, 3D confocal microscopy images (available in FABA2 database: http://www.bpni.bio.keio.ac.jp/chordate/faba2/2.2/top.html) and histological section images, we determine the criteria to define stages covering the following five periods: larva, adhesion, tail absorption, body axis rotation, and juvenile. As stages 1-26 were defined in our previous study (Hotta et al., 2007), covering the development from zygote to hatched larva, subsequent 12 distinct stages (from stage 26 to stage 37) were determined for these periods. These stages are easily recognizable analyzing in vivo or fixed whole mount embryos at steromicroscope, and represented the base to construct a new anatomical ontology. More than 200 anatomical terms (entities) associated to references were manually annotated. Utilizing as base the previously reported embryonic ontology (Brozovic et al., 2015), we were able to define a "start to end table" describing the start stage and the end stage of each entity. These data are now linked each other and interactively manageable as TunicAnatO (Tunicate Anatomical and developmental Ontology) database. TunicAnatO standardizes the anatomical ontology and developmental stages in solitary ascidians and provides the information for comparative anatomy as well as guideline for future studies of tunicates.

Talk 50
The ontology of larval and metamorphosis stages in Ciona. II. Anatomy

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Among tunicates, the solitary ascidians Ciona intestinalis and Ciona robusta represent elective model species for researchers on Evo-Devo. The two species are very close from a phylogenetic point of view, and share many developmental and anatomical features. To facilitate studies on these species, the tunicate community is working in updating the current database systems, in order to provide different types of data (genomic, proteomic, transcriptomic, anatomical, developmental) and relate them to each other. Advanced model organism databases represent anatomy via a hierarchical textual ontology defined for each developmental stage. Since the description for Ciona genus was limited to the embryonic stages up to late tailbud stage, we created an Open Biomedical Ontologies (OBO)-compliant anatomical ontology for the larval and metamorphosis stages, based on complete series of confocal and histological sections, and on timelapse data. In the new ontology, we defined a vocabulary of more than 200 anatomical entities organized in a hierarchical way, reporting a precise definition of each term and possible synonyms. Basing on literature and our morphological observations, we included for each entity the developmental stages of appearance and end, specifying if they were deduced from cell lineage, histological/confocal recognition, or organ functionality. Many terms reported in the old ontology were corrected or updated. Importantly, for each entity we also added the main references, taking into account both classical anatomical texts and the more updated and technologically advanced publications. This anatomical ontology represents an implementable resource and an important tool for the tunicate scientific community. Together with the embryonic ontology, it will allow to correlate each other most of events occurring during Ciona development.

#Round table discussion: Databases

Chair: Patrick Lemaire

Talk 51, Poster/Demo 10
ANISEED 2017: an updated version of the tunicate database with revised orthology, extension to RNA-seq data and APIs

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ANISEED (Ascidian Network for In Situ Expression and Embryological Data) is a major database system for the genetic control and anatomy of ascidian embryos. Over the past two years, the system has been enriched in several aspects, which will be made available online in the summer 2017.

First, existing tools, functionalities and datasets have been updated. The system now uses the https protocol and an automated back-up procedure. The current Gbrowse genome browser is being progressively replaced by the new-generation epigenome browser from Washington University (Zhou et al., 2011), starting with Ciona robusta. Genome annotations and orthology relationships have also been added for 4 new species representing major tunicate clades: Phallusia fumigata (phlebobranchians), Halocynthia aurantium (pyurid stolidobranchians), Molgula occidentalis and Molgula occulta (molgulid stolidobranchians), raising the number of covered species to nine. Article biocuration is also being continued.

Second, we implemented an improved gene homology pipeline to identify orthologs and paralogs within all 9 ascidian species and with vertebrates and other deuterostomes. These relationships are available from each Gene card and displayed in form of interactive phylogenetic trees using Phylocanvas (http://phylocanvas.org/). These refined orthology relationships were also used to rename coding genes according to the guidelines for the nomenclature of tunicate genetic elements (Stolfi et al., 2015).

Third, we extended the database Chado schema to store and represent new types of high-throughput genomic information, starting with RNA-seq data. We developed a module for the representation of individual RNA-seq experiments in wild-type and experimentally perturbed conditions, which are displayed using interactive charts accessible from the expression page for each coding gene. To be able to compare experiments from different labs, we implemented a Python pipeline for the normalization of raw data obtained from GEO.

Last, we are developing two client software, which communicate with ANISEED via dedicated APIs. The first tool, "3D Cloud Embryos" allows to interactively explore segmented virtual high-throughput imaging data, onto which gene expression profiles can be projected (See abstract by Leggio et al.). The second tool, "My Local Aniseed", provides tunicate scientists with a solution to store and organize their experimental data (currently limited to in situ hybridization data, including 2-color experiments) on their local hard-disk, using a format that is compatible with ANISEED, and making use of the same ontologies. This tool both facilitates the organization of personal private expression data and their subsequent submission to ANISEED.

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Talk 52
First Ascidiacea World Database Editor Workshop

Marc Rius[1,2,*], Arjan Gittenberger[3], Gretchen Lambert[4], Françoise Monniot[5], Rosana Moreira da Rocha[6], Noa Shenkar[7], Billie J Swalla[4], and Xavier Turon[8]

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This presentation will present the outcomes of the World Register of Marine Species (WoRMS, http://www.marinespecies.org) Ascidiacea editors workshop which was held at the Flanders Marine Institute in Oostende, Belgium between the 15th and 17th February 2016. The meeting brought together all of the WoRMS Ascidiacea editors for the first time and included a special guest, namely the world-renowned ascidian taxonomist Françoise Monniot. She shared both her extensive knowledge and a database that comprises decades of taxonomic research compiled by both her and Claude Monniot. The workshop provided an invaluable opportunity to discuss more efficient ways to coordinate the work among the editors, as well as between the editors and the WoRMS Data Management Team based at the Flanders Marine Institute. The editors learned how to use the full set of online tools available, which has allowed for an increasingly effective editing of the content of the Ascidiacea World Database (one of the Global Species Databases of WoRMS, see http://www.marinespecies.org/ascidiacea/). In addition, many species records and taxonomic issues (e.g. missing authorities, homonyms) were resolved during the workshop. Finally, species distributions were revised and a total of 714 PDFs uploaded, which ensures easier access to species distribution information and primary literature for both users and editors. This workshop therefore marks the start of a comprehensive revision of the Ascidiacea World Database.

Notes:

Further information can be found:
http://www.marinespecies.org/news.php?p=show&id=4483
http://www.marinespecies.org/docs/activities/2015/Ascidiacea_Workshop_Report_Final.pdf
http://depts.washington.edu/fhl/tidebites/Vol32/
http://depts.washington.edu/ascidian/AN77.pdf

#Round table discussion: Functional genomics methods & resources

Chair: Alberto Stolfi

Talk 53
Establishment of CRISPR based genome editing in *Oikopleura* for gene functional study

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The Larvacean *Oikopleura dioica* has a short life cycle of 6 days and can be routinely cultured for many (more than 100) generations in facility (Bouquet et al., 2009; Nishida, 2008). It also features the smallest Chordate genome and a phylogenetic location at the base of Chordates evolution (Delsuc et al., 2006; Nishida, 2008; Seo et al., 2001), making it an interesting organism for studies of chordate development and genome evolution (Denoeud et al., 2010; Kugler et al., 2011; Lemaire, 2011). However, due to lack of forward genetic screen and transgenic production, gene function analysis in this organism remains limited . Though recently, morpholino and RNAi / DNAi have been successfully used in this organism, introduction of gene knock out is still highly desirable (Omotezako et al., 2013, 2015; Sagane et al., 2010). However, application of state of the art genome editing techniques, like TALEN and Crispr, has not been reported in this organism.

Here we report the establishment and optimization of Crispr based genome editing in *Oikopleura dioica*. First, we discovered that, a certain type of genomic sites can be targeted by Crispr-induced-mutagenesis in *Oikopleura*. Second, we optimized the Crispr protocol and developed mutant screen methods. Through which we obtained a somatic mutation rate of 8.3 ~ 58% at 5 different target sites, and a germ line transmission rate of 13% of adult animals in the making of a *Brachyury* mutant line. Third, we used Illumina RNA-seq to analyse the gene expression in *Brachyury* mutant embryos immediately after hatching, and detected 121 genes up-regulated together with 18 down-regulated. In situ hybridization of a part of the up-regulated genes suggests that most of them are specifically or predominantly expressed in the notochord.

Our work established a Crispr based genome editing method in *Oikopleura dioica* and presented its application in a case screen for *Brachyury* downstream genes (Nibu et al., 2013). Given the short life cycle of *Oikopleura* and availability of Crispr and other tools in *Ciona* (Sasaki et al., 2014; Stolfi and Christiaen, 2012), this method would benefit the gene functional studies in *Oikopleura* as well as comparative molecular development between the two tunicate species.

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POSTERS

Allorecognition and immunity

Poster 11

The innate immune system of Styela clava as seen by an inorganic chemist

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Tunicates have been used as models for understanding cell-mediated and humoral immunity. We have recently reported that Clavanin A, one member of a family of antimicrobial peptides (AMPs) produced by the solitary tunicate Styela clava, utilizes Zn^{2+} ions to potentiate its antimicrobial activity not only by reducing the concentration at which the peptide inhibits the growth of bacteria but also by increasing the rate of killing.1 Overall, these studies identified a remarkable new mechanism by which zinc contributes to the immune response in the tunicate S. clava. A second member of this family of AMPs, Clavanin C, has a copper binding motif, known as the ATCUN motif. In AMPs from other organisms, the ATCUN motif has been shown to be important in their antimicrobial role. 2,3 Whether copper binding is of importance to the antimicrobial activity of Clavanin C remains to be demonstrated. Herein, we will present our recent results on the rich inorganic chemistry of these tunicate peptides.

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Poster 12

The complement system of Botryllus schlosseri.

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Among the various effector mechanisms involved in immune responses, the complement system is one of the most ancient, deeply-rooted and important for its ability to orchestrate different cells and factors of both innate and adaptive immunity. The comprehension of its roots in the evolution is useful to understand how the main complement-related proteins had changed in order to adapt to new environmental conditions and life-cycles or, in the case of vertebrates, to interact with the adaptive immunity. In this context, data from organisms evolutionary close to vertebrates, such as tunicates, are of primary importance for a better understanding of the changes in immune responses associated with the invertebrate-vertebrate transition.

In our model tunicate Botryllus schlosseri we have described a lectin and alternative pathway of complement system activation very similar to those of Vertebrates. All the complement-related genes such as c3, bf, ficolin, mbl and masp are transcribed by morula cells, the immunocytes in immunomodulation and cytotoxic responses. Functional data suggest a complement-related cross-talk between morula cells and phagocytes immunocyte during the immune response. When B. schlosseri hemocytes are incubated with yeast (Saccharomyces cerevisiae) cells, there is an overexpression of C3 by morula cell that led to increase of phagocytosis that is prevented in the presence of the C3 inhibitor, compstatin.

In the next future, we will focus our efforts on the regulation of complement system in tunicates to shed new light on the complement system function in a pre-adaptive immunity scenario.

Poster 13
Evolutionary origin of the mammalian Hematopoietic and Immune systems found in a Colonial Chordate.

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To gain insight into the evolutionary relationship between vertebrate and invertebrate hematopoietic system, we have characterized the immune system and cell populations of the colonial tunicate *Botryllus schlosseri*. *B. schlosseri* belongs to a group considered the closest living invertebrate relative of vertebrates, it has bidirectional blood cell flow through an interconnected vasculature. To isolate and characterize the *Botryllus* cell populations we adapted Fluorescence-Activated Cell Sorting (FACS). We used Cytof Mass Cytometry to scan 50 diverse antibodies. Antibodies that differentially bind to *B. schlosseri* cells, in combination with lectins and fluorescent reagents activated by enzymes, were used to isolate live *B. schlosseri* cell types. Additionally, we used mouse serum against the *Botryllus* Histocompatibility Factor and analysis of cell size, granularity and auto fluorescence to isolate 34 cell populations. We prepared libraries from these populations for RNAseq, and analyzed their gene expression. This analysis revealed cell population homolog to mammalian hematopoietic stem cells, which upon transplantation, migrated to stem cell niche and differentiated into other cell lineages. Interestingly, we have shown that this niche is homolog to mammalian bone marrow stromal cells. Using functional immunological assays for cytotoxicity and phagocytosis we characterized 3 different phagocytic cell-types. One of these demonstrated transcriptional and functional features resembling myeloid cells in vertebrates. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells. Our data suggests that the common ancestor of tunicates and vertebrates had a true hematopoietic myeloid lineage, while the cytotoxic cells may result from a convergent evolutionary mechanism.

Poster 14

Study on Marine ascidian *Didemnum bistratum* and its antibacterial and anticancer properties

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The marine environment contains bioactive compounds that could serve as useful lead structure for the development of new classes of drugs. Ascidians are sessile marine animal with great source to produce bioactive molecules with notable therapeutic application. It is essential to study their active biomolecules, pharmacological properties when considering their pharmaceutical applications. This present study, examines Indian ascidian *Didemnum bistratum* were collected in Tuticorin coastal waters. The chemical diversity of MeOH crude extracts determined by GC-MS method and yielded 234 metabolites in *Didemnum bistratum*. The *Didemnum bistratum* shows the promising result of zone of inhibition in antibacterial activity revealed the maximum zone of inhibition activity for Methicillin-resistant *Staphylococcus aureus* (MRSA) (9.66±0.57 mm) and the minimum zone was measured against *E. coli* (1.33±0.57 mm). The crude extracts various concentrations (50 to 1000 µg/ml) were used to check the cell viability reduction and it has shown the cytotoxicity against A549 Cell line (IC_{50} 400µg/ml). The result showed promising anti-cancer activity against A549 Cell line (lung carcinoma cancer). The further bioassays, compound sepration and structural idenitification of compounds lead for Indian ascidians are potential resource in marine drugs.

Key words: Ascidian, Antibacterial, GC-MS, Cytotoxicity, Anti-Cancer

Cell and developmental biology

Poster 15

The Botryllus schlosseri embryogenesis timeline reveals heterochrony between solitary and colonial ascidian embryogenesis

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Solitary ascidians, like Ciona intestinalis, have been used for over a century as a model system of embryological studies. These species are oviparous producing a large number of eggs which are released and fertilized outside the parent body. C. intestinalis embryos develop rapidly and reach the larva stage in about 20 hours (20°C). The larvae then settle and metamorphose into sessile zooids in a process that takes several days. On the other hand colonial ascidians like Botryllus schlosseri are ovoviviparous, were fertilized eggs are developed inside the parent body. These embryos are connected to the zooid body via a cup-like placenta, and develop into larvae within a week (20o C). The B. schlosseri larvae, which possess both typical larval organs and prospective juvenile organs, are released into the seawater where they settle and metamorphose rapidly into juvenile zooids. The development of the Botryllus embryos within their parent body limits the ability to study embryogenesis in colonial ascidians. To counter this we combined in vitro culturing of B. schlosseri embryos, confocal and time-lapse microscopy to characterize the developmental stages of B. schlosseri embryos. When we compared the timeline of embryo development between B. schlosseri and C. intestinalis we found that the relative proportions between developmental stages was significantly different. This heterochrony of development reflects the different maturation timing of anatomical structures between solitary and colonial ascidians.

