Cover and back cover designed by
Laurel Hiebert – Drawings modified from Lahille, M.F., 1890.
Contributions à l’étude anatomique et taxonomique
10th International Tunicate Meeting
July 7 - 12, 2019
Villefranche sur Mer, France

Organizing Committee
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Rémi Dumollard
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Antonietta Spagnuolo (Stazione Zoologica Anton Dohrn – Italy)
Bob Zeller (San Diego State University – USA)
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From the Airport: Bus service from the airport Nice Côte d’Azur to Villefranche (15 km).

By Train: Frequent service between the stations of Nice and Villefranche-sur-Mer (Take bus service or Taxi, see details below).

By car: Exit from the autoroute A8 at “Nice - Promenade des Anglais” onto the “Promenade des Anglais” continue along the coast road towards Monaco. Alternatively, exit the autoroute A8 at “Nice Est”, follow the signs toward the Port (of Nice) and from the Port take the “Basse Corniche” towards Monaco.

By Taxi or UBER: You can find many taxis at both airport terminals, which can take you to Villefranche. Price is around 60€. Remember to have cash ready to pay the driver (cash points and exchange bureaux are handily located in both airport terminals). For the return is cheaper to call a taxi in Villefranche-sur-Mer. UBER works very well and it is cheaper.

By Bus: Ligne d’Azur, the city’s bus operator, runs dedicated airport services, lines 98 start at Terminal 2, before picking up passengers at Terminal 1. An Aéro ticket costs 6€ one-way and can be purchased on board. The 98 runs frequently, three times an hour - the first at 05:50 and the last 23:50. The ticket is valid for 74 minutes and allows for one connecting voyage on any urban bus. To go to Villefranche sur Mer, take the bus 98, and get off at the bus stop “Promenade des Arts”. When you arrive at “Promenade des art” take the bus 81 (frequently 2 time an hour) in direction “Port de Saint Jean”. After 15mn you arrive at Villefranche sur Mer and get off at the bus stop “Saint Esteve” if you stay at the Flore Hotel. To go to the Laboratory LBDV, the workshop and your hotel, follow the Avenue of “Général de Gaulle” and continue along the “Darse port” then towards the “Station Zoologique”. For the return, take the bus at bus stop “OCTROI” and drop off at the terminal station “Garibaldi”, and take the return bus at the “Lycée Massena” bus stop.
Getting around Villefranche sur Mer

The Citadel
INFORMATION ON THE MEETING

Registration
The Registration desk is situated next to the lecture hall and will be open Sunday 7th from 5pm to 8pm and Monday 8th starting from 8:15am.

Wifi internet access
Wifi is available at the venue, you can login without a password.

Oral Presentations
20 minutes: 15 min talk and 5 min discussion (Talks)
40 minutes: 35 min talk and 5 min discussion (Plenary of Session 9)
1 hour: 45 min talk and 15 min discussion (Plenary lectures)
Speakers will be asked to upload their presentation from their USB pen on a PC or Mac provided in the lecture hall. The speakers are kindly requested to avoid the use of their computers for the presentation, if strictly necessary the lecture hall is provided with VGA and HDMI plugs.

Posters
The posters (format A0) will be displayed at the marine station IMEV in the Jules Barrois building (see map). Poster boards will be numbered as in the program.
The poster can be hung the same day either during the lunch break or before the poster session. The poster should be removed at the end of each poster session.
A list with the names of all the 10th ITM participant will be available by the lecture hall, if you wish someone in particular to visit your poster, you have to write the poster number by her/his name.

Lunches and coffee breaks
Coffee breaks and lunch boxes will be served outside the lecture hall and can be consumed in the surrounding area or at the beach.

Gala dinner
The closing dinner will be served in the citadel at the gazebo.

Afternoon off
In the afternoon of Wednesday 10th there won't be any lectures or sessions. Details about the two programmed activities, i.e. the visit to the Monaco Oceanographic Museum and the diving in the Villefranche bay will be given during the meeting. For those who are not participating at these two events, the area offers several amenities: tourist material will be included in the conference bag, and the organizers will be happy to direct you to points of interests (beaches, museums, hiking trails, etc.).

10th ITM T-Shirts
The T-shirt of the 10th ITM will be available for purchase everyday at the entrance of the lecture hall.
<table>
<thead>
<tr>
<th></th>
<th>SUNDAY JULY 7TH</th>
<th>MONDAY JULY 8TH</th>
<th>TUESDAY JULY 9TH</th>
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<tr>
<td>8:15</td>
<td>REGISTRATION</td>
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<td>9:00</td>
<td>OPENING REMARKS</td>
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<td>SESSION 5</td>
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<td>9:15</td>
<td>SESSION 1 ECOLOGY</td>
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<td>BEGINNING OF</td>
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<td>10:15</td>
<td>COFFEE BREAK</td>
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<td>EMBRYOGENESIS</td>
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<td>LUNCH BREAK</td>
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<td>12:20 LUNCH</td>
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<td>14:00</td>
<td>WORKSHOP WASHU (MARINE STATION IMEV - GALERIENS BLDG.)</td>
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<td>FROM DEVELOPMENT TO GENOMICS</td>
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<td>LATE EMBRYO AND LARVAE</td>
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<td>17:00</td>
<td>REGISTRATION (CITTADELLE DE VILLE-FRANCHE SUR MER)</td>
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<td>17:40 POSTER SESSION 1 ODD NUMBERS (MARINE STATION IMEV - J.BARROIS BLDG.)</td>
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<td>Time</td>
<td>Wednesday</td>
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<td>9:00</td>
<td>SESSION 8</td>
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<td>NERVOUS SYSTEM DEVELOPMENT</td>
<td>NON TUNICATE AQUATIC MODELS</td>
<td>PLENARY LECTURE PATRICK LEMAIRE</td>
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<td>COFFEE BREAK</td>
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<td>10:40</td>
<td>SESSION 8 NERVOUS SYSTEM DEVELOPMENT</td>
<td>10:40 SESSION 9 NON TUNICATE AQUATIC MODELS</td>
<td>10:20 SESSION 12 GENE REGULATION</td>
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<td>11:40</td>
<td>PLENARY LECTURE STEVE WILSON</td>
<td>11:20 SESSION 10 DEVELOPMENTAL AND CELL BIOLOGY</td>
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<td>12:40</td>
<td>EXCURSION - DIVING</td>
<td>12:20 LUNCH BREAK</td>
<td>12:20 LUNCH BREAK</td>
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<td>14:00</td>
<td>SEUSSION 10 DEVELOPMENTAL AND CELL BIOLOGY</td>
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<td>COFFEE BREAK</td>
<td>14:40 GROUP PHOTO</td>
<td>14:40 GROUP PHOTO</td>
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<tr>
<td>15:20</td>
<td>SESSION 11 POST-EMBRYONIC DEVELOPMENT</td>
<td>15:10 ROUND TABLES GENERAL DISCUSSION</td>
<td>15:10 ROUND TABLES GENERAL DISCUSSION</td>
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<tr>
<td>17:00</td>
<td>POSTER SESSION 2 EVEN NUMBERS (MARINE STATION IMEV - J.BARROIS BLDG.)</td>
<td>16:50 CLOSING REMARKS</td>
<td>16:50 CLOSING REMARKS</td>
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<td>19:30</td>
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<td>19:30 GALA DINNER</td>
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PROGRAM

Sunday, July 7, 2019
Marine Station IMEV (Tregouboff Room);
14:00 - 17:00 Workshop WashU, ANISEED Genome Browser
Citadelle de Villefranche-sur-mer
17:00 - 20:00 REGISTRATION

Monday, July 8, 2019
Citadelle de Villefranche-sur-mer
8:15 - 9:00 REGISTRATION
9:00 - 9:15 OPENING REMARKS - Remi Dumollard and Stefano Tiozzo
9:15 - 10:15 Session 1 - ECOLOGY (chair: Marie Nydam)
9:15 - 9:35 T1 Xavier Turon: “Local microbiome of a global invader”
9:55-10:15 T3 Vittoria Roncalli: “Transcriptomics of the developmental response of Oikopleura dioica to diatom-bloom derived biotoxins”
10:15 - 10:35 COFFEE BREAK
10:35 - 11:35 Session 2 - IMAGING AND MODELLING (chair: Alex McDougall)
10:35 - 10:55 T4 Benoit Godard: “Tension driven engulfment of non-mitotic by mitotic tissue determines cell division orientation in ascidian embryos”
11:15 - 11:35 T6 Emmanuel Faure: “MorphoNet: An interactive online morphological browser to explore complex multi-scale data”
11:35 - 12:35 Session 3 - ASEXUAL REPRODUCTION, REGENERATION, AND AGING (chairs: William Jeffery, Anthony De Tomaso)
11:55 - 12:15 T8 Megan Wilson: “Epigenetic regulation of Botrylloides leachii whole body regeneration”
12:15 - 12:35 T9 Susannah H. Kassmer: “Primordial Blasts, a population of blood-borne stem cells responsible for whole body regeneration in a basal chordate”
12:35 - 14:00 LUNCH BREAK
14:00 - 15:20 Session 3 - ASEXUAL REPRODUCTION, REGENERATION, AND AGING (chairs: William Jeffery, Anthony De Tomaso)
14:00 - 14:20 T10 Marta Scelzo: “Novel budding mode in Polyandrocarpa zorritensis: a model for comparative studies
on asexual development and whole body regeneration”

14:20 - 14:40  
**T11 Bill Jeffery:** “Dying to Regenerate: Apoptosis, Wnt Signaling, and Polarity of Body Regeneration in Ciona intestinalis”

14:40 - 15:00  
**T12 Delany Rodriguez:** “Aging and Senescence Effects on the Extracorporeal Vasculature of Botryllus schlosseri”

15:00 - 15:20  
**T13 Alexandre Alié:** “Evo-Devo approaches to non-embryonic developments in colonial tunicates”

15:20 - 15:40  
**COFFEE BREAK**

15:40 - 17:00  
**Session 4 - FROM DEVELOPMENT TO GENOMICS (chair: Kaoru Imai)**

15:40-16:00  
**T14 Chiara Anselmi:** “Molecular and Morphological Atlas of Botryllus schlosseri Developmental Pathways”

16:00-16:20  
**T15 Elijah Lowe:** “Identification of key morphogenesis genes in Molgulidae with alternate body plans and their interspecific hybrid embryos”