Poster 16
DDR is required for behavior during convergent extension of the Ciona embryonic tail

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Convergent extension is a key developmental process that contributes significantly to the rearrangement of cells in the developing embryo. During this process, the body of the organism narrows along one axis while subsequently elongating along another perpendicular axis. Ciona undergoes convergent extension in the tail by intercalating the two medial rows of epidermal cells into one. In the epidermal cells of the tail, this process causes several morphological differences over time starting with a change in cell shape, volume and sphericity. When the morphological changes are tracked in embryos over time, we observe that there is a significant reduction in the sphericity of the epidermal cells in the tail. This difference is even more striking when the medial cells are compared to the lateral cells of the tail epidermis. Discoidin Domain Receptor (DDR), a collagen binding receptor tyrosine kinase, is expressed in the epithelial cells of the tailbud. By expressing a dominant negative form of DDR in the epidermis using the epidermis specific EfnB1 enhancer, the epithelial tail cells look more rounded possibly due to loss of adhesion leading to distinctly altered phenotypes. Though dnDDR affected cells appear smaller, it is still unknown how the processes of convergent extension are affected in the presence of dnDDR during development. When we compare the number of cells in a defined area in wild type and dnDDR affected embryos, we see that while the number of cells within the defined area of wild type embryos decreases as cells undergo morphologic changes, the number of cells in a defined region of dnDDR expressing embryos remains constant over time. . Based on the initial results, we believe that DDR is required for epidermal cell behavior and may arrest the cell cycle to allow for rearrangement during development.

Poster 17
Molecular Mechanisms Behind Germline Stem Cell Parasitism

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Cell competition (CC) is a universal, quality control process in which cells compare their relative fitness (e.g., growth rates) with winner cells contributing to further development, while suboptimal cells are outcompeted. This process has been studied in organisms ranging from fungi to humans, and has both developmental and disease relevance. For example, during cancer development neoplastic cells can function as supercompetitors in a tissue, outcompeting wild-type cells and driving tumor growth. Despite recent data the molecular mechanisms that underlie this process are not well understood. We are utilizing the unique biology of the colonial tunicate, Botryllus schlosseri, which has germline stem cells (GSCs) with genetically determined competitive phenotypes- when GSCs of two individuals are mixed, one genotype will outcompete the other, solely contributing to the germline of subsequent generations. Termed stem cell parasitism (SCP) this clonal dominance is heritable, stable and repeatable. We hypothesize that SCP is partially due to differential homing of winners and losers to the germline niche, which is supported by in vitro migration assays. Furthermore, qPCR results build upon previous work from the lab indicating a role in CC for the S1P-S1PR migration pathway as well as other novel pathways involved in cell:cell communication. Moreover, live-imaging of transplanted GSCs from winner/loser genotypes reveal that winner and loser GSCs both arrive in the developing germline niche, suggesting a role for direct interaction between winner/loser GSCs within the niche. Based on these preliminary results we propose that the basis of GSC competition resides in a combination of variation in homing ability and cell competition for niche occupancy. Current results will be presented.

Poster 18
TailbudProfiler: developing an automatic image analysis software for quantifying Ascidian tailbud morphogenesis

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Tailbud is a shared and prominent structural feature of most chordate embryos. During this period, the chordate embryo drastically changes in shape by elongating its tail along main body axis, eventually becoming a tadpole-type larva. The mechanism of tailbud morphogenesis is then evolutionary important to understand. Although the consisting organs — external epidermis, neural tube, notochord, paired muscles, trunk mesenchyme and ventral endoderm — are thought to influence the tailbud shape, the quantitative aspect of those changes and the underlying mechanisms are still obscure. To describe this phenomenon precisely, a semi-automated analysis tool has been developed (Muraoka et al, in preparation); based on conventional stereo-microscopy images, it aimed at quantitively measuring different morphological parameters derived from the embryo shape, and proved useful in elucidating the interaction between the genetic and mechanical processes for the ascidian tailbud embryo. However, this method relied on manual, subjective placement of the anterior-posterior edges of tailbud embryo in individual images, making it a bottleneck for high-throughput, image-based genome-wide screens, as well as reducing the reproducibility of the process. We have therefore developed an automatic image analysis software, TailbudProfiler, by fully modifying the previous analysis tool and especially automatizing the critical head, tail and neck points placement step. To this end, a newly developed algorithm for recognizing tail/trunk edges by analysing the embryo outline was incorporated into TailbudProfiler, and we are now testing its precision and accuracy in comparison with the previous semiautomated method. Our goal is, then, to produce an automatic and objective analysis pipeline to quantify relevant morphometric parameters and track their evolution during development, using for instance live imaging techniques. This approach would ultimately allow to easily analyse larger datasets in order to define standards for wild type embryos and identify statistically significant phenotype variations when conducting chemical screen and functional genomics studies.

Poster 19
Bisphenol A specifically disrupts the formation of pigmented cells in Phallusia mammillata

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Bisphenol A (BPA) (a widely used molecule in plastic materials) possesses endocrine-disrupting potential in vertebrates/humans and can bind to several nuclear receptors (NRs) to induce the transcription of target genes. More recently, BPA was found to exert neurodevelopmental toxicity to Ciona embryos as well as in fish and mammals. Despite all these studies, no clear mode-of-action has been proposed so far.

Tadpoles of Phallusia mammillata (a European solitary tunicate) have a prototypical chordate body plan with a centralized brain and a dorsal nerve cord. The simple brain of ascidian tadpoles consist in a brain vesicle, hosting two pigmented cells (ocellus and otolith), and a visceral ganglion, containing motor neurons extending into the nerve cord.

We demonstrate here that BPA induces neurodevelopmental toxicity in the ascidian Phallusia mammillata, by disrupting pigmented cells development. The 2 pigmented cells are 2 abutting a11.193 blastomeres which start to be pigmented at 17 hpf (16°C). Timelapse imaging shows that melanization dynamic is different in the ocellus and otolith, and that the otolith forms on the dorsal side of brain vesicle migrating then towards the ventral side, at the same time as trunk elongation and palps formation.

By analyzing brain and pigmented cells gene regulatory networks (GRNs) we find that BPA do not disrupt neither pigmented cells nor brain specification GRNs. By ISH and promoter analysis we show that, among the 17 NRs present in the ascidian genome, 4 are expressed in the ascidian brain vesicle (VDR/PXRα, ERR, PPAR and COUP-TF). We now attempt to decipher the involvement of these 4 NRs in PSO formation and in the phenotype induced by BPA.

Poster 20

Mechanism of CAMP (cortical array of microtubule in posterior vegetal region) formation during cytoplasmic and cortical reorganizations in the acidian agg

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In ascidian eggs, maternal determinants localized to the cytoskeletal domain called myoplasm move to the future posterior side by two phases in cytoskeleton dependent manners1),2). This movement is called cytoplasmic and cortical reorganization. The second phase of reorganization is microtubule dependent, but its mechanism is unclear due to the insufficient technique for the microtubule observation3),4). To reveal the whole mechanism of cytoplasmic and cortical reorganization, a more thorough description of microtubules is crucial. We previously reported two novel microtubule structures during first cell cycle by using modified Scale 5) , named G1T0, as the pretreatment reagent for immunostaining. One is the short microtubule meshwork existed in the relatively broad cortical area of unfertilized egg. The other one is the cortical microtubule array designated as CAMP (cortical array of microtubules in posterior vegetal region), which appears posterior-vegetal cortex during second phase of reorganization. The CAMP was suggested to be important for the second phase of reorganization.

The cortical microtubule array during first cell cycle such as CAMP has also been reported in frog and zebrafish eggs6),7) . In these animals, cortical microtubule array contributes to determining the embryonic axis by transporting cytoplasmic factors7),8) . In the frog egg, the cortical microtubule arrays arise from several microtubule sources including sperm aster and neighboring cytoplasm8). The transport of vesicles and the cytoplasmic rotation are dependent on microtubule motor proteins, such as kinesin8) . In addition, it has been reported that microtubule array was observed in the artificially activated egg in both animals 6),7) .

In this study, we investigated newly identified microtubule structures in more detail. The microtubule meshwork in the unfertilized egg remained its position and configuration until 10 minutes post fertilization (mpf), independent of the cortical contraction of the first phase of reorganization. At 15 mpf, short microtubules were shifted and accumulated in the vegetal pole, where the sperm aster started to grow. At 30 mpf, the sperm aster had already moved to the equatorial region of the posterior cortex, and poorly aligned faint microtubule bundles could be detected on the vegetal cortex. This poor array could be a source of CAMP. When the egg was treated with ciliobrevin, an inhibitor of cytoplasmic dynein, from 10 mpf to 45 mpf, CAMP did not form and the second reorganization was stopped. On the other hand, in the egg artificially activated by calcium ionophore A23187, CAMP-like cortical microtubule array was formed on 60 min after treatment. These results indicated that the formation of CAMP was dependent on dynein and calcium signaling.

Our results suggested that the cortical microtubule array in frog, zebrafish, and ascidian egg during first cell cycle had many common features and functions. Moreover, the embryonic axis determination mechanism using cortical microtubule array could be a conserved mechanism for animal development at least in chordate. Studies on the formation of cortical microtubule array and the transportation of maternal determinants will reveal the entire mechanism of axis formation.

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Poster 21
Modular development in Symplegma brakenhielmi and the evolution of coloniality in styelid ascidians

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Colonial animals are biological systems composed of discrete units (zooids) that are physiologically interconnected. Colonial assemblies are engineered by the modular development of each zooid and have diversified into many distinct sessile life forms. In the Styelidae morphological and molecular evidence suggest a solitary ancestor for the family, with one or several transitions to coloniality within the family. Colonial Botryllus and Botrylloides are well-studied genera that exhibit colony-wide developmental mechanisms that regulate synchronous and orchestrated cycles of budding and turnover of zooids. The origins of modular developmental mechanisms that facilitated the evolution of coloniality in this group remain unclear. To reconstruct ancestral states of coloniality we studied Symplegma brakenhielmi, a sister taxon of the botryllids. S. brakenhielmi zooids are embedded in a common tunic and present a similar vascular system as the brotryllids, however development and turnover of zooids occurs asynchronously and in a more independent manner. We classified S. brakenhielmi blood cells by morphology using cytohistological assays and transmission electron microscopy. We found eleven types of hemocytes similar to previously reported (e.g. hemoblasts, macrophage like cell, storage cells). We observed considerable variations in characteristics of precursors cells, and in their proportions during regeneration. These results suggest a continuous hematopoiesis, and hemocyte differentiation processes in the blood of the colonies. In vivo observations of zooid regression in S. brakenhielmi colonies indicates the involvement of macrophage like cells suggesting programmed cell death (PCD) as a main process of zooid turnover. We tested colony response to external perturbations by systemic bud or zooid removals. In most cases, vascular tissues of perturbed colonies merged at the sites of zooid regeneration. We observed an accelerated development of buds after original buds were removed from the colony. Our observations suggest that zooids are recycled after resorption, and that development time and lifespan of zooids in the colony are controlled by endogenous mechanisms of the colony. Thus, colonies act as self-regulating systems that respond to perturbations by altering the development of its modules. Modular communication, programmed cell death, and recycling of tissues mediated by macrophage like cells, represent some of the important developmental processes of colonial evolution in the styelid ascidians.

Poster 22
Identifying the Mechanism Driving Anterior-Posterior Differences in Gene Expression in the *Ciona* Notochord

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The larval notochord of Ciona intestinalis consists of two separate cell lineages; the primary notochord, comprised of the anterior 32 cells, and the secondary notochord, comprised of the posterior 8. The primary and secondary notochord cells both express Brachyury and intercalate into a single-file rod, but there are both quantitative and qualitative differences in morphogenetic behavior between these two cell types. Here we report that the putative ECM component fibulin-like shows strikingly differential expression between the primary and secondary notochord, with strong expression in the secondary notochord, and almost no expression in the primary notochord. We have identified a cis-regulatory module that partially recapitulates the fibulin-like expression pattern, and are dissecting it as an entry point towards understanding the regulatory basis for secondary-specific expression. Our initial analysis indicates that there is considerable redundancy and considerable use of negative regulation in the fibulin-like CRM.

Poster 23

Spatio-Temporal Relationship between Dynamics of Actin Cytoskeleton and Cell Stiffness in Early Ascidian Embryo Development.

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Mechanical properties in cells play important roles for animal morphogenesis. However, the molecular basis for regulating cell stiffness during animal development has not been well known due to a lack of the direct measurement. We investigated the spatial-temporal change in the surface topography and stiffness (Young's modulus) of ascidian embryos at an early stage of development - from 32-cells to 112-cells stages - by using atomic force microscopy (AFM). During these stages, two rounds of cell division occur in ectodermal lineages. We found that the stiffness of cells increases before cell division and then gradually decreasesto the level of the interphase. Moreover, we found that treatment of actin polymerization inhibitor, Latrunculin A, suppresses these changes of cell stiffness. To compare the actin dynamics with the change of stiffness during ectodermal cell division, we investigated the densities of F-actin both the cell membrane (corresponding to cortical F-actin) and intracellular regions (corresponding to cytoplasmic F-actin) during two rounds of ectodermal cell division by live-imaging of F-actin-binding protein, lifeact-GFP. The change of the cortical F-actin densities correlates with the change of the stiffness: the cortical F-actin increases before cell division and decreases after cell division. However, the cytoplasmic F-actin shows negative correlation to the stiffness. These results suggest that the cortical F-actin is directly related to the stiffness, and that F-actin turns over between the membrane and cytoplasmic regions during cell division in early ectodermal cells.

Poster 24
The molecular signatures of development in Botryllus schlosseri

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The colonial chordate, Botryllus schlosseri, possesses two developmental pathways to build its adult body plan. 1) Embryogenesis: follows from sexual reproduction, in which a single fertilized eggs divides and differentiates to form the larval stage (a tadpole) that settles and then undergoes metamorphosis into an oozoid. 2) Blastogenesis: a weekly asexual process whereby secondary buds differentiate to form primary buds which in turn grow and becomes a mature zooid as the existing zooid is resorbed. Multiple biological replicates at many stages of development in both of these pathways have had transcriptome libraries prepared and undergone RNA-seq to build an atlas of gene expression profiles. These are compared to the embryogenesis in Ciona and other organisms from an evolutionary point of view and contrasted with blastogenesis. Using some of our hundreds of other sequenced RNA-seq libraries from Botryllus we can compare developmental gene sets with those of other processes such as allogeneic resorption, and to use our cell and tissue specific libraries to deconvolve which tissues may dominate or originate in different developmental stages. We couple the molecular signatures with anatomical data from microscopy to provide a comprehensive picture of how Botryllus schlosseri is able to grow into its adult form in two different ways.

Poster 25
Dynamic roles of cellular protrusions during neural tube closure in Ciona

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In Ciona, the neural tube forms by fusing the lateral borders of the neural plate and the surface ectoderm from the posterior side of the embryo toward its anterior side in a process called zippering. It is similar to neural tube closure in vertebrates. We have developed live two-color two-photon microscopy technique in order to study neural tube closure in Ciona. This technique has allowed us to visualize highly dynamic cellular protrusions emanating from the future dorsal neural tube cells as well as from the surface ectoderm both in front and behind the zippering fork. These cellular protrusions, which are either lamellipodia or filopodia, are essential in mice since in their absence, neural tube closure is impaired. However, due to the complexity of the process in the mouse embryo, the role of the protrusion dynamics has not been assessed. Since neural tube closure in Ciona is a simplified version of what is occurring in higher vertebrates, we propose to evaluate the role of the cellular protrusion dynamics during neural tube closure by taking advantage of the genetic toolbox available in Ciona to disrupt gene function as well as by using the high resolution live imaging technique developed in the laboratory.

Poster 26
Onecut gene regulatory network in chordate development and evolution

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Onecut genes (OC) have been identified in all major groups of metazoans and are expressed in the nervous system and in some endodermal derived tissues. Their function in liver and pancreas differentiation in mammals has been quite well studied, while almost nothing is known about their function in neurogenesis and eye formation in chordates.

By using the tunicate Ciona intestinalis and vertebrate zebrafish Danio rerio as model systems, we studied the role and the genetic cascade of OC genes in photoreceptor cells and in eye formation during chordate evolution. The Ciona genome contains a single OC gene, while the analysis of the zebrafish genome revealed the presence of five OC orthologues OC1, OC2, OClike and two gene copies of OC3, named OC3a and OC3b. To acquire novel insights into the degree of OC genes functional conservation across chordates, we performed OC targeted perturbation by transgenic approach in Ciona and OC morpholino-mediated knockdown in zebrafish. By differential transcriptomic analyses on Ciona OC transgenic embryos we set the ground for the identification of OC target genes. The analysis of three OC morphant phenotypes in zebrafish revealed a OC conserved role in eye specification. These data insert a new piece in the genetic cascade controlling the specification of the ocellus and eye structures and highlight a conserved and important role played by OC genes in this process during chordate evolution.

Poster 27

Homeobox genes play an important role in the development of oikoplastic epithelium of Larvaceans.

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The complex filter-feeding house and highly organized trunk epithelium of Larvaceans are evolutionary novelties that appeared in early tunicates evolution. Appearance of new organs is not possible without adaptation of genetic network and regulatory circuits. The goal of our work was to know the genes that govern the formation of the oikoplastic epithelium during embryo and larval development. We found that many homeobox genes have expression pattern in the trunk epithelium and many of them have been duplicated. We presume that the molecular mechanisms that definite epithelium developmental program must have included at least some conserved transcription factors secondarily recruited for this new purpose. We performed a functional study of prop-genes (prop gene of vertebrates plays an important role in the pituitary gland development) using dsRNA – injections into unfertilized eggs. The results have shown that propA and propB are playing important role in the morphogenesis of middle trunk epithelium: the absence of their functional RNA leads to the malformation of dorsal epithelium, anterior rosette and nuclei disorganization around the dorsal midline. The knockdown of prop genes also abolished the post-metamorphosis expression of oikosin41a gene in the Anterior rosette field, confirming their importance for cell differentiation in this region. Many homeobox genes involved in the oikoplastic epithelium development are duplicated, and both duplicates are expressed in a similar region of the epithelium. This suggests that the epithelial expression preceded the gene duplication events and were not required for the house emergence. If the functional divergence of duplicated homeobox genes represents more than subfunctionalization, they may have played a role in the complexification of the oikoplastic epithelium and of the house architecture.

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Poster 28

Chemical genetics reveal the role of 14-3-3 ea in Ciona notochord tubulogenesis

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Chemical genetics has been an effective approach to identify key molecules underlying diverse biological processes in cultured cells. However, only a few attempts have been made to apply chemical genetics in developmental biology. Here we used chemical genetics to examine the early development of ascidian Ciona intestinalis. Phenotypic screening identified that UTKO1, an inhibitor of cell migration we developed previously [1], selectively inhibited notochord tubulogenesis. This result prompted us to elucidate the molecular mechanisms of notochord tubulogenesis by using UTKO1. For this, we attempted to identify the target protein of UTKO1 using Biotin-UTKO1. Since UTKO1 targets to 14-3-3 proteins in human cells, we investigated whether UTKO1 bounds to any of Ciona 14-3-3 isoforms: 14-3-3 ea, eb, g1, and g2. These proteins were purified from E. Coli as GST fusion proteins, mixed with Biotin-UTKO1, and pulled down by avidin beads. As a result, 14-3-3 ea was highly pulled down by Biotin-UTKO1, and its requirement in notochord tubulogenesis was also confirmed by morpholino knockdown analysis. Since 14- 3-3 proteins are adapter proteins that interact with various client proteins, UTKO1 phenotype was thought to be caused by the inhibition of this interaction. One candidate for 14-3-3 ea client protein is Ezrin/Radixin/Moesin (ERM), because it is specifically expressed in notochord cells [2], and plays essential roles in notochord tubulogenesis [3]. Indeed, we showed the direct interaction of ERM with 14-3- 3 ea at UTKO1 binding site, and this interaction is abrogated by UTKO1. Taken together, our chemical genetics approach will open up novel mechanisms of notochord tubulogenesis in terms of the interaction between 14-3-3 ea and ERM.

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Poster 29

Morphometry of Tailbud Embryo for Revealed Distinct Role on Tail Curving by Myosin-Dependent Mechanical Tension at Ventral Midline Epidermis.

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Although the ascidian tail elongates with changing overall morphology, it is still unclear how different morphological parameters change during tail formation. To quantify the shape of developing ascidian tailbud embryos and elucidate the correlation among different morphological parameters, we developed a semi-automated multi-parametric analysis tool. We acquired images of tailbud embryos with conventional stereo-microscope, and extracted 9 morphometric parameters for further analysis. Principal component analysis (PCA) was applied for these 9 parameters and three principal components analysis (PC1-3) explained over 95% of the total variance in tailbud shape. “Tail/Trunk ratio”, “tail length”, “tail width”, “tail area” and “tail sharpness” were associated with PC1, “tail curvature” preferentially associated to PC2, and PC3 was related to “trunk area”. Interestingly, inhibition of ROCK, an upstream regulator of myosin II activity, during tail formation induced statistically significant difference on PC2 but not on PC1, indicating its influence on tail curving rather than tail elongation. We further found that myosin II is phosphorylated in epidermal cells of ventral midline in mid-tailbud stage embryos. Finally, we found by using UV-laser cutting that this myosin accumulation was accompanied with increased tension along the ventral epidermal midline, pointing to the possibility that tension in the ventral epidermis induces tail curving.

Together, these results suggest that the curving shape of the developing tailbud embryo is regulated by myosin-dependent tension generated by a small population of epidermal cells. Our approach will help to unravel the interaction between the genetic and mechanical processes by which the ascidian embryo shape.

Poster 30
Two distinct clocks cooperatively control zygotic genome activation in the ascidian embryo Izumi

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The maternal-to-zygotic transition is an embryonic stage that all animal embryos pass through. One of the major events during this process is the activation of the zygotic genome. In Ciona intestinalis embryos at the 8- to 16-cell stages, maternal transcription factors directly activate zygotic gene expression; Tcf7/β-catenin activates genes in the vegetal hemisphere, and Gata.a activates genes in the animal hemisphere. The initial transcriptional quiescence is important to enable the establishment of the initial differential gene expression patterns along the animal-vegetal axis by the maternal factors. Following this quiescence, the third cell division separates the animal and vegetal hemispheres into distinct blastomeres. In the present study, we found that maternal Tcf7/β-catenin activates its targets strictly at the 16-cell stage, while maternal Gata.a begins to activate its targets weakly at the 8-cell stage and strongly at the 16-cell stage. We provide evidence that global transcription is gradually relieved until the 8- and 16-cell stages, and that the nuclear translocation of β-catenin is regulated by the absolute time after fertilization. Thus, in Ciona embryos, two distinct clock mechanisms cooperatively determine the timing of the zygotic genome activation.

Poster 31
Induced vascular regression is mediated by changes in the extracellular matrix, apoptosis and coordinated cell extrusion

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To investigate the physical role of the extracellular matrix in vascular homeostasis we are using the basal chordate Botryllus schlosseri, which has a large, transparent extracorporeal vascular network encompassing areas >100 cm2 that expand radially away from the bodies. Using in situ hybridization, we determined that the collagen crosslinking enzyme lysyl oxidase 1 (LOX1) is highly expressed in all vascular cells. In vivo inhibition of LOX1 by specific inhibitor, ß-aminopropionitrile (BAPN), causes a rapid regression of the entire vascular bed, with some vessels regressing >10 mm within 16 hours without bleeding. Transmission electron microscopy reveals that BAPN treatment disrupts the collagen ultrastructure in the basement membrane. Disruption of the basement membrane triggers apoptosis of vascular cells, which are then removed from the vascular wall by blood-borne phagocytes. Apoptosis, engulfment and regression occur in a coordinated manner that maintains vessel integrity, with no loss of barrier function, and is reversible. To investigate if Integrins are sensing the disruption of collagen fibers, we inhibited the Integrin signaling pathway using pharmacological antagonists of FAK, Ras, Raf, and MAPK. Inhibition of integrin signal transduction caused vascular regression similar to BAPN treatment. This suggests the presence of regulatory mechanisms linking physical changes to a homeostatic, tissue level response. We are developing a new method to further dissect how vascular epithelial cells are mechanosensing their environment by growing animals on a stretchable surfaces. This will allow us to elongate blood vessels mechanically and to assess their response and adjustments to forces applied directly to the blood vessels. The ability to manipulate physical characteristics in vivo and assess outcome in real time provides a new model to dissect mechanotransduction pathways.

Poster 32
Transcriptional regulation of a hedgehog gene in the ventral nerve cord of the Ciona intestinalis larva

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In vertebrate embryos, Sonic hedgehog is produced in axial tissues, including the notochord, prechordal plate, and floor plate, and plays important roles in patterning of the central nervous system
(CNS) and somites. The ascidian Ciona intestinalis has two genes encoding hedgehog (hedgehog.a and
hedgehog.b). Transcripts of hedgehog.a are only present as maternal RNA in embryos, while hedgehog.b is expressed in a group of cells at the anterior endoderm-neuroectoderm boundary at the larval stage and in the ventral row of embryonic nerve cord cells, which is the homolog of the vertebrate floor plate, at the tailbud stage. Neither hedgehog.a nor hedgehog.b is expressed in the notochord1. By comparing genome sequences between two Ciona species, we found two highly conserved regions in the first intron of hedgehog.b, while no highly conserved sequences were detected in the upstream intergenic region. Functional analysis of the upstream region and first intron of hedgehog.b in vivo by using a fluorescence reporter revealed that the 2.6-kb upstream region is sufficient to recapitulate endogenous expression patterns at both tailbud and larval stages. The highly conserved regions in the first intron were shown to have enhancer activities as well as silencer activities that suppress ectopic expression. Serial deletions of a 200-bp segment in the 2.6-kb upstream region revealed redundancy of multiple cis-regulatory elements dispersed within the 2.6-kb upstream region. To further dissect the 2.6-kb region, either the 5' or 3' half of this region was removed from the reporter construct and the reporter expression was examined in electroporated embryos. The results suggest that the 3' half of the region contains cis-regulatory sequences that activate transcription in the ventral nerve cord. Interestingly, combination of the 2.6-kb upstream region and first intron was shown to confer the ectopic expression of the reporter gene in the endodermal strand, suggesting that the ventral nerve cord and the endodermal strand share a part of their gene regulatory programs. This is particularly interesting because the endodermal strand occupy a similar embryonic position to the vertebrate hypocord and the hypocord shares expression of several genes with the floor plate, including sonic hedgehog. We further discuss evolution and regulation of hedgehog gene expression in the axial structures in chordates.

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Poster 33
Morphological abnormalities in Ciona intestinalis embryos exposed to polycyclic aromatic hydrocarbons

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Polycyclic aromatic hydrocarbon (PAH) refers to organic compounds that have two or more aromatic rings and are composed of only carbon and hydrogen atoms. PAHs are common environmental pollutants produced by not only natural phenomena such as volcanic eruptions but also human activity such as combustion of fossil fuels in power plants and automobiles. PAHs released to the atmosphere diffuse widely and shift to inland water and ocean due to rainfall and sedimentation. PAHs are metabolized by a pathway mediated by the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor. Many PAHs were reported to show carcinogenicity, endocrine disrupting ability, and teratogenicity in laboratory animals. In order to know the influence of PAHs on ecosystems, it is essential to know the influence of PAHs on invertebrates. However, few studies have been done on the toxicity of PAHs to invertebrates. Therefore in this study we investigated the effect of PAHs on the development of the ascidian Ciona intestinalis, an excellent model organism employed in developmental biology and toxicology.

We tested the developmental toxicity of three PAHs (dibenzothiophene, fluorene, and phenanthrene) and two compounds related to PAHs (beta-naphthoflavone and alpha-naphthoflavone). Betanaphthoflavone is an agonist of AHR. Alpha-naphthoflavone is an inhibitor of cytochrome P450, an enzyme involved in xenobiotic metabolism and induced by AHR. Exposure of embryos to dibenzothiophene, fluorene, or phenanthrene from the 1-cell stage to the swimming larva stage resulted in increased incidence of morphological abnormalities in a concentration-dependent manner. When swimming larvae were exposed to dibenzothiophene, fluorene, phenanthrene, or alpha-naphthoflavone, they developed into juveniles with a degenerated tail larger than normal. These results suggest that Ciona embryos and larvae are sensitive to PAHs and serve as a model to study the effects of PAHs on the development of invertebrates.

Poster 34
Asymmetric cell division in Ciona notochord tapering

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Tapered body parts are common in nature, but little is known about the mechanisms by which taper arises. The notochord of Ciona embryos form a tapered rod of axial mesoderm where cells near the anterior and posterior ends of the notochord are progressively smaller in volume than cells in the middle. Previous work has implicated asymmetric cell division as a possible player in Ciona notochord tapering, however this was never tested fully. We have used a genetic fate mapping approach that relies on the clonal propagation of electroporated transgenes to identify sibling cell pairs for all notochord cells immediately after all the relevant divisions as well as after the notochord intercalates into a single-file rod. We then measured cell volumes in 3D and compared sibling cell volumes to directly quantify the asymmetry of each division. We find that cells fated to be near the anterior or posterior ends of the intercalated notochord divide to become smaller than their siblings that are more fated to be near the middle of the notochord. This occurs in two consecutive divisions such that relatively small asymmetries in each round of division lead to robust volume asymmetries in postmitotic notochord cells. Our data show that asymmetric cell division is the primary driver of volume differences along the AP axis of the intercalated notochord, and we find no evidence of regionally controlled cell shrinking or swelling also being involved. We also find evidence that smaller cells may be preferentially sorted towards the anterior and posterior ends during intercalation based on their volumes, but that this tendency is relatively modest. Our work now focuses on uncovering the molecular mechanisms that control these asymmetric cell divisions.