16:20-16:40  
**T16 Billie Swalla:** “Tailless Molgulid Ascidians have a Notochord “Ball” that never converges or extends”

16:40-17:00  
**T17 Brad Davidson:** “Differential drift among enhancer elements in the tunicate cardiopharyngeal gene network”

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**Tuesday, July 9, 2019**

_Citadelle de Villefranche-sur-mer_

9:00 - 10:40  
**Session 5 - BEGINNING OF EMBRYOGENESIS (chair: Takahito Nishikata)**

9:00 - 9:20  
**T18 Masaki Matsuo:** “Protein phosphatase 2A is essential to maintain meiotic arrest, and to prevent Ca2+ burst at spawning and eventual parthenogenesis in the larvacean Oikopleura dioica”

9:20 - 9:40  
**T19 Marianne Roca:** “The Spindle Assembly Checkpoint in Phallusia mammillata embryos”

9:40 - 10:00  
**T20 Toshiyuki Goto** “Analyses of spatio-temporal interaction between microtubule and ER or maternal mRNAs in Ciona egg using new high resolution double staining methods”

10:00 - 10:20  
**T21 Silvia Caballero Mancebo** “Actin-dependent contraction drives ooplasmic segregation in ascidian oocytes”

10:20 - 10:40  
**T22 Yana Mikhaleva:** “Gap junction-dependent coordination of intercellular calcium signalling during early embryogenesis of Oikopleura dioica”

10:40 - 11:00  
**COFFEE BREAK**

11:00 - 12:20  
**Session 6 - EVOLUTION, SYSTEMATICS AND TAXONOMY (chairs: Rosana Rocha, Xavier Turon)**

11:00 - 11:20  
**T23 Benyamin Rosenthal:** “Evolutionary Origin of the Mammalian Hematopoietic and Immune Systems Found in a Colonial Chordate”

11:20 - 11:40  
**T24 Federica Montesanto:** “Further revelations on Ciona taxonomic complexity”

11:40 - 12:00  
**T25 Maria Casso:** “Single zooids, multiple loci: Population genomics of a global invader”
<table>
<thead>
<tr>
<th>Time</th>
<th>Session/Panel</th>
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<tbody>
<tr>
<td>12:00 - 12:20</td>
<td>T26 Jacques Piette: “The thaliacean Salpa fusiformis: the return of sessile ascidians to pelagic life”</td>
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<tr>
<td>12:20 - 14:00</td>
<td>LUNCH BREAK</td>
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<td>14:00 - 15:20</td>
<td>Session 6 - EVOLUTION, SYSTEMATICS AND TAXONOMY</td>
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<td>(chairs: Rosana Rocha, Xavier Turon)</td>
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<td>14:00 - 14:20</td>
<td>T27 Marie Nydam: “Phylogeny of the Botryllid Ascidians”</td>
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<td>14:40 - 15:00</td>
<td>T29 Rosana Rocha: “Diplosoma listerianum: an interesting model of cryptic speciation”</td>
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<td>15:00 - 15:20</td>
<td>T30 Katrin Braun: “Phylogenetic analysis of phenotypic characters of Tunicata supports basal Appendicularia and monophyletic Asciidacea”</td>
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<td>15:20 - 16:00</td>
<td>Session 7 - LATE EMBRYO AND LARVAE (chairs: Ute Rothbächer, Bo Dong)</td>
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<td>15:40 - 16:00</td>
<td>T32 Bo Dong: “Coordination of asymmetrical notochord contractility and epithelia cell proliferation drives tail bending in Ciona embryogenesis”</td>
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<tr>
<td>15:20 - 16:00</td>
<td>T30 Katrin Braun: “Phylogenetic analysis of phenotypic characters of Tunicata supports basal Appendicularia and monophyletic Asciidacea”</td>
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<td>COFFEE BREAK</td>
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<td>16:20 - 17:20</td>
<td>Session 7 - LATE EMBRYO AND LARVAE (chairs: Ute Rothbächer, Bo Dong)</td>
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<td>16:20 - 16:40</td>
<td>T33 Yuji Mizotani: “Pulsatile Transport of basal factors toward apical domain during tubulogenesis, directed by the interaction between 14-3-3ζα and ERM”</td>
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<td>16:40 - 17:00</td>
<td>T34 Hiroki Nishida: “Neurula rotation and left-right asymmetry in ascidian embryos: Ciliary movements and the vitelline membrane signal”</td>
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<td>17:00 - 17:20</td>
<td>T35 Steve Irvine: “The effects of high water temperature on developmental processes in C. intestinalis: limits to embryonic robustness”</td>
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Marine Station IMEV - (Jules Barrois Bldg.)