Poster 35
Transcriptional targets of Xbp1 in the Ciona notochord.

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In all members of the Chordate phylum, the notochord plays a major role in providing structural support and patterning signals to the developing embryo. For these reasons, we are interested in elucidating the essential complement of evolutionarily conserved transcription factors expressed during notochord development in Ciona, and in studying their respective functions. We have previously reported the identification of Ciona Xbp1 (Ci-Xbp1) and its sustained expression during notochord formation. Xbp1 genes have been reported in several chordates; however, their role in notochord formation is yet to be determined. Here we provide evidence of the transcriptional dependence of Ci-Xbp1 upon Ciona Brachyury (Ci-Bra), a major regulator of notochord formation in all chordates. We also report the identification of several Ci-Xbp1-downstream genes, including 43 notochord genes, 23 of which were first identified through this study. These notochord genes include extracellular matrix components, anion exchange transporters and cell adhesion molecules, many of which are conserved in higher chordates.

Of note, Ci-Xbp1 also appears to be influencing transcription of Ci-Bra itself, through a positive feedback loop. These results uncover a relevant connection between these two transcription factors that are both crucial players in a variety of cellular processes, such as tumorigenesis, in higher chordates.

Poster 36
Induced Cytoplasmic Aster in ascidian Ciona intestinalis egg

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Plant cells and most of the animal egg have no centrosome. In the animal development, mitotic apparatus of first cell division is organized by the centrosome, which is derived from the fertilized sperm. However, mouse and some rodent sperms have no centrosome. In the mouse unfertilized egg, there are many asters, designated as cytoplasmic aster (CA), which is developed without centrosome1) . These CAs can be incorporated into the mitotic apparatus of first several rounds of cell cycle. CA in mouse egg is a unique structure. Most of the vertebrate egg, including human, bovine, and frog egg has no CA. However, in the extract of frog egg, the addition of taxol could induce microtubule assembly, which resembled to CA2) . In addition, when the human and bovine egg was treated with Paclitaxel followed by the parthenogenetic development, CA was appeared3),4). These results suggested that many vertebrate egg had an ability to make CA even though they did not show any CA in normal development. In order to find another example of the CA induction, we used the ascidian, Ciona intestinalis, egg. The ascidian is a chordate, and C. intestinalis is one of the well-studied model animals for understanding the vertebrate development. When the unfertilized egg was treated with 10 μM Paclitaxel for one hour, many CAs were formed in the peripheral region (Fig. 1). This CAs were quickly disappeared (within 5 min) when the drug was washed out. When the CA-induced unfertilized egg was fertilized in the Paclitaxel containing sea water, fertilization was normally occurred and CAs did not show obvious change. Up to 10 minutes post fertilization (mpf), CAs gradually moved closer to the cortex, independent of the cortical contraction of the first phase of reorganization. In addition, no CA was observed interacting with the sperm aster. This is the first report of the CA induction in ascidian egg. This ascidian CA has several advantages; easy to handle, quickly induced, large-scale production, and less ethical problem. It could be a good experimental model system for studying the function of CA. In chordate development, some eggs form CAs and the other prevent the formation of CA. It is very interesting to understand why and how these various developmental mechanisms could be evolved among chordate. Figure. 1 Paclitaxel induced CA in the unfertilized ascidian Ciona intestinalis egg. Scale bar, 20 μm.

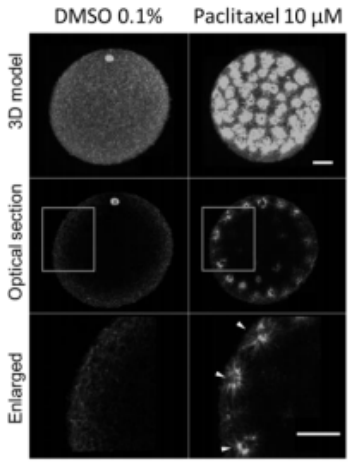

Figure. 1 Paclitaxel induced CA in the unfertilized ascidian Ciona intestinalis egg. Scale bar, 20 μm

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Poster 37
Evolutionary and Developmental analysis of germline formation in simple chordate embryos Tao

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In sexually reproducing animals, correct specification of the germ cell lineage during development is critical for transmission of the genetic information over generations. Previous studies demonstrated that, in contrast to conserved molecules and mechanisms for somatic cell development, the primordial germ cell (PGC) segregation from somatic cells is governed in part by non-homolog molecules across various species[1]. In particular, it is of interest to understand how and why genes responsible for keeping the PGC identity during early development through regulation of analogous transcriptional repression, such as PEM in Halocynthia roretzi, Pgc in Drosophila, and PIE-1 in C. elegans, can be easily replaced in evolution[2-4]. In our current study, we are interested in studying the specification of PGC in two closely-related ascidian species, Halocynthia roretzi and Ciona savignyi, as their PEM genes may harbor certain unpredictable divergences in terms of function or functional domains, according to the previous studies[2]. Specifically, we are focusing on comparing the mechanisms and functional domain applied by the germline transcriptional repressor PEM from these two representative ascidian species. Here, we are also keen on working with a basal Urochordate animal Oikopleura dioica, in which PEM is not present in its genome and also the process of germ cell specification remains unclear. We are examining the molecular mechanisms by which Oikopleura dioica mediates germline formation. Thus, our study aims to provide more comparative information to pinpoint whether and how the mechanisms for PGC specification might be different even within closely-related Tunicata lineages.

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Poster 74

TCF is dispensable for cardiopharyngeal fate specification in *Ciona*

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In vertebrates, canonical Wnt signaling first activates cardiogenic mesoderm and then inhibits the formation of cardiomyocytes. I am studying the roles for Wnt signaling in the cardiopharyngeal lineage of *Ciona robusta*. *Ciona* possesses two pairs of cardiopharyngeal precursors called trunk ventral cells (TVCs). They divide asymmetrically forming the first heart precursors and secondary TVCs (sTVCs), the latter giving rise to the second heart precursors and pharyngeal muscle precursors, known as atrial siphon muscle (ASM) precursors. Several Wnt components, such as TCF, Dkk, and Frizled receptors, are up-regulated in TVCs and their progeny. To reveal quantitative effects of TCF on heart vs. ASM specification, I conducted lineage-specific RNA sequencing experiments on sorted TVC progeny in TCF loss-of-function conditions using the CRISPR/Cas9 system. Analysis shows no significant overlap between TCF targets and heart or ASM genes. Later in development, Wnt-related genes, such as Frizzled4, LRP4/8, and two Wnt ligands show restricted expression in FHPs. I used the CRISPR/Cas9 system to knock out the function of Frizzled4 and LRP4/8 and did not detect differences in MHC2 expression in the juvenile heart. This result suggests that Wnt signaling does not have a late role in the heart insofar as our methods were able to detect.

Poster 75

Regulation of cardiopharyngeal development – moving beyond transcription

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In vertebrate embryos, both head muscles and the chambered heart arise from a common pool of progenitor cells in the cardiopharyngeal field. This developmental connection explains why cardiac and craniofacial congenital defects are often linked, as in DiGeorge syndrome. Similarly, in the simple chordate model Ciona, multipotent cardiopharyngeal progenitors go through asymmetric divisions giving rise to both Atrial Siphon Muscle (ASM) and heart precursors. These multipotent progenitors, the trunk ventral cells, display multi-lineage transcriptional priming: they express a combination of early ASM- and heartspecific regulators that become restricted to their corresponding precursors following asymmetric divisions. However, heart progenitors need to clear gene products that pertain to the ASM program very rapidly after the asymmetric divisions, and vice versa. We hypothesize that regulation beyond transcription, e.g. translation, protein modifications, and degradation, is a crucial component of this process. A recent single-cell RNA-seq analysis has identified ASM and heart specific markers which also contains ubiquitination-related enzymes and RNA-binding proteins. I have validated these markers with respect to restricted expression in either the heart or the ASM precursors. I then conducted lineage-specific loss-of-function analyses using the CRISPR/Cas9 system on 6 of 15 candidate regulators. Knock-out of RNF149, which contains an uncharacterized protease-associated (PA) domain, results in an ASM cell behavior defect and downregulation of the ASM marker Ebf. The molecular underpinnings of this phenotype remain to be elucidated. Characterization of RNF149 and other post-transcriptional regulators require development of small-sample proteomics approaches which I will develop by experimentally converting the entire cardiopharyngeal lineage into either heart or ASM progenitors to obtain homogeneous cell populations for proteomics analysis.

Genetics and genomics

Poster 38
An update of the ascidian biocuration: new data, new tools and prospective

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Bioself Communication (www.bioself-communication.com) was developed to provide in a sustainable manner biocuration and scientific communication in the ascidian community. Bioself is dedicated to train researchers, developers and biocurators on the access, the use and the annotation of ascidian resources and tools. To that end, Bioself provides several training and services in different domains: Definition, extension and publication of standards (controlled vocabulary and ontologies) ; Organization of data collection, storage and processing ; Data annotation (cis-regulatory region, expression data) ; Data quality control ; Database design, etc... The Lemaire lab funded first missions of Bioself to take in charge ANISEED biocuration.

I will present in this poster an update of new data available in ANISEED in addition to new annotation tools. Bioself is looking for new collaborations and will be pleased to provide its expertise to the ascidian community.

Poster 39
Evolution of SL trans-splicing in the deuterostomes

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SL trans-splicing in the deuterostomes was first identified in the tunicate chordate Ciona intestinalis and has since been found in all tunicate species examined. Because tunicates are the sister group of vertebrates, this finding has suggested that vertebrates, in which SL trans-splicing appears to be absent, may have descended from a transsplicing deuterostome ancestor. Evaluation of this possibility requires careful assessment of the presence/absence of transsplicing in other major deuterostome lineages. We defined a stringent and practical criterion for judging the presence/apparent absence of SL trans-splicing based on high-throughput 5'-RACE sequence data, and on this basis assessed the SL trans-splicing status of the best-characterized species in each of the major deuterostome clades Echinodermata (Strongylocentrotus purpuratus), Hemichordata (Saccoglossus kowalevskii), and Chordata (represented by Cephalochordata (Branchiostoma floridae), and Agnatha (Petromyzon marinus). Our results indicate the apparent absence of SL trans-splicing in all of these species. This supports an evolutionary scenario in which vertebrates did not arise from an SL-trans-splicing ancestor but, rather, that SL trans-splicing arose within the tunicate lineage after the tunicate/vertebrate divergence. We discuss the implications of these findings for evolution within the deuterostomes, and for the larger-scale evolution of SL trans-splicing in the eukaryotes as a whole

Poster 40
Resource project of *Ciona intestinalis* for supporting Tunicate community

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We have developed the systematic material sharing system for facilitating your experiments. This project, named National BioResource Project (NBRP) provides transgenic and mutant lines, DNA constructs, and wild types of *Ciona intestinalis*. This presentation is purposed to evoke your interest in this project and promote your requests.

Transgenic and mutant lines. Transgenic lines are valuable markers for tissues and organs because they express fluorescent protein in the non-mosaic fashion. In *Ciona*, many transgenic lines have been created with transposon-based technologies, and these lines are available from NBRP. In addition to the marker lines for larval tissues, organ markers in the juvenile/adult body are present whose labeling has not been achieved by electroporations of DNA constructs. For abroad laboratories, we can provide alive dry sperm of transgenic lines that can be easily used by mixing it with wild type eggs. Mutants are particularly useful for elucidating functions of genes. In *Ciona*, some mutants have been made based on transposon-based mutagenesis and genome editing technologies. These mutants are also available from NBRP.

DNA constructs. Expression vectors are routinely used for analyzing gene functions and labeling organelles, cells, tissues, and organs. These DNA constructs are invaluable resources for *Ciona* because we can introduce them easily by electroporation. NBRP have collected expression vectors associated with the transgenic lines, and the vector information has been databased that includes their sequences and references. Recently, knockouts of *Ciona* genes with TALENs and CRISPR/Cas9 were reported. The technologies have enabled us to address gene functions quickly in G0 generation. TALEN and CRISPR/Cas9 expression vectors created in Japan have also been collected in our resource, and their basic information including the mutation frequencies has been databased. We are waiting for your requests for these materials.

Wild type (closed colony). Unfortunately, tunicate community does not have a standard wild type strain that assures the reproducibility of experiments. NBRP has started a new project for circumventing this issue. First, we cultivate closed colonies of wild types. Closed colonies are the populations maintained by crossing within the populations. This restricts genetic variations appearing in the families, thus assuring more reproducible experiments than those done with unknown populations collected from the ocean every time you do experiments. We are sequencing the genomes of the closed colonies in order to characterize the variations in the families. The genome sequences will allow us to know the potential variations in your genes-of-interest to deduce its effect on your experiments, and to design oligonucleotides and genome editing constructs that can bind to the genome without mismatches. Living, matured wild type (closed colony) adults can be delivered to abroad countries in a few days while keeping their health. We wish your use of our wild type (closed colony) to standardize experiments in *Ciona*.

Order system and information. You can order resources through our online shopping system and can pay fees with a credit card. Materials Transfer Agreement is necessary for your first orders of the materials. The URL for the shopping and databases are as follows:

Transgenic lines, mutants and DNA constructs; http://marinebio.nbrp.jp/ciona/
Genome Editing; http://marinebio.nbrp.jp/ciona/forwardToKnockOutAction.do
Wild type (closed colony); http://marinebio.nbrp.jp/wild.jsp
Genome sequence; http://ghost.zool.kyoto-u.ac.jp/cgi-bin/gb2/gbrowse/kh/

Poster 41
The Role of the Transcription Factor NFAT5 in Notochord Development and Tubulogenesis

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In all chordates, from ascidians to humans, the notochord acts as a fundamental source of support and patterning signals for the developing body plan. In ascidians, the steps required for notochord formation can be clearly visualized because the embryos are translucent and develop very rapidly, and the notochord is composed by only 40 cells. Of note, the last steps of notochord development in ascidians involve the formation of intercellular pockets that will eventually coalesce, forming a lumen. Lumen formation, or tubulogenesis, is an evolutionarily conserved process that is reiteratively used by widely different organisms to form tubular structures, and is required during the morphogenesis of a variety of organs. Our laboratory has shown that the transcription factor NFAT5 (Nuclear Factor of Activated T Cells 5) is expressed in the developing Ciona notochord. In vertebrates, NFAT5 is expressed in the central most regions of the intervertebral discs, which are formed by notochord-derived cells, and is required to maintain cell homeostasis and osmolarity by activating genes that counteract hypertonic stress. Despite the information available on its role in the survival of notochord-derived cells, the function of NFAT5 in notochord formation is still unknown. Based upon our preliminary results, we hypothesized that Ciona NFAT5 (CiNFAT5) functions in notochord development by controlling the formation of intercellular pockets and consequent tubulogenesis. To test this hypothesis, we are determining the identities of genes that are transcriptionally regulated by Ci-NFAT5. To this aim, we have isolated Ciona notochord cells through fluorescence-activated cell sorting (FACS) from control embryos and from embryos expressing a repressor form of Ci-NFAT5. We are currently analyzing the transcriptomes of these cells through single-cell RNA-Seq. These experiments should provide the first insight into the role that NFAT5 plays in notochord development and tubulogenesis, and expand the knowledge of the effectors used by NFAT5 to ensure the integrity of the notochord-derived cells of the intervertebral discs.

Poster 42
Advancing CRISPR-Cas Technologies for Effective Gene Perturbations in the Ascidian Ciona robusta

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Eliminating or silencing a gene's level of activity, whether by antisense morpholinos, TALE nucleases or RNAi, is one of the classic approaches developmental biologists employ to determine a gene's function. A prokaryotic adaptive immune system that protects against invading viral DNA, called the CRISPR-Cas System, was recently adapted for use in eukaryotic cells. This technology has been established in several model organisms as a powerful and efficient tool for knocking out or knocking down the function of a gene of interest. This simple system requires the coexpression of Cas9 nuclease and a specifically designed guide RNA that in combination targets and cleaves a sequence of interest introducing an indel-forming double-stranded break. Several groups have now shown that CRISPR-Cas functions with fidelity and efficiency Ciona robusta. Previously, we designed and built guide-RNAs (gRNAs) that targeted the transmembrane domain-coding region of the Tyrosinase (Tyr) gene. Co-expressing these guide-RNAs and the Cas9 nuclease in the pigmented sensory cells produced albino embryos suggesting that the system functioned as a genome editor.

Here, we show that CRISPR-Cas mediated genomic knock-ins can be efficiently generated in Ciona. Electroporating a tissue-specific transgene driving Cas9 and a U6- or H1-driven gRNA together with a specifically designed homology-directed-repair template results in gene-specific patterns of fluorescence consistent with a targeted genomic insertion. Sequencing PCR products derived from single embryos validates correct genomic editing. Genome editing efficiency at the Tyr locus is increased when gRNAs flanked by ribozymes are specifically expressed in these cells suggesting that RNA Pol II-driven gRNAs are more effective than RNA Pol III gRNAs.

Finally, we show that a nuclease-dead Cas9 fused to a repression domain is an effective transcriptional repression system that can be deployed against any gene of interest without modifying the genome. We believe that these variations to CRISPR-Cas technology will be invaluable tools for illuminating the genetic regulation of nervous system development in Ciona.

Poster 43
Single-cell transcriptome analysis in Ciona intestinalis

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Cell-specific gene expression during animal development has typically been studied using tissue or whole-organism techniques such as in situ hybridization. Over the past decade this has been supplemented with genome-wide quantitative techniques, including EST sequencing, microarrays and RNA-Seq, which support the discovery of novel factors by measuring the expression of all genes simultaneously and allow for quantifiable comparisons between conditions - at different stages of development or in different cell types. In general, these methods have required biological material to be pooled, thus limiting wider applicability within developmental biology. More recently, RNA-Seq techniques have emerged that can work with small quantities of RNA, even at the level of the single cell, making it possible to study gene expression variability across different cell types within or across individual embryos or to follow the profile of gene expression in the developing embryo at the level of individual cells.

We have developed and tested single cell RNA-Seq using dissected cells from the embryo of Ciona intestinalis whose invariant lineage allows us to provide distinct gene expression on each cell type. We will present data quality and reliability across our biological replicates at the 16-cell stage and highlight our success in discovering known and novel patterns of cell-specific expression.

Neurobiology

Poster 44
The ascidian Phallusia mammillata as a system model to study the neurodevelopmental toxicity of endocrine-disrupting chemicals

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Endocrine-disrupting chemicals (EDCs) are compounds able to mimic, antagonize, or modify normal hormonal activity. EDCs can interfere with the endocrine system through two main pathways: the genomic pathway, via binding to nuclear receptors; or the non-genomic pathway, via binding to cell membrane receptors. Numerous studies showed that brain formation in ascidians is sensitive to toxic insults especially from endocrine disruptors. Together with the simplicity of the neural complex of ascidian larvae, such sensitivity makes the ascidian embryo a favorable model to study neurodevelopmental defects induced by toxic compounds.

Phallusia mammillata (a European solitary tunicate) tadpole larvae bear a central nervous system composed of a sensory/brain vesicle comprising gravity and light sensing pigment sensory organs (PSO), and visceral/motor ganglion homologous to the vertebrate diencephalon and hindbrain respectively.

Different EDCs can induce brain malformations easily observed in Phallusia tadpole larvae. To better characterize the neural phenotypes induced by EDCs, the following endpoints were quantitatively assessed: trunk length/width ratio, otolith-ocellus area and otolith-ocellus distance. After setting up endpoints in non-exposed versus BPA-exposed embryos, the phenotypes of β-estradiol-3-benzoate and other known EDCs was studied. Our analysis using multiple endpoints allows us to characterize more specific phenotypes to discriminate the phenotypes induced by different classes of compounds.

Poster 45
A second complete connectome: the larval CNS of the ascidian Ciona intestinalis

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There is growing acknowledgement that the comprehensive compilation of synaptic circuits densely reconstructed in model brains is now both a technologically feasible and scientifically enabling possibility in neurobiology, much as 30 years ago genomics was in molecular biology and genetics. Such circuit information from electron microscopy will eventually be required to understand how differences between brains underlie differences in behaviour, or to identify common synaptic circuits in different species. The diminutive brains of invertebrates, especially those of larval stages with few cells, are Nature's bounty for connectomic analyses. Pride of place for the first report of a complete connectome goes to C. elegans (White et al., 1986). Drosophila also supports powerful genetic methods and interesting behaviours, especially for vision, olfaction and courtship, but its neurons branch profusely to yield many slender neurites that require special methods to reconstruct at EM level. Pursuing a suitable alternative of an interesting simple brain in a model species, one more closely related to vertebrates, we have used serial section EM to compile a complete densely reconstructed connectome for the CNS in the tadpole larva of Ciona intestinalis. The dorsal, tubular CNS of the ~1- mm tadpole larva of this a sibling vertebrate is built on a similar plan to vertebrate brains, its ~330 cells distributed rostrocaudally in three centres: a brain vesicle, motor ganglion and caudal nerve cord. Although early neural development is well studied, detailed cellular organization of the swimming larva's CNS is hitherto unreported. Myelin and glia are both lacking; most were formerly unknown. Neurons are simple unipolar tubes with at most a single dendrite and few synapses. In one larva (Ryan et al., 2016, eLife) 177 neurons formed 6618 CNS synapses including 1772 neuromuscular junctions, augmented by 1206 gap junctions. Some synapses are bidirectional, others form reciprocal or serial motifs; 922 are polyadic. Axo-axonal synapses predominate. Most neurons have ciliary organelles, and many features lack structural specialization. Some neuron types show homology with vertebrate neurons, as revealed by their similar circuits. I will discuss the value of this new connectome, and how knowledge of it can open this tiny new model invertebrate brain to future neurobiological study in a basal chordate. Support: NSERC (Ottawa).

Poster 46

Alternative methods for miR-7 knockdown in Ciona

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MicroRNAs are small, non-coding RNAs of 21-23 nucleotides that regulate gene expression at posttranscriptional level (1). miR-7 mature sequence is evolutionary conserved across Bilateria (2). During embryonic development miR-7 neural expression was reported in mammals and teleosts as well as in animals as distantly related as annelids. The first in vivo studies were performed on fly and demonstrated that miR-7 mutants develop defects in sensory structures only under stressful conditions (4). In humans, the majority of studies concerning this miRNA were focused on its role in cancer, where miR-7 seemds to be context-specific as evidence exists for both its oncogenic and tumor suppressor roles (3). Moreover, in neural stem cells, miR-7 plays a crucial role in synapse formation as its overexpression induced a significant increase of synapsin gene expression in the derived neurons (5). A miR-7 sequence, identical to human one, is also present in the ascidian Ciona intestinalis genome and its neural expression appears to be conserved. In Ciona tailbuds and larvae, mature transcripts were found in the sensory vesicle. To characterize miR-7 function, we started performing knockdown experiments by injecting the commercial AntagomiR-7 (Dharmacon) and Peptide Nucleic Acids (PNAs).

PNAs are synthetic mimics of natural oligonucleotides, in which each ribose phosphate unit is replaced by an aminoethylglycine unit. PNAs bind complementary DNA and RNA strands with excellent affinity and sequence specificity, and exhibit greater mismatch selectivity compared to natural oligonucleotides.

The hatching ratio of control and injected samples was not affected by anti miR-7 injections. Injected embryos displayed similar morphology to controls. In situ hybridization experiments revealed that although the nervous system was differentiated properly, the expression of Ci-syn was drastically reduced in injected embryos. Synapsins are neuronal proteins involved in neurotransmitter release, neurite elongation and synapse formation (6). Therefore, our first results are consistent with what reported in human, rising the hypothesis that the neural role of miR-7 is conserved between vertebrates and ascidians.

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Notes

Poster 47
Signaling through the T-type Ca2+ channel CAV3 alters gene expression during neural tube closure.

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The formation of the central nervous system is a defining event in chordate development. In ascidians, 40 cells make up the neural plate. During neurulation, the neural plate invaginates and the neural fold edges fuse at the dorsal midline to make the neural tube. During tube closure, neural cells must separate from neighboring epidermis and adhere to neural cells in the opposing folds.

Previously we described the Ciona savignyi mutant line bugeye, which carries a defect in CAV3, the T-type calcium channel (1). Bugeye mutants exhibit an open and protruding anterior neural tube. Neural induction, neural fold formation and posterior neural tube closure appear normal in this mutant.
Interestingly, the phenotype manifests several hours after the end of neurulation, suggesting a failure to fuse the anterior neural tube.

Recently we isolated a Ciona robusta mutant line with an identical phenotype. This mutant is in the same complementation group (as determined by crossing to C. savignyi bugeye). However, we find no change in CAV3 expression in the C. robusta mutant. We sequenced the CAV3 gene in the C. robusta mutant and found two mutated amino acids in a conserved domain of CAV3. The sequence mutation and phenotype suggest a functional null. We have performed RNAseq analysis on both the C. savignyi and C. robusta lines by isolating RNA from mutant embryos and their phenotypically normal siblings. Using the Ilumina High Output NextSeq500, we obtained 50 million paired-end reads per triplicate sample. We found 42 genes upregulated and 52 genes downregulated in C. robusta bugeye mutants and 41 upregulated and 18 downregulated genes in C. savignyi (FDR <0.05). Using qRT-PCR, we validated the differential expression of select genes. Differentially expressed genes fall into several categories, including extracellular matrix proteins (e.g., fibronectin-like proteins), cell adhesion (e.g., ependymin, selectin) proteins and transcription factors (e.g., Sp1, zinc finger containing proteins).

We hypothesize that calcium signaling through CAV3 may serve as a checkpoint in neural tube closure, acting as a cue for the neural cells to fuse and begin their next developmental program. We are currently characterizing differentially expressed genes in bugeye and wild-type embryos using CRISPR knockouts and by overexpression in the nervous system. These results should enhance our understanding of CAV3 function during neural tube closure.

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Poster 48
Localization analysis of cionin, cholecystokinin/gastrin ortholog, and its receptor in ascidian, Ciona intestinalis

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Cholecystokinin (CCK) and gastrin comprise a peptide family in vertebrates. These peptides share sulfated tyrosine residues and the tetrapeptide consensus sequence (Trp-Met-Asp-Phe-NH2) at the Cterminus, suggesting that they are derived from a common ancestral gene. Moreover, they share two paralogous CCK receptors (CCKRs). Cionin is a CCK/gastrin family peptide that was isolated and identified from the neural complex in the ascidian, Ciona intestinalis (Johnsen and Rehfeld, 1990). It shares sequence features with vertebrate CCK/gastrin family peptides. In vertebrates, treatment with cionin induces gallbladder contractions and gastric acid secretions, which are typical CCK and gastrin functions, respectively. Previously, we identified two authentic cionin receptors (CioRs) that are orthologous to vertebrate CCKRs (Sekiguchi et al., 2012). This demonstrated that the molecular characteristics of CCK/gastrin family peptides and their receptors are conserved in chordates. However, the evolution of the biological role of the CCK/gastrin family remains unclear due to the lack of functional information on cionin in C. intestinalis.

In the present study, we analyzed the tissue distribution of mRNA of cionin and two CioRs using quantitative PCR. The transcript of cionin gene was exclusively expressed in the neural complex. CioR1 and CioR2 mRNA were predominantly expressed in the neural complex and moderately expressed in the endostyle, stomach, intestine, and ovary. Interestingly, the expression level of cionin in the digestive tract was lower than that in the neural complex, unlike the vertebrate CCK/gastrin family peptide gene. These issues suggested that cionin is released from the central nervous system and acts in peripheral organs via nervous or humoral pathways. Then, to evaluate the localization of cionin mRNA in the neural complex, we conducted in situ hybridization analysis using whole and sectioned neural complex specimens. Cionin mRNA was detected in the anterior part and surface layer of the cerebral ganglion. Furthermore, immunohistochemical analysis using serial sections of the neural complex confirmed that the cionin peptide was expressed in the cell bodies of the anterior part of the cerebral ganglion and neural fibers throughout the cerebral ganglion. On the other hand, in situ hybridization of CioR1 mRNA revealed expression throughout the cerebral ganglion. Finally, to characterize the cioninergic neurons, we compared cionin mRNA localization with Ci-vesicular acetylcholine transporter (Ci-VACHT) mRNA, which is a marker gene of cholinergic neurons. Ci-VACHT mRNA localization was detected mainly in the middle and posterior parts of the cerebral ganglion. This expression pattern partially overlapped with that of CioR1 mRNA, suggesting that cioninergic neurons interact with cholinergic neurons.

In this study, we clarified the localization of cionin and CioR1. These data will give us a clue as to the physiological function of cionin in C. intestinalis.