17:40 - 19:40  POSTER SESSION 1 - Odd numbers

Wednesday, July 10, 2019
Citadelle de Villefranche-sur-mer

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 8 - NERVOUS SYSTEM DEVELOPMENT AND ANATOMY</th>
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<td>(chairs: Bob Zeller, Clare Hudson)</td>
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<tr>
<td>9:00 - 10:20</td>
<td>T36 Alberto Stolfi: “Transcriptional states underlying neuron subtype-specific traits in the Motor Ganglion of Ciona”</td>
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<tr>
<td>9:00 - 9:20</td>
<td>T37: Kouhei Oonuma: “Left-right asymmetric development of cells in the larval brain of Ciona”</td>
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<tr>
<td>9:20 - 9:40</td>
<td>T38: Bob Zeller: “Jagged acts as a lateral inducer to specify caudal epidermal sensory neuron fate in Ciona”</td>
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<tr>
<td>10:00 - 10:20</td>
<td>T40: Nanako Okawa: “In vivo calcium-imaging reveals a possible role of the GnRH system in larval swimming of Ciona”</td>
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<tr>
<td>10:00 - 10:20</td>
<td>T41: Nanako Okawa: “In vivo calcium-imaging reveals a possible role of the GnRH system in larval swimming of Ciona”</td>
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<td>COFFEE BREAK</td>
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<td>10:40 - 11:40</td>
<td>Session 8 - NERVOUS SYSTEM DEVELOPMENT AND ANATOMY</td>
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<td>(chairs: Bob Zeller, Clare Hudson)</td>
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</table>
10:40 - 11:00  T40 Izumi Oda-Ishii: “Transcriptional regulatory mechanisms of Tbx6b by Zic-ra (Macho1) through secondary binding motif sites”

11:00 - 11:20  T41 Kerriane Ryan: “Stereotypy and variation among the larval motor circuits”

11:20 - 11:40  T42 Marios Chatzigeorgiou: “Ciona intestinalis as a neuroethology model”

11:40 - 12:40  PLENARY LECTURE

T43 Steve Wilson: “Brain asymmetry – from genes to circuits and behavior”

14:00  FREE TIME (EXCURSION and DIVING)

Thursday, July, 11, 2019
Citadelle de Villefranche-sur-mer

9:00 - 10:20  Session 9 - NON TUNICATE AQUATIC MODELS (chairs: William Smith, Alexandre Alie)

9:00 - 9:40  T44 (PLENARY) Carl-Philipp Heisenberg: “Mechanosensation of tight junctions by ZO-1 phase separation and flow”

9:40 - 10:00  T45 Amro Hamdoun: “Patterning of transport function: How transporters are integrated in early developmental programs of the sea urchin”

10:00 - 10:20  T46 Alex Venn: “The Highs and Lows of pH regulation in Corals and Symbiotic Cnidarians”

10:20 - 10:40  COFFEE BREAK


10:40 - 11:00  T47 Eric Rottinger: “Regeneration is a partial redeployment of the embryonic gene regulatory network”

11:00 - 11:20  T48 Ina Arnone: “Gene regulatory network approaches for gut patterning and evolution”

11:20 -12:20  Session 10 -DEVELOPMENTAL AND CELL BIOLOGY (chairs: Hiroki Nishida, Lionel Christiaen)


11:40 - 12:00  T50 Geraldine Williaume: “ephrin-mediated “damping” of FGF signaling underlies the spatial precision of ascidian neural induction”

12:00 - 12:20  T51 Rose Jacobson: “Shp2 Phosphatase is Required for Normal Embryogenesis in C. intestinalis”

12:20 - 14:00  LUNCH BREAK

14:00 - 15:00  Session 10 -DEVELOPMENTAL AND CELL BIOLOGY (chairs: Hiroki Nishida, Lionel Christiaen)

14:00 - 14:20  T52 Christina Cota: “Mitotic Kinases choreograph FGF receptor storage and redistribution during cardiopharyngeal cell”

14:20 - 14:40  T53 Alfonso Ferrández-Roldán: “Braveheart, Oikopleura dioica a cardiogenic loser, but not a heartless chordate”

14:40 - 15:00  T54 Fan Zeng: “A fine analysis of the Ciona larval sensory adhesive organ”

15:00 - 15:20  COFFEE BREAK
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<tr>
<th>Time</th>
<th>Session 11 POST-EMBRYONIC DEVELOPMENT (chair: Cristian Cañestro)</th>
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<tr>
<td>15:40 - 16:00</td>
<td>T56 Christopher Johnson: “Molecular profile and function of the axial columnar cells of Ciona papillae”</td>
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<td>16:00 - 16:20</td>
<td>T57 Xiaoming Zhang: “miR-4055 regulates Ciona sensory organ morphogenesis through AKT-MAPK signaling pathway”</td>
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<td>16:20 - 16:40</td>
<td>T58 Maiki Wakai: “Ca2+ signaling contributes to the beginning of metamorphosis via mechanical stimuli in palps”</td>
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**Marine Station IMEV - (Jules Barrois Bldg.)**