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Poster 49
Developmental profiling of the Central Pattern Generator in the Ascidian Swimming Larva

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The brain of vertebrates consists of neural circuits with an astronomical number of neurons. In contrast, the central nervous system of ascidians contains only about 300 cells and thus provides us with a simple model to investigate how neurons differentiate and connect each other to construct neural circuits. Central pattern generator (CPG) is a neural circuit that produces rhythmic movements such as mastication, respiration and locomotion. For locomotion, vertebrates show rhythmic and alternate contractions of skeletal muscle caused by CPGs located in the spinal cord. Ascidian larvae also show typical rhythmic tail movement during their swimming. This movement has been suggested to be driven by a neural circuit that may be homologous to the spinal CPG of vertebrates. In this study, we observed in detail development and differentiation of a putative CPG for larval tail movements of the ascidian Ciona intestinalis. Previous studies reported that the five pairs of motor neurons (MNs) for contraction of larval tail muscles are existed in the motor ganglion1. We proposed a hypothesis that these MNs and two pairs of inhibitory interneurons called anterior caudal inhibitory neurons (ACINs) are the core components of the CPG for ascidian tail movement2. We first observed differentiation of these neurons with several neuronal markers such as VACHT, a marker for cholinergic neurons, and VGAT, a marker for GABA/glycinergic neurons. At the mid tailbud stage (12-hour post fertilization, hpf), three pairs of MNs precursor cells began to express VACHT. Then, they extended their axons posteriorly and additional two pairs of cells in the anterior region of the motor ganglion started to express VACHT. There were no VGAT-positive cells in the tail at this point. This result indicates that differentiation of MNs precedes that of ACINs. The precursor cells of ACINs began to express VGAT at 16 hpf and then extended the contralateral axon terminals at 18 to 20 hpf. Based on these observations, we suspected that the development of CPG for larval tail movement is divided into two phases. In the first phase from 12 to 16 hpf, larvae have only motor neurons and solely show nonrhythmic tail movement. In the second phase from 16 to 20 hpf, larvae become to have all neurons for CPG and are able to rhythmically move the tail. To assess this hypothesis, we examined tail movements from 12 to 20 hpf. The tail movements first observed were a single or double flicking without rhythmic oscillation. Rhythmic tail movement began from 16 hpf and this period corresponds to the commencement of ACIN differentiation. These findings together support the hypothesis that MNs and ACINs are essential components of the CPG for swimming locomotion of the ascidian larva.

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Poster 50

SoxB1 plays a crucial role in development of the anterior caudal inhibitory neurons in the Ciona intestinalis larva

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The simple central nervous system of the ascidian Ciona intestinalis larva contains a central pattern generator (CPG) neural circuit for swimming. The CPG has been proposed to contain two major components: several bilateral pairs of cholinergic motor neurons in the motor ganglion and two bilateral pairs of GABAergic/glycinergic inhibitory interneurons (anterior caudal inhibitory neurons; ACINs) in the anterior part of the tail nerve cord1 . The organization of the CPG in the ascidian larva seems simpler but resembles that of the spinal CPGs for vertebrate locomotion, so it could serve as a model for the development and function of chordate CPGs with single-cell resolution. To investigate the developmental mechanism of ACINs, we have analyzed the cis-regulatory region of the vesicular GABA/glycine transporter gene (CiVGAT), a specific marker for GABAergic/glycinergic neurons and identified SoxB1 as a candidate transcription factor regulating ACIN development. Cell linage analyses using DiI inferred that ACINs derive from A11.116 cells, which had been thought to solely give rise to glial ependymal cells of the nerve cord2 . Knockdown of SoxB1 resulted in superfluous ACINs, suggesting that an inhibitory role of SoxB1 in ACIN development. To further confirm the inhibitory role, SoxB1 tagged with a red fluorescence protein was overexpressed in the descendants of the A11.116 cells under the control of the Hox5 promoter. As expected, forced expression of the SoxB1-mCherry fusion protein throughout the anterior lateral nerve cord cells resulted in the loss of ACINs. Comparison of the primary structure between Ciona SoxB1 and mammalian Sox2 reveals that the HMG DNA-binding domain, including two nuclear localization signal (NLS) sequences, is highly conserved whereas the C-terminal region, which is known to contain a transcriptional activator domain, is less conserved. A mutant SoxB1 protein in which one of the NLS sequences was deleted mislocalized in the cytoplasm but retained activity to inhibit ACIN differentiation. In contrast, deletion of the C-terminal region from Ciona SoxB1 resulted in the loss of the inhibitory effect on ACIN differentiation while this mutant protein was properly localized in the nucleus. Furthemore, a SoxB1 mutant lacking the HMG domain exhibited neither nuclear localization nor inhibitory effect on ACIN differentiation. These results suggest that SoxB1 suppresses differentiation of ACINs and that this activity requires the C-terminal and HMG domains.

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Post-embryonic development and physiology

Poster 51

The SHARK gene *cymric* may be involved in tail loss in the ascidian *Molgula occulta*

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Ascidians share several developmental features with the vertebrates including pharyngeal slits, an endostyle, and a notochord inside a functional larval tail. During typical ascidian development ten notochord precursor cells divide twice to make forty notochord cells and then converge and extend to create a notochord that becomes flanked by muscle to make a swimming tail. The molgulids are a monophyletic clade of ascidians in which a tailless phenotype has independently evolved multiple times. We are searching for the molecular basis of this tail loss by investigating the differential gene expression of two species of molgulids, *Molgula oculata* and *Molgula occulta*. *M. oculata* has the tailed phenotype, but *M. occulta* has lost the tail and notochord, developing twenty notochord cells that do not converge and extend. The two species can be hybridized: if the eggs of the tailless species are used, then some of the resulting hybrids have twenty notochord cells that will converge and extend into a non-functional half tail. We have sequenced the genomes and developmental transcriptomes of these two species and the hybrid, and are searching for differential gene expression of notochord related genes to identify the developmental mechanisms responsible for the loss of the tailed phenotype. We are using the transcriptomes to identify potential genes, then using qPCR and *in situ* hybridization to confirm spatio-temporal expression. We first investigated *cymric,* a SHARK tyrosine kinase located in the myoplasm in *M. oculata*. *Cymric* is altered in the tailless *M. occulta* (Swalla et al. 1993); the tailed *M. oculata* makes the full transcript, but the tailless *M. occulta* transcript is missing the tyrosine kinase domain. Genome analysis confirms the transcriptome findings; the *M. occulta cymric* gene lacks the exons for the tyrosine kinase domain. We are currently investigating whether other tailless species also have an altered *cymric* gene, and our preliminary results suggest that the altered *cymric* is not the original mutation causing taillessness in molgulid ascidians, but rather a secondary effect observed in *Molgula occulta*.

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Poster 52
AMPA glutamate receptors are essential for sensory-organ formation and morphogenesis in the basal chordate

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AMPA-type glutamate receptors (GluAs) mediate fast excitatory transmission in the vertebrate central nervous system (CNS), and their function has been extensively studied in the mature mammalian brain. However, GluA expression begins very early in developing embryos, suggesting that they may also have unidentified developmental roles. Here, we identify novel developmental roles for GluAs in the ascidian Ciona intestinalis. Mammals express Ca2+-permeable GluAs (Ca-P GluAs) and Ca2+-impermeable GluAs (Ca-I GluAs), by combining subunits derived from four gene. In contrast, ascidians have a single gluA gene (8). Taking advantage of the simple genomic GluA organization in ascidians, we knocked down (KD) GluAs in Ciona and observed severe impairments in formation of the ocellus, a photoreceptive organ used during the swimming stage, and in resorption of the tail and body axis rotation during metamorphosis to the adult stage. These defects could be rescued by injection of KD-resistant GluAs. GluA KD phenotypes could also be reproduced by expressing a GluA mutant that dominantly inhibits glutamate-evoked currents. These results suggest that, in addition to their role in synaptic communication in mature animals, GluAs also have critical developmental functions.

Poster 53
ABC-transporter activity and autocrine stimulation by 12-S-HETE are required for germ cell migration towards sphingosine-1-phosphate in Botryllus schlosseri

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The colonial ascidian Botryllus schlosseri provides a unique model to study germline stem cell biology, as it continuously regenerates entire bodies, including all somatic tissues and the germ line, during repetitive cycles of asexual reproduction that occur each week. The source of the germline in these asexually-derived individuals are germ cell precursors (GCP) and long-lived germline stem cells (GSC) that migrate to new germline niches as they develop. We have previously shown that homing and migration of germ cells in Botryllus is directed by sphingosine-1-phosphate (S1P) signaling. Here, we show that ABC-transporters such as abcc1 and abcb1 are highly expressed on GSC and GCP. To investigate the role of ABC transporter activity in germ cell migration, GSC and GCP were prospectively isolated by flow cytometry, and subjected to transwell migration assays. Inhibition of ABC-transporter activity leads to a reduction in the migratory response towards low concentrations of S1P in vitro. In vivo, inhibition of ABCC1, ABCB1 or both leads to substantial failure of germ cell homing to secondary bud niches. In mammals, ABCC1 and ABCB1 are known to export lipid-signaling molecules such as leukotrienes and prostaglandins from the cytoplasm. These molecules are derived from arachidonic acid, which is produced by Phospholipase A2 (PLA2). We found that in Botryllus, PLA2 activity is required for migration of germ cells towards low concentrations of S1P, as well as homing of germ cells to secondary buds in vivo. This suggests that production and export of arachidonic acid-derived eicosanoids is required for migration of germ cells towards low concentrations of S1P. Arachidonic acid is metabolized to prostaglandins by cyclooxygenases (Cox), or to leukotrienes by Lipoxygenase 5 (Lox-5). The enzyme 12-Lipoxygenase (Lox-12) metabolizes arachidonic acid to 12-hydroperoxyeicosatetraenoic acid, which may then be metabolized to 12-Hydroxyeicosatetraenoic acid (12-HETE) and to hepoxilins. In vitro, inhibition of Lox-12 significantly reduced migration of Botryllus germ cells towards S1P, whereas inhibition of Cox1, Cox2 or Lox-5 did not affect germ cell migration. The addition of 12-s-HETE rescues migration towards S1P in the presence of inhibitors of ABCC1, ABCB1, PLA2 or Lox-12. In vivo, inhibition of PLA2 or Lox-12 inhibits homing of germ cells to secondary buds. In conclusion, autocrine stimulation by 12-S-HETE and ACB-transporter activity are required for migration of germ cells towards low concentrations of S1P. In future experiments, we aim to identify the receptor for 12-S-HETE and assess its expression in germ cells and its role in finetuning the migratory activity of germ cells in Botryllus.

Poster 54
Regeneration in Botryllus Schlosseri Coincides with Proliferation of Blood Cells

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Regeneration is the ability for an animal to replace damaged or lost tissues, and research in this field will provide insight that may have important biomedical implications regarding regeneration in humans. Upon removal of all bodies from the colony, the tunicate Botryllus schlosseri maintains the capacity to form a new body, including the heart, digestive tract, nervous system and germline, from nothing but remaining extracorporeal vasculature. This process serves as an ideal model to research the molecular mechanisms that underlie robust regeneration. We determined the localization of actively dividing cells using an antibody against phosphorylated histone H3 during early regeneration, and observed that proliferation primarily occurs in circulating cells located inside vessel ampullae.
We are currently testing the role of cell proliferation during the initial stages of regeneration by blocking cell division with hydroxyurea and mitomycin C. Blood borne proliferating cells also express Integrin-alpha-6, which we have shown in previous studies to be expressed by germline stem cells, and these cells appear to home to sites where early regeneration occurs and the first differentiated tissues are formed. We have yet to determine if these cells are necessary for regeneration, however, in ongoing experiments we are developing cell labeling strategies, including EdU, to determine their contribution to regeneration. We are also testing whether the S1P pathway, which directs homing of germ cells to developing asexual buds, is involved in recruiting germline stem cells to regeneration niches.

Poster 55
Polyandrocarpa zorritensis: a model for comparative studies on budding and whole body regeneration

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In colonial ascidians zooids can propagate asexually and regenerate their entire body through varied forms of budding. Buds can rise from different part of the body, involving different kinds of epithelia and/or mobile putative stem cells. In the Styelidae family we find species that cover heterogeneous budding ontologies. In species belonging to the Botryllinae sub-family, such as *Botryllus spp*. and *Symplegma spp*., the bud origin and morphology is described and the molecular processes involved in bud formation are currently under investigation. In contrast, in more phylogenetically distant species such as *Polyandrocarpa zorritensis*, the budding/regeneration are less or not described at all.
In order to characterize the budding in this species, we established a laboratory culture of *P. zorritensis* that allowed maintaining a constant source of asexually propagating zooids. Through *in vivo*, histological and ultrastructural observations, coupled with proliferation assay, we described the early bud development, and showed a budding process quite different from other Styelidae. This allowed us to define a staging system to collect a temporal series of transcriptomes. In addition, in order to investigate the potential role of hemoblasts in the regenerative process, we started to morphologically and molecularly characterize the population of *P.zorritensis* blood cells.
The first observations already showed a budding process quite different from other Styelidae species and further investigations will help to lay comparisons between related ascidian species in order to better understand the molecular mechanisms involved in budding and regeneration and to track their evolution among the group.

Systematics, ecology and evolution

Poster 56
Phylogeny of Tunicata – a morphology based analysis

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Being one of the three major chordate taxa tunicates possibly are closest living relatives to vertebrates. However, tunicate phylogeny remains ambiguous, because molecular phylogenetic studies of the interrelationships of tunicate taxa are contradictory. Furthermore former phylogenetic studies based on morphological characters are limited due to taxon sampling and characters chosen. In order to elucidate chordate evolution we reconstruct the phylogenetic relationships of 19 tunicate families, belonging to all five major tunicate taxa. Our final character matrix includes 80 characters and 45 tunicate and 5 outgroup species. In addition to classical tunicate characters (e.g. structure of branchial basket, body division, colony formation, position of gonads), that traditionally were used in ascidian taxonomy, we focussed on neuroanatomical characters. The latter played a major role in pylogenetic considerations in many other taxa.

Our results show that neuroanatomical characters are quite similar within tunicates in some aspects, but differ considerably in others. The phylogenetic relevance of some characters present in salps such as the absence of the brain-associated neural gland, conspicuous motoneurons, serotonergic neurons, and lateral nerve fiber tracts in the brain is difficult to ascertain, because these characters might be the result of the more active planktonic lifestyle in opposition to a sessile mode of life in ascidians. Our findings help to reconstruct the ground pattern of the last common tunicate ancestor and therefore allow us to draw conclusions on chordate evolution.

Poster 57

Anthropogenic factors influencing invasive ascidian establishment in natural environments Mediterranean Sea

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Marine environments, especially along the coastal zone, are constantly impacted by invasive species1 . Invasive ascidians (Chordata, Tunicata) are well-known for their ability to rapidly overgrow any available substrate2 . While the majority of studies have investigated the factors contributing to the successful establishment of ascidians on artificial substrates3 , the anthropogenic factors that contribute to such establishment on natural substrates have rarely been addressed. Here, we conducted underwater field surveys at eight natural sites along the Israeli Mediterranean coast in order to provide an analysis of biotic and a-biotic factors contributing to these establishment. The findings revealed that sites exposed to extended sewage-spill events demonstrated a reduction in native ascidian species. Moreover, a higher invasive ascidian abundance was recorded at sites located near international ports. Understanding these factors is essential for planned monitoring and protection of areas receptive to invasion, and for developing effective tools for the management and control of invasive species in natural environments.