**Friday, July 12, 2019**  
*Citadelle de Villefranche-sur-mer*

**PLENARY LECTURE**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 12 - GENE REGULATION (chairs: Antonietta Spagnuolo, Sebastien Darras)</th>
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<tr>
<td>9:00 - 10:00</td>
<td>T59 Patrick Lemaire: “Extreme morphogenetic canalization of ascidian embryonic development despite high genetic variability”</td>
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<tr>
<td>10:00 - 10:20</td>
<td>COFFEE BREAK</td>
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<tr>
<td>10:20 - 12:20</td>
<td>T60 Emma Farley: “Highly Conserved Enhancer Grammar”</td>
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<tr>
<td>10:40 - 11:00</td>
<td>T61 Cristelle Dantec: “Comparative genomic analysis: Identification of novel genes in ascidians”</td>
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<tr>
<td>11:00 - 11:20</td>
<td>T62 Kotaro Shimai: “Working towards a comprehensive and quantitative Ciona notochord gene regulatory network”</td>
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<tr>
<td>11:20 - 11:40</td>
<td>T63 Claudia Racioppo: “Combinatorial chromatin dynamics foster accurate cardiopharyngeal fate choices”</td>
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<td>T64 Shin-ichi Tokuhira: “Foxd acts as an activator and a repressor for patterning along the animal-vegetal axis in early embryos”</td>
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<td>12:00 - 12:20</td>
<td>T65 Boqi Liu: “Foxg is required for the palp formation in ascidian embryos”</td>
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<td>LUNCH BREAK</td>
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<td>14:00 - 14:40</td>
<td>T66 Takehiro Kusakabe: “Evolution of developmental programs for the midline structures in chordates: insights from gene regulation in the floor plate and hypochord homologues of Ciona embryos”</td>
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<td>T67 Burcu Vitrinel: “Discovering post-transcriptional regulators of heart development”</td>
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<td>15:10 - 16:50</td>
<td>ROUND TABLE - GENERAL DISCUSSION</td>
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<td>CLOSING REMARKS - Remi Dumollard and Stefano Tiozzo</td>
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<td>19:30 - 24:00</td>
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TALKS
ABSTRACTS
T1-T67
T1
LOCAL MICROBIOME OF A GLOBAL INVADER

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Ascidians harbour in their tunics a high diversity of prokaryote symbionts, which may play an important role in the species’ biology and survival. The global invader Didemnum vexillum provides a unique opportunity to assess the patterns of variability of ascidian microbiomes and, in particular, whether they remain stable as the host species colonises new habitats or whether they shift according to local environment. We have analysed, using 16S amplicon sequencing, the microbiome of 60 D. vexillum colonies belonging to 2 native (Japan) and 10 introduced populations spawning the known range of the species. We found 2,984 zero-radius OTUs (ZO-OTUs) in the ascidians, belonging to 36 bacterial and 3 archaeal phyla. The ascidian microbiome had a markedly different composition from surrounding seawater. We found a high-abundance but low-diversity core microbiome in ascidians, as only 8 ZOTUs (0.27%) were present in all colonies, while 47 (1.58%) were present in all localities. However, these ZOTUs comprised 71.31 and 89.60%, respectively, of the total number of reads obtained. The variable component is quantitatively much less important but comprises a highly diverse assemblage. This component differs within localities (0.59 average Bray-Curtis distance), but more so between localities (0.70) and between recognized genetic groups (0.74). Each colony had an average of 11.95% exclusive ZOTUs, while each locality had a mean of 22.05% exclusive ZOTUs. The combination of a quantitatively dominant core component and a highly diverse variable fraction in the microbiome of D. vexillum can contribute to the success of this global invader in different environments.
T2
USING SOLITARY ASCIDIANS TO ASSESS MICROPLASTIC AND PHYTHALATE PLASTICIZERS POLLUTION IN MARINE ENVIRONMENTS

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Marine plastic pollution has become an emerging threat to marine ecosystems worldwide. Studying the interaction of microplastic (MP) with marine organisms is crucial for understanding the effect that MP and plastic additives may have on oceanic environments. However, knowledge regarding the magnitude and effects of these pollutants in general, and particularly in the Eastern Mediterranean Sea and the tropical Red Sea, is still lacking. Here we examined the levels of common plastic additives, phthalate acid esters (PAEs), and MP in two widely-distributed ascidians, Herdmania momus and Microcosmus exasperatus, sampled along the Mediterranean and Red Sea coasts of Israel. High levels of Dibutyl phthalate (DBP) and Bis (2-ethylhexyl) phthalate (DEHP), were found in ascidians at the majority of sampling sites, reaching mean concentrations of 5064 ± 1806 ng/g dry weight and 9095 ± 4574 ng/g dry weight for DBP and DEHP respectively. MP particles were discovered at all the studied sites, with mean concentrations of 1.37 ± 1.29 particles per individual. The high levels of pollutants found at the studied sites emphasize the need for further research into the magnitude and effects of MP and PAEs in the Eastern Mediterranean and the Red Sea. The use of widely-distributed ascidian species that are able to create large aggregates and interfere with native biota present fundamental opportunities for their use in environmental monitoring. Together with the developed methodology for PAE quantification in marine invertebrates this study demonstrates a strong potential for the use of ascidians for assessing microplastic and plastic additives pollution worldwide.
T3
TRANSCRIPTOMICS OF THE DEVELOPMENTAL RESPONSE OF OIKOPLEURA DIOICA TO DIATOM-BLOOM DERIVED BIOTOXINS

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Understanding the impact that biotoxins derived from microalgal blooms might have on the development of appendicularians, one of the most abundant components of the mesozooplankton, is of prime ecological interest, especially upon a possible future intensification of blooms associated to ocean warming and acidification. Our work, after performing treatments with polyunsaturated-aldehydes and extracts from oxylipin-producing diatoms, reveals that embryo development of the appendicularian Oikopleura dioica is compromised by these biotoxins, even at concentrations in the same range than those measured after blooms. Analyses of developmental gene markers reveals a novel “golf ball” phenotype caused by diatom biotoxins, which includes blockage of morphogenesis, midline convergence and tail elongation. The genetic response of embryos exposed to biotoxins includes global silencing of zygotic transcription of developmental genes, and a rapid upregulation of some defensome genes. We are currently performing a transcriptomic approach by RNA-seq to reveal differential gene expression profiles underlying the phenotypes caused by these biotoxins. Our results reveals that some of these differences can be already detected at the 8-cell stage, much earlier than the onset of most of the zygotic transcription. Finally, our study aims to provide a catalogue of gene markers that could be used as molecular biosensors to monitor the genetic stress of natural populations exposed to microalgal blooms.

Reference
T4
TENSION DRIVEN ENGULFMENT OF NON-MITOTIC BY MITOTIC TISSUE DETERMINES CELL DIVISION ORIENTATION IN ASCIDIAN EMBRYOS

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Even though the invariant cleavage pattern of ascidians has been described for more than a century [1], the mechanism that generates this pattern is still unknown. Clues come from the observations that the invariant cleavage pattern relies on mitotic cell shape, which is modulated by cell cycle asynchrony between the animal and vegetal hemispheres [2]. This suggests the presence of spatial and temporal anisotropic tension distribution within the embryo with the mitotic cell as the source of mechanical forces. Indeed, extensive studies in various model systems have demonstrated that mitotic cell rounding arises from an increase of the actomyosin cortical tension and hydrostatic pressure [3,4]. In order to decipher the mechanics of ascidian blastula, we conducted direct measures of the cell surface tension and furthermore we manipulate the tension distribution within the embryo.

We found that the apical surface tension decreases when cells round up in mitosis (without change of the cell volume), rather than increasing as would have been predicted. Conversely, non-mitotic cells possess higher surface tension than mitotic cells and this is associated with an accumulation of P-myosin at the apical cortex and at the adjacent apical junctions, which both lose P-myosin in mitosis. Next, we show that the shape of the mitotic cells is influenced by tension generated by non-mitotic cells with which they are in contact. Finally, by manipulating the spatial or temporal distribution of tension within the embryo we were able to change the shape of mitotic cell leading to a change in spindle orientation and hence cleavage plane.

In conclusion, we report the first case of mitotic cells that do not obey the classical mechanism of mitotic cell rounding. As a consequence of their lower apical surface tension, the mitotic cells of the ascidian blastula integrate all the mechanical forces present within the embryo. Therefore the remarkable fine-tuning of tension distribution in space and time sustains the evolutionary conserved invariant cleavage pattern of ascidian.

References
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3. Thery and Bornens, HSFP J., 2008
4. Champion et al., Trends in Cell Biology, 2017
T5
DIFFERENTIAL EXPRESSION OF CADHERIN2 PATTERNS
RHOA AND MYOSIN II ACTIVITY TO DRIVE ZIPPERING
AND NEURAL TUBE CLOSURE IN A SIMPLE CHORDATE.

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Dynamic control of force generation at tissue boundaries underlies many forms of morphogenesis, but these mechanisms remain poorly understood. Here, we address this question in the context of zippering and neural tube closure in the basal chordate, *Ciona robusta*. During zippering, the neural folds meet and fuse from posterior to anterior, replacing heterotypic junctions between neural and epidermal cells (Ne/Epi junctions) with homotypic Ne/Ne and Epi/Epi junctions. We previously established that zippering involves spatiotemporal patterning of Myosin II activity: high along the Ne/Epi junctions ahead of the zipper, and low along Ne/Ne and Epi/Epi junctions behind the zipper. Here we show how this pattern of activity is shaped by neural-specific expression and homotypic localization of a classical cadherin, Cadherin2. Cadherin2 accumulates along homotypic Ne/Ne junctions, but is absent from heterotypic Ne/Epi junctions, where RhoA and Myosin II are locally activated. Equalizing Cadherin2 expression across the Ne/Epi boundary inhibits RhoA and Myosin II activation and zipper progression, while creating ectopic Cadherin2 expression boundaries in neural or epidermal domains is sufficient to direct RhoA and Myosin II activity to those boundaries. Cadherin2 directs RhoA activity to heterotypic junctions by sequestering the Rho GTPase activating protein, GAP21/23, to homotypic junctions and away from heterotypic junctions. Ahead of the zipper, sequestration of GAP21/23 to Ne/Ne junctions redirects RhoA and Myosin II activity to heterotypic Ne/Epi junctions; behind the zipper, Cadherin2 recruits GAP-21/23 to newly-formed Ne/Ne junctions to inactivate RhoA and Myosin II. Together, these actions couple local junction exchange and RhoA/Myosin II activity to dynamically maintain tissue level contractile asymmetry that is essential for zipper progression.
The last 20 years have seen astonishing progress in technological and experimental approaches in life sciences. Novel types of - frequently large-scale - data have brought unprecedented insight into fundamental biological processes and concepts, and helped bridge scales of analysis. These innovations, however, generate a deluge of experimentally produced data whose analysis, sharing, and interpretation necessitate the development of advanced informatics and computational tools. A remarkable technological revolution in biology has been brought by the next-generation genome sequencing, which led to the development of the concept of genome browsers. These browsers allow research communities to share data, findings, to collaborate in the creation of growing online databases, and above all permit researchers to make sense of tremendously large amounts of raw data.