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Poster 58
Filter Architecture: An Interdisciplinary Approach to the Tunic Structure of the Appendicularian

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With the suggested presentation the interdisciplinary work within the Berlin-based interdisciplinary research cluster „Image Knowledge Gestaltung“ is introduced, where biologists, materials scientists, media theorists, and cultural historians work together on the tunicate appendicularian Oikopleura diocia. The house of the appendicularian is a peculiar structure that neither is a part of the animal in a narrow sense – it is not an organ – nor can it be considered as some kind of animal shelter or external tool as there is a specific physiological function and thermodynamic relation between the animal and its filter system. The question how the tunicate house can be described is investigated in an interdisciplinary cooperation between biomaterials' science and cultural history. In contrast to conceptions like »animal architecture« (Mike Hansell), »extended phenotype« (Richard Dawkins) or »extended organism« (J. Scott Turner), which combine biology with mechanics and thermodynamics, a more radical approach is suggested that is taking the common notion of the tunic filter ›architecture‹ or ›structure‹ seriously. In the 1960s Austrian architects like Walter Pircher, Hans Hollein and Haus Rucker Co. developed temporal and mobile pneumatic housing structures in order to challenge conventional notions of architecture as static and immobile. In reference to these and similar operative filter architectures, we describe the filter house of the tunicate Oikopleura diocia as an operative system that is blurring the clear distinction between ›animate‹ and ›inanimate‹.

Poster 59
Fouling ascidians in the Galapagos on artificial surfaces

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From April 24-May 4, 2016 a group from the Smithsonian Environmental Research Center (SERC) Invasions Lab surveyed a number of floating docks and pilings and numerous settlement panels both uncaged and caged, on two of the Galapagos Islands: Santa Cruz and Baltra. The panels had been submerged for either 3 months or 14 months. Studies were concentrated on several sites on the Santa Cruz Island because this is the location of the main town, Puerto Ayora, and has the most boat and ship traffic. The airport is located on the small adjacent Baltra Island and there is frequent boat traffic there, as well as a large Navy base and dock. Ascidians were the dominant foulers on most panels, especially the caged ones, with a dramatic difference in coverage between caged and uncaged which is presumed to result from differential predation pressure. Since the panels were all suspended with none touching the benthos, the predators are hypothesized to be primarily fish. There were schools of small fish around all the docks, and though they were not identified they were observed to be constantly nipping at the dock surfaces. While this is not the first fouling study to compare caged and uncaged surfaces, it does show that similar studies should include both treatments of settlement panels. The most common ascidians were Didemnum perlucidum, Ascidia sydneiensis, an Ascidia sp. with long tunic spines, 2 small pink Pyura spp. one of which has long siphonal spines, two unidentified didemnids, Diplosoma cf. listerianum, Polyclinum constellatum, Polyandrocarpa zorritensis, and Styela canopus. Several colonies of Botrylloides giganteum were retrieved from plates on the Baltra Navy dock. Other common foulers included Botrylloides nigrum and a few small colonies of Symplegma sp. Two large specimens of the (presumed native) species Halocynthia dumosa (~6 cm) (usually incorrectly referred to as H. hispida) were collected from the Baltra Is. Navy floating dock and one by SCUBA at 40 ft off Bartolome Is. by Greg Ruiz. A complete species list will be presented, including new records for the Galapagos. I thank SERC for their support for this study.

Poster 60
Collection and husbandry of colonial and solitary ascidians

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Ascidians have been used for decades as model organisms in a range of research fields including developmental and evolutionary biology, population genetics and toxicology. These research efforts largely rely on a secure, stable and high quality supply of live ascidians in the laboratory. Several culture methods have been published, in particular for *Ciona* species (Cirino *et al*, 2002; Hendrickson *et al*, 2004; Joly *et al*, 2007). However, it remains challenging for many laboratories to supply a large number of mature individuals on a daily basis, which hinders a thorough application of transgenic methods such as TALEN and CRISPR/Cas9.
Here, we describe methods to collect and breed on artificial supports both *in situ* and *ex situ* several species of ascidians including *Ciona* spp., *Phallusia mammillata* and *Botryllus schlosseri*. We present standardized methods: 1) to culture these species from fertilization until they develop into mature individuals that produce sperm and eggs; 2) to maintain fertile adult individuals; and 3) to induce regeneration for the production of clonal stocks in colonial species. Such methods can also be successfully applied for other ascidian species, notably Styelidae such as *Polyandrocarpa* sp. and *Styela* sp., but also Aplousobranchia like *Clavelina*. Services including ascidian culture and supply are available in the UPMC/CNRS marine stations of Banyuls-sur-Mer, Roscoff and Villefranche-sur-Mer in the context of the national research infrastructure EMBRC-France (http://www.embrc-france.fr/en).

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Poster 61

Color variation in an introduced ascidian reveals two distinct genetic lineages

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Color variation among marine invertebrates is a common phenomenon, typically attributed to intraspecies variation. However, molecular analyses have provided insight into the genetic basis of color variation, and have revealed numerous cases of cryptic speciation. The colonial ascidian Distaplia bermudensis is believed to be native to the Bermuda Islands but exhibits a global spread, with introduced populations in the Mediterranean Sea, Eastern Atlantic, and Western Atlantic, including along the North Carolina (NC) coast. D. bermudensis is characterized by a huge diversity of color morphologies, with tunics in varying shades of white, orange, pink, and purple, and zooids ranging from clear to opaque. However, morphological examination of the zooids had not revealed any significant differences among the morphotypes. In order to determine whether the color variation observed in NC D. bermudensis had a genetic basis, we sampled 12 different color morphs of D. bermudensis from Wilmington, NC and sequenced fragments of the mitochondrial Cytochrome C Oxidase Subunit I (COI) and 18S rRNA genes. Analyses of both the COI and 18S rRNA genes revealed two distinct, well-supported lineages of D. bermudensis that corresponded with distinct color combinations in the live animals (the pattern was unobservable in formalin fixed samples). Lineage A was formed by colonies with dark tunics and clearer zooids, while colonies with lighter tunics and more opaque zooids made up lineage B. These results suggest that two cryptic species may co-exist in NC. Further research will investigate larval morphology and alternative approaches to classic taxonomical observations (e.g. chemotype or microbial symbiont characterization) to further elucidate this potential speciation.

Poster 62

Dismantling the genetic pathway of retinoic acid metabolism and signaling in *Oikopleura dioica*

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Gene loss is an important evolutionary force that generates genetic diversity and shapes the evolution of genomes and species. Outside bacteria and yeast, however, the understanding of the evolutionary impact of gene loss remains elusive, especially in the evolution of complex metazoan species. In this work, using the dismantling of the retinoic acid metabolic gene network (RA-MGN) in the larvacean *Oikopleura dioica* as a case study, we combine approaches of comparative genomics, phylogenetics, biochemistry and developmental biology to investigate the mutational robustness associated to biased patterns of gene loss (Martí-Solans et al., 2016). We demonstrate the absence of alternative pathways for RA-synthesis in *O. dioica*, which suggests that gene losses of RA-MGN were not compensated by mutational robustness, but occurred in a scenario of regressive evolution. This work illustrates how the identification of patterns of gene co-elimination – in our case five losses (*Rdh10*, *Rdh16*, *Bco1*, *Aldh1a* and *Cyp26*)– is a useful strategy to recognize gene network modules associated to distinct functions. Our work also illustrates how the identification of survival genes helps to recognize events of neofunctionalization and ancestral functions. Thus, the survival and extensive duplication of *Cco* and *RdhE2* in *O. dioica* correlated with the acquisition of complex compartmentalization of expression domains in the digestive system and a process of enzymatic neofunctionalization of the *Cco*, while the surviving *Aldh8* could be related to its ancestral housekeeping role against toxic aldehydes.

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Poster 63
Effects of exposure to microplastics on the development and metamorphosis of Ciona robusta

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Microplastics are plastic particles produced to be of small size, as sandblasting media, virgin pellet, cosmetics (Fendall and Sewell, 2009) or that originate from degradation of bigger plastic debris, as polyester fibers, polyethylene plastic bags and polystyrene particles from buoys and floats (Browne et al., 2011; Davidson, 2012; O'Brine and Thompson, 2010). An incredible amount of plastic reaches the marine environment and its ecological impacts are of particular interest. In fact, after ingestion, they may cause damage and block of feeding appendages and digestive system, can migrate from alimentary canal to other body tissues, limit the food intake and transfer pollutants in living organisms (Cole et al., 2013). We evaluated microplastics effects on development and metamorphosis of the solitary ascidian Ciona robusta. We used microbeads with 10 μm diameter made of polystyrene, because it is highly stable with a negligible styrene migration (Cohen et al., 2002). We tested four different concentrations (0.125, 1.25, 12.5 and 25 μg/ml) and analyzed the effects on larval development and survival. Then we exposed attached larvae for five days to test the effects on metamorphosis. Juvenile survival was calculated exposing metamorphosed individuals to microplastics for eight days. We also estimated the ingestion rate as the number of particles ingested in one minute and the expulsion capability measuring the localization of particles in stomach or fecal pellets after 24 hours. Metamorphosis was the process most affected by the microplastics. In fact, the percentage of individuals that completed metamorphosis was statistically lower in all the exposed groups, compared to control. Feeding individuals were also fed with microalgae. The co-ingestion of algae and beads probably reduced the amount of algae assimilated by the juveniles. Moreover, they might spend energy to expel microplastics thus reducing the energy available for the metamorphosis. After 8 days, juvenile survival was not affected, even if the particles persisted longer in the stomach of individuals exposed to the highest concentration, suggesting that further detrimental effects could be observed after a longer exposure.

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Poster 64
The Biodiversity and distribution of Ascidians (Tunicates: Urochordata) from Irish waters

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Invasive ascidian communities are extremely threatening to global biodiversity. While invasive ascidians have been recorded in Ireland, causing problems for the shellfish industry the diversity and distribution of ascidians in Ireland are poorly known. Here we report 18 native ascidian species distributed across five families and nine genera along with four non- indigenous species recorded from the West and South of Ireland. One Pacific species, *Didemnum candium* (Savigny, 1816) was discovered Galway waters for the first time. Solitary ascidians, *Ascidia mentula*, *Ascidiella aspersa, Corella eumoyata* were most abundant in Galway locations while *Aplidium* sp and *Botryllus schlosseri* were dominant in Cork collection sites. The systematics of ascidian fauna employed the integration of morphological characteristics, spicule shape and molecular markers (mtCOI and 18S rDNA). We have recorded extensive biofouling of the invasive solitary ascidians *A. aspersa*, *C. eumoyata* along with other ascidians on a blue mussel (*Mytilus edulis*) shellfish farm at Killary harbour (west coast of Ireland), greatly reducing mussel production. Biofouling also causes an increase in maintenance costs and significant economic losses for aquaculture farmers. Chemically mediated interactions between invasive ascidians and shellfish will be investigated to detect defense metabolites and provide novel information about the biochemical pathway involved in the chemical defense.

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Poster 65
New tools for taxonomy training in Ascidians

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One of the greatest current threats to the study of biodiversity is the loss of taxonomic expertise. Ascidians are model organisms in many research areas ranging from genetics, evolution of development, environment quality indication to bioinvasion. Accurate identification of species if of major concern as exemplified by the difficulties in interpreting variation of results in the study of Ciona embryonic development, because of the confusing between two cryptic species: C. intestinalis and C. robusta (Brunetti et al., 2015). As the number of experts declines, it becomes increasingly difficult to train the next generation of taxonomists as well as to disseminate knowledge of the basic methods required to study poorly known taxa. A major challenge in teaching the taxonomy of ascidians is to show the tricks of the trade that are buried in hard-to-obtain taxonomic papers or simply unpublished. Dissemination of this information is complicated by the fact that many of the methods are best conveyed with hands-on demonstrations and in-person training. As a first step to confront these challenges, we have developed a series of six "how to" videos focused on basic techniques for collection, preservation, dissection and identification of ascidians (http://bocasarts.weebly.com). These videos aim to help students and researchers to prepare material that is useful for identification and taxonomic research. Associated protocols provide more detailed information on the best ways to observe the anatomy and biology of the organisms. Transcripts in both English and Spanish are provided to broaden accessibility of the information. We have also developed a graphical user interface for an illustrated multilingual glossary (https://stricollections.org/marina/glossary, Collin et al. 2016) using the Symbiota Software Project biodiversity platform. For use as a translation tool for technical terms in taxonomy and systematics, the GUI is Open Source and could be implemented in any Symbiota Portal. We are populating the TaxaGloss with illustrations, photographs and translations from our biodiversity work on ascidians in Bocas del Toro, Panama. Many taxonomists have collaborated with the translation of terms in seven languages by now and we encourage other researchers to join us in this effort to make the glossary available in other languages and to disseminate good practices in ascidian taxonomy.

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Poster 66
How to make lemonade from invasive ascidians

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The Mediterranean coastline has been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current environmental stressors on the marine fauna, and of the possible potential of marine organisms as bio-indicators of environmental health. Sessile marine organisms such as ascidians may provide invaluable information regarding a wide scale of pollutants and stress factors over time, in contrast to common chemical assays. As highly efficient filter feeders, ascidians are well known for their ability to accumulate heavy metals, and to concentrate harmful compounds that are present in low or even only trace concentrations in the water column. The wide geographic distribution of invasive ascidians in both polluted and pristine habitats make them ideal candidates for bio-monitoring a wide variety of marine habitats, and investigating the pathological effects of a variety of stressors. The main objectives of the current study are to explore the potential use of the solitary invasive species *Phallusia nigra*, *Styela plicata*, *Microcosmus exesperatus*, and *Herdmania momus* as biological indicators for micro-plastic, phthalates, and heavy-metals. By combining a suit of analytical methods including chemical, physiological, histological and proteomic analysis we aim to locate and describe 'hot-spots' of polluted zones along the Mediterranean and Red Sea coasts of Israel, and further understand the physiological effect of pollutants on benthic invertebrates.

Poster 67
Evolution of the dead – diversification of extracorporal materials of tunicates

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The name-giving feature of the taxon Tunicata, the tunic constitutes the unique evolutionary acquisition of the ability to secrete cellulose within the animal kingdom. Globally, cellulose is the most common macromolecule of biogenic origin making up, for example, a chemically rather inert component of cell walls in plants and bacteria. In ascidians the tunic constitutes an external tissue that is supplied with blood vessels and amoeboid cells, serving mainly passive functions, such as protection, stability, anchoring, or substrate for camouflaging organisms. The tunic, however is also present in early life-history stages, where its presence might have severe evolutionary consequences, impacting life-history strategies of the entire organism. In appendicularians, we see the tunic evolutionarily transformed into a complex, multifunctional, external house that is regularly cast aside. Here, like in plants, cellulose fibers perform their intricate functions as a dead material. In order to elucidate diverse functional aspects, we investigated a level mediating between light- and electron microscopy to bridge the gap between structural and molecular understanding. Our interest in the (pre-)formation of the house structures forms the basis for a technical understanding of the fluid mechanics of the expanded house and the unfolding process of this intricate external structure. Moreover, the appendicularian house serves as a focus in an interdisciplinary effort to use this research object as an example/metaphor for the different aspects of filter operations in a broader sense.