The last decade has seen a new wave of technological revolution in life sciences: novel microscopy and tomography approaches have opened the way to quantitative approaches in morphogenesis and morphodynamic research. Large volumes of raw and reconstructed 3D or 3D+time data are currently available, often covering several hours in the evolution of dynamic processes. These data cover a remarkably broad range of phenomena, species, and scales, ranging from the reconstruction of entire animals or organs from MRI images down to the single-cell resolution segmentation of developing embryos imaged through, for instance, light-sheet microscopy. High volumes of morphological information need to be shared, categorized and quantified in order to achieve a better understanding of the fundamental role of shape and shape changes in biological structures. But the field misses both the equivalent of the genome browsers and of standardized formats to describe and share morphological and quantitative or qualitative geometrical properties.

We have therefore adapted the philosophy underlying the creation of genome browsers to imaging datasets. The result is MorphoNet, a novel class of online morphological browsers, which can be used both for research and dissemination. MorphoNet allows to explore complex segmented and meshed natural or simulated structures, onto which various morphological or genetic data can be projected. As a companion to the browser, we also introduce an easy-to-use and human-readable universal format to describe and exchange morphological data and associated information. This format can be used to describe...
a broad range of datasets (from developing embryos to termite nests) acquired using diverse imaging technologies and will greatly help researchers carry out quantitative analyses on their systems and share their results within and between multidisciplinary scientific communities.

During this presentation, I will give a brief overview of MorphoNet (Fig. 1) and discuss a specific use case of application on tunicates. We have efficiently used MorphoNet for multidisciplinary research within the ascidian community, with the reconstruction of more than 10 Phallusia mammillata embryos acquired on light sheet microscopy.
Colonial ascidians are the only known chordates that can undergo whole-body regeneration (WBR). This dramatic developmental process allows a minute isolated fragment of vascular system to restore a functional animal in as little as 10 days.

Following the initial injury of the colony, healing of the lesion takes place followed by the remodeling and condensation of the vascular system. After this initiation phase, the establishment and development of multiple regeneration niches will invariably lead to the regeneration of one single zooid.

Intriguingly, the origin of the stem-like cells responsible for WBR remain elusive and regeneration establishes exclusively within the vascular system. Similarly, while the remodeling of the vascular system has been morphologically described, which facets of this process are required for WBR to succeed remains unknown. In addition, we have observed some variability in the pace of WBR, potentially reflecting an underlying disparity in the organization of the vascular system.

To explore these dependencies, we have set out to characterize the involvement of the various components of colony during the initiation of WBR. To study this dramatic process, our laboratory is using an interdisciplinary approach combining biology, microscopy and computer science. By perturbing and modulating the regenerating vascular system with targeted micro-surgeries, we are dissecting the initiation of WBR in Botrylloides leachii.
EPIGENETIC REGULATION OF *BOTRYLLOIDES LEACHII* WHOLE BODY REGENERATION

Lisa Zondag, Rebecca Clarke, and Megan J Wilson

Developmental Biology and Genomics group. Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin. Affiliation

The colonial tunicate Botrylloides leachii is exceptional at regenerating from a piece of vascular tunic after loss of all adults from the colony. Previous transcriptome analyses indicate a brief period of healing before regeneration of a new adult (zooid) in as little as 8-10 days. However, there is little understanding of how the resulting changes to gene expression, required to drive regeneration, are initiated and how the overall process is regulated. Rapid changes to transcription often occur in response to chromatin changes, mediated by histone modifications such as histone acetylation. Here, we investigated 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.

Through our transcriptome data, we identified and quantified the expression levels of HDAC and histone acetyltransferase (HAT) enzymes during whole body regeneration (WBR). To determine if HDAC activity is required for WBR, we inhibited its action using valproic acid (VPA) and Trichostatin A (TSA). HDAC inhibition prevented the final morphological changes normally associated with WBR and resulted in aberrant gene expression. *B. leachii* genes including Slit2, TGF-b, Piwi and Fzd4 all showed altered mRNA levels upon HDAC inhibition in comparison to the control samples. Additionally, atypical expression of Bl_Piwi was found in immunocytes upon HDAC inhibition.

Together, these results show that HDAC function, specifically HDAC I/IIa class enzymes, are vital for *B. leachii* to undergo WBR successfully.
PRIMORDIAL BLASTS, A POPULATION OF BLOOD BORNE STEM CELLS RESPONSIBLE FOR WHOLE BODY REGENERATION IN A BASAL CHORDATE

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The invertebrate chordate Botrylloides leachii regenerates entire new bodies from small fragments of blood vessels. Here, we show that during early stages of regeneration, proliferation occurs only in small, blood borne cells. These proliferating blood cells express integrin-alpha-6, pou3 and vasa and are present in the blood of uninjured colonies. Elimination of these proliferating blood cells with Mitomycin C (MMC) blocks regeneration. Injection of 1 single prospectively isolated Integrin-alpha-6 (IA6)-positive cell is capable of rescuing whole body regeneration. Upon transplantation of Edu-labeled IA6+ cells into MMC treated recipients, Edu label is detected in regenerating tissues. Inhibitors of either Notch or canonical Wnt signaling block regeneration and proliferation of pou3+ stem cells. We propose that ia6+pou3+ cells are pluripotent, proliferating stem cells that are present in the blood of B. leachii and are responsible for whole body regeneration. We named these cells Primordial Blasts - based on the fact that they are undifferentiated, constantly dividing, express germline multipotency genes and give rise to somatic regenerating tissues.
T10
NOVEL BUDDING MODE IN POLYANDROCARPA ZORRITENSIS: A MODEL FOR COMPARATIVE STUDIES ON ASEXUAL DEVELOPMENT AND WHOLE BODY REGENERATION

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In colonial ascidians the capacity to build an adult body via non-embryonic development (NED), i.e., asexual budding and whole body regeneration, has been gained and lost several times across the whole subphylum. A recent phylogeny of the family Styelidae revealed an independent acquisition of NED in the colonial species Polyandrocarpa zorritensis and highlighted an undescribed budding mode that we named “vasal budding”.