Poster 68
Ascidians as bio-indicators of micro-plastic and phthalates in marine environments

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Worldwide occurrence of micro-plastic in the marine environment is a major aspect of plastic pollution and an increasing threat to marine organisms and ecosystems. Ingested by different organisms, these particles can cause mechanical damage to tissue and release toxic chemicals into organism. One group of such chemicals is phthalate plasticizers, added to plastics during manufacture to soften and increase flexibility. Phthalates are found in many products and are potentially harmful, are known to bio-accumulate in organisms, and have been associated with endocrine disrupting effects. Therefore, scientific methods aimed at assessing phthalates accumulation in organisms will contribute to our understanding of its eco-toxicological impact. Our goal is to investigate the potential use of solitary ascidians (Chordata, Ascidiacea) as in-situ biological indicators of micro-plastic and phthalates. As sessile filter feeders, they filter high volumes of seawater and retain particulate matter.
Ascidians have a wide global distribution in both polluted and pristine waters, and some are very successful invasive species. As so, they make ideal candidates for monitoring microplastic and their additives in a wide variety of marine environments. Our objectives are: (1) Develop analytical methods for detection of micro-plastic and phthalates in solitary ascidians; (2) Investigate the ability of solitary ascidians to accumulate micro-plastic and phthalates in polluted vs. non-polluted sites, and (3) Specify the organs in which micro-plastic and phthalates accumulate in the organism's body. By developing methods to detect and quantify micro-plastic and plastic additives, along with verifying the use of ascidians as suitable bioindicators for micro-plastic and phthalates, this study will present a new and applicable tool for bio-monitoring these contaminates in the marine environment.

Poster 69
Histone deacetylase activity is required for Botrylloides leachii whole body regeneration.

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The colonial tunicate Botrylloides leachii is exceptional at regenerating from a piece of vascular tunic after loss of all adults from the colony. This regeneration process rapid, going from vascular tunic to new zooid in as little as 8 days. Transcriptome analysis of regenerating fragments revealed the range of gene expression changes that occur over the time course of regeneration. However, there is still little understanding of how rapid changes to gene expression required to drive regeneration are initiated and how the regeneration process is regulated. We investigated the expression and function of a group of key epigenetic modifiers, histone deacetylases (HDAC) that are known to play an important role in many biological processes such as development, healing and regeneration.
We identified several B. leachii HDAC genes, two of which were expressed during whole body regeneration (WBR). To determine if histone deacetylation was required for WBR, we inhibited its activity through valproic acid (VPA) and Trichostatin A (TSA). Inhibition of HDAC activity prevented regeneration, along with altering the expression of genes linked to regeneration in comparison to controls. Here we show that HDAC activity, specifically HDAC I/IIa enzymes, is vital for B. leachii to successfully complete whole body regeneration.

Poster 71
Whispering cell communication may constrain the evolution of ascidian embryonic morphogenesis

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A small fraction of animal embryos, including ascidians, develop with an invariant, stereotyped pattern of cell divisions. Using light-sheet microscopy and automated cell segmentation and tracking, we constructed a whole-embryo atlas of *P. mammillata* cell behaviors between the 64-cell and initial tailbud stages. This 4D description of embryogenesis tracks the positions, shapes and cell contacts of each of 1304 cells with a 2-minute temporal resolution and across 671 cell divisions. Quantitative analysis of this geometrical description confirms the bilaterality and high level of stereotypy of ascidian development, and was used to systematically identify fate restriction events up to the end of gastrulation, without the need for any molecular information.

We found that most fate restriction events identified are associated with either unequal mother cell divisions, or distinct lifespans of daughter cells, providing temporal boundaries for the cues driving fate restriction. These cues could be provided by juxtacrine cell inductions, whose success depends on the area of contacts between inducing and induced cells. Finally, we show that temporally and spatially scattered juxtacrine inductions impose geometrical constraints on most embryonic cells, which could explain the high level of geometric stereotypy of ascidian embryos.

Poster 72
Integrative taxonomy of the genus Didemnum (Ascidiacea: Didemnidae): combining morphological and molecular data

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We reconstructed evolutionary relationships among species in the genus Didemnum using morphological and molecular data. We used 59 characters describing colony external morphology, spicules, zooids and larval anatomy of 34 species, and 27 species were sequenced for the mitochondrial marker CO1. We used tunicate-specific primers (Stefaniak et al. 2009) and Didemnum-specific primers designed for this study. Cystodytes sp. was used to root the trees. The resulting morphology-based maximum parsimony consensus tree was compared with the maximum likelihood molecular tree with nodes supported by bootstrap (1000 replicates).

Topologies of the trees were different except for the clade that included D. ligulum and D. multispirale. The molecular analysis recovered the following groups of sister-species with strong support: D. cuculliferum + D. fragile, D. vexillum + D. psammatodes, D. apersum + D. perlucidum, D. cineraceum + D. vanderhorsti + Didemnum sp. The pairs D. ligulum + D. multispirale and D. cineraceum + D. vanderhorsti include species with very similar colonial and zooid morphologies, with the main difference being the gemmiparous condition of the larvae of one species in each pair. Didemnum cuculliferum and D. fragile both occur in the western Pacific and have similar larval morphologies. The other two pairs include species with wide geographical distributions and many cases of introduction. While D. vexillum is found in cold waters, D. psammatodes is tropical, and both form large colonies, often with hanging lobes. Didemnum apersum and D. perlucidum are white didemnids found in warm waters.

The morphological data shows that many characters are homoplastic, even important characters for taxonomy such as the size and gemmiparous condition of the larva, and number of ectodermal ampullae, which evolved many times. CO1 was unable to resolve the relationship among clades within Didemnum, but it is an important tool for species delimitation and to identify closely related species.

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Poster 73
Transcriptional regulation of *Raldh2* in the *Ciona intestinalis* embryo

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Localized action of retinoic acid is necessary for coordinated pattern of organogenesis during embryonic and post-embryonic development of the ascidian *Ciona intestinalis* (Sasakura et al., 2012; Yoshida et al., 2017). Expression of the retinaldehyde dehydrogenase-encoding gene *Raldh2* (*Aldh1A1*) is restricted to a few anterior tail muscle cells in the *C. intestinalis* embryo (Nagatomo and Fujiwara, 2003). Although similar expression patterns of *Raldh2* orthologs are conserved among vertebrate embryos, it remains unknown how this spatial pattern of expression is regulated.

We have found that a 1.6-kb upstream region of *Raldh2* activated reporter gene expression mainly in six anterior muscle cells of the *C. intestinalis* embryo. Two of them derive from B7.5 blastomere, while the other four derive from B7.8 blastomere (Fig. 1A). Among four B7.8-line cells, the level of expression of endogenous *Raldh2* is the strongest in cell #3 (Fig. 1A). In contrast, the level of expression of *Raldh2* reporter genes was equally strong in all of the four cells. Deletion analyses revealed that different regions are required for transcriptional activation in B7.5-line and B7.8-line cells (Fig. 1B). We found four E-boxes within the 1.6-kb upstream region (Fig. 1B). Mutation of one of them diminished the expression of reporter gene only in the B7.5-line cells. Disruption of all the E-boxes caused severe reduction of the reporter gene expression. The transcription factor Mesp, which is expressed in B7.5-line cells, bound these E-boxes. A dominant-negative form of Mesp suppressed the expression of the reporter gene and endogenous *Raldh2* in B7.5-line cells, but not in B7.8-line cells. These results indicate that distinct lineage-specific regulatory pathways are responsible for transcriptional activation of *Raldh2* in B7.5-line and B7.8-line muscle cells.

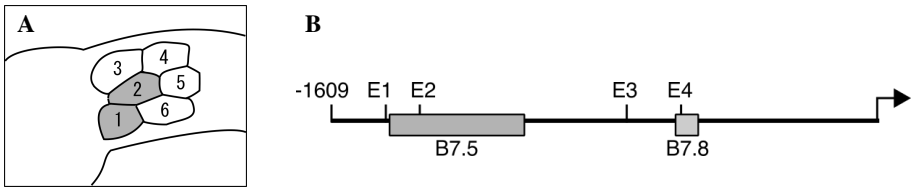

Fig. 1. (**A**) Schematic diagram of the anterior tail muscle cells. The anterior is oriented to the left, with the dorsal side up. Cells #1 and #2 (shaded) derive from B7.5. Cells #3~6 derive from B7.8. Cells #1~3 strongly express *Raldh2*, while cells #4~6 weakly express *Raldh2*. (**B**) The upstream region of *Raldh2*. An arrow indicates the transcription start site. Four E-boxes are indicated (E1~E4). Grey boxes indicate the regions required for activation in B7.5-line or B7.8-line cells.

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Khashayar Razghandi

Poster 56
Phylogeny of Tunicata – a morphology based analysis
Katrin Braun

Poster 57
Anthropogenic factors influencing invasive ascidian establishment in natural environments Mediterranean Sea
Mey-Tal Gewing

Poster 58

Filter Architecture: An Interdisciplinary Approach to the Tunic Structure of the Appendicularian
Susanne Jany, Khashayar Rhazghandi

Poster 59
Fouling ascidians in the Galapagos on artificial surfaces
Gretchen Lambert

Poster 60
Collection and husbandry of colonial and solitary ascidians
Régis LASBLEIZ

Poster 61
Color variation in an introduced ascidian reveals two distinct genetic lineages
Susanna López-Legentil

Poster 62
Dismantling the genetic pathway of retinoic acid metabolism and signaling in *Oikopleura dioica*
Josep Martí-Solans

Poster 63
Effects of exposure to microplastics on the development and metamorphosis of Ciona robusta
S. Messinetti

Poster 64
The Biodiversity and distribution of Ascidians (Tunicates: Urochordata) from Irish waters
Satheesh Kumar Palanisamy

Poster 65
New tools for taxonomy training in Ascidians
Rosana M. Rocha

Poster 66
How to make lemonade from invasive ascidians
Noa Shenkar

Poster 67
Evolution of the dead – diversification of extracorporal materials of tunicates
Stach, T

Poster 68
Ascidians as bio-indicators of micro-plastic and phthalates in marine environments
Gal Vered

Poster 69

Histone deacetylase activity is required for Botrylloides leachii whole body regeneration.
Megan J Wilson

Poster 71
Whispering cell communication may constrain the evolution of ascidian embryonic morphogenesis
Patrick Lemaire

Poster 72
Integrative taxonomy of the genus Didemnum (Ascidiacea: Didemnidae): combining morphological and molecular data
Rosana Moreira da Rocha

Poster 73
Transcriptional regulation of *Raldh2* in the *Ciona intestinalis* embryo
Shigeki Fujiwara

INFORMATION

Instructions for presentations

Oral Presentations

Rosenthal Pavilion

Each speaker, except for keynote lectures, has 25 minutes: 20 minutes talk and 5 minutes for questions and answers.

Chair persons will be instructed to signal after 18 minutes, stand-up after 20 minutes, and not take questions after 25 minutes, in order for the program to unfold smoothly.

We kindly ask speakers to make every effort to stay on time.

Poster presentations

Rosenthal Pavilion

Poster session I: 5:15pm - 7:15pm Tuesday, July 18th.

ODD numbered posters

Poster session II: 5:15pm - 7:15pm Thursday, July 20th.

EVEN numbered posters

Poster boards are 8' x 4' and will accommodate two posters on either side.
Poster numbers will be indicated on the boards.
Pushpins will be provided.
Set-up Posters for session I as early as possible.
Remove posters for session I on Wednesday 19th, 12pm at the latest.
Set-up posters for session II as soon as possible after Wednesday 19th, 12pm.

Registration

https://2017-tunicate-meeting.bio.nyu.edu/registration/

Includes attendance to all sessions, including breakfasts, coffee breaks and 1 drink ticket at the bar for each poster session (2 green tickets total).

Faculty, principal investigator, group leader: $150

Student, Post-doctoral fellow: $100
optional Welcome reception: $32 (1 white ticket for the drink)
optional lunch box Monday/Tuesday: $35 (2 blue tickets)
optional lunch box Thursday/Friday: $35 (2 blue tickets)
optional Farewell dinner: $69 (sign-up by Wednesday 7/21) (1 red ticket for the drink)

In addition to these tickets and your name tag, you will be provided with star sticker to help break the ice and indicate if you are:
- A keynote speaker: red star
- An organizer or scientific committee member: blue star
- A local person: green star
- A speaker: silver star
- A poster presenter: gold star

As shown below:

VENUE

The meeting will be held at the NYU Kimmel Center, which is located on the South Side of Washington Square Park, in Manhattan, New York City.

Plenary and poster sessions, as well as coffee breaks, the welcome reception and the farewell dinner will all be held in the Rosenthal Pavilion.

A breakout room, Kimmel Center #905/907, will be available during the meeting for discussions, and lunch (*if you prefer not to eat your lunch box outside in the Washington Square Park*).

The round table discussion will be help in Kimmel Center room 914 on Friday, July 21st.

Address of the conference
Kimmel Center
60 Washington Square S, New York, NY 10012

Address of the NYU dorm housing
University Hall, 110 East 14th Street, New York, NY 10024

WIFI at NYU
eduroam works with your institutional email address and your usual password at your instituion

The login and password for the network "nyuguest" changes weekly and will be provided at the registration desk.

TRANSPORTATION

From JFK

- Cheapest: subway, Airtrain to Jamaica then line E (blue) to West 4. This is very close to the venue at NYU Kimmel Center.
Other lines work too.
- Uber obviously works, but I've heard the cost to and from the airport can fluctuate.
- Taxi: use the yellow cab at the dedicated station, there is a flat fare to Manhattan, DO NOT ACCEPT SOLICITATIONS.
- If you want a pre-ordered cab, I often use Dial7, which is quite reliable. This is more convenient to leave from Manhattan to the airport

From La Guardia Airport

I would take a yellow cab, it is fast and cheaper than from JGK, although no flat fare.

From Newark

- There is a train to Penn Station (34th street in Manhattan) then take Blue or Orange lines to West 4th.
- Cab is more convenient but more expensive (~$80)

Taxi drivers expect tips (usually ~15%-20%).
They should all accept credit cards.

The best direction to give them a cross street (e.g. La Guardia and 4th is the x for the Kimmel Center).

For the public transportation system, MTA is the go-to website

Acknowledgements

Organizers

Lionel Christiaen

Anna Di Gregorio

Scientific committee

Federico Brown

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Clare Hudson

Takehiro Kusakabe

Patrick Lemaire

Filomena Ristoratore

Noa Shenkar

Billie Swalla

Website and more

Delphine Dauga

Administrative support

Sandra Kim

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