We characterized the asexual life cycle and the morphology of the early stages of P. zorritensis budding. During vasal budding, the new zooids origin from protrusions of the adult epidermis in vascularized areas defined as budding nests. The budding nest can alternatively develop into a round-shaped structure, the spherule, which represents a dormant state, able to survive low temperatures and harsh environmental conditions.

The bud arises through a folding of the epithelia of the vessel with the contribution of undifferentiated mesenchymal cells. In order to assess the involvement of these two tissues we analyzed the proliferation dynamic via EdU labelling. We characterized the hemocyte morphotypes and identified a population of undifferentiated cells, the hemoblasts, known as putative stem cells in other ascidian species.

In conclusion, to understand the mechanisms of NED and their evolution we started from a robust phylogenetic framework and selected relevant species to compare. The description of NED in P. zorritensis provides the foundation for future comparative studies on plasticity of budding and whole body regeneration in tunicates.
Regeneration in *Ciona intestinalis* is polarized according to the body axis: severed basal parts regenerate distal structures, such as the siphons and neural complex, but severed distal parts do not replace basal structures. This phenomenon, termed distal regeneration, involves the activity of pluripotent stem cells residing in branchial sac vasculature. In response to distal injury, branchial sac stem cells (BSC) are induced to proliferate, the progenitor cells migrate to the sites of injury and form a blastema, which replaces missing distal tissues and organs. Progenitor cell migration is specific: only injured body parts are targeted. We have used microsurgical, pharmacological, and molecular analysis to investigate the mechanisms of distal regeneration. Apoptosis at injury sites is one of the earliest events in distal regeneration. Blocking apoptosis with caspase inhibitors interferes with BSC activation and prevents distal regeneration. Furthermore, apoptosis, blastema formation, and BSC proliferation are themselves polarized. When animals are bisected across the middle, apoptosis at the injury site, BSC proliferation, and blastema formation occur in the regenerating basal parts but not in the non-regenerating distal parts, although both parts share the same injury site and contain a large portion of the branchial sac. These results show that apoptosis is required to induce BSC proliferation and that BSC are necessary but not sufficient for distal regeneration. Microarray-based gene expression, regeneration rescue assays, and gene knockdowns indicate that apoptosis activates Wnt signaling at injury sites, which in turn triggers BSC proliferation and mobilizes the migration and distal targeting of progenitor cells.

Supported by NIH and EMBRC-France
AGING AND SENESCENCE EFFECTS ON THE EXTRACORPOREAL VASCULATURE OF BOTRYLLUS SCHLOSSERI

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Ageing causes a progressive deterioration of both the structure and function of vascular cells that triggers several age-related vascular diseases. The extra-corporeal vasculature of the colonial ascidian \textit{Botryllus schlosseri} interconnects all individuals, but due to weekly turnover of the zooids inherent in asexual reproduction in this species, is the only persistent organ in an individual, thus the only one to age. Characteristics of vascular aging include narrowing of the vessel and decreased blood flow. Interestingly, we also see predictable age-related changes in vascular cell morphology, including major remodeling of the cytoskeleton and basement membrane as well as changes in cell shape. We have recently developed a new quantitative approach (computer program) to track these changes at high resolution. In addition, using these phenotypic markers we can identify the genetic basis of senescence. To initiate these studies we have isolated multiple \textit{Botryllus} genotypes at specific ages and carried out mRNA-seq. Using differential expression analyses we have identified key genes involved in aging. The products of these genes are involved in multiple processes, including regulation of actin cytoskeleton, apoptosis, ATP metabolism, DNA/RNA repair, among others. Current results will be discussed.
EVO-DEVO APPROACHES TO NON-EMBRYONIC DEVELOPMENTS IN COLONIAL TUNICATES

Alexandre Alié, Marta Scelzo, Maria Mandela Prünster, Sonia Lotito, Lorenzo Ricci, Manon Boosten, Stefano Tiozzo

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Beside embryogenesis, to build an adult body colonial tunicates adopt non-embryonic developments (NED), i.e. asexual propagation and whole body regeneration. NED were acquired independently at least seven times across the tunicates. In different species NED starts from non-homologous somatic cells and tissues but nevertheless lead to similar adult body plans. The possibility to compare within the same group, non-homologous tissues that converge to common ontogenetic stages makes tunicates an excellent case study to deeper explore the molecular mechanisms underlying evolution of NED in metazoans.

In the recent years we used the colonial ascidian Botryllus schlosseri as a model to investigate the partial co-option of embryonic mechanisms during NED within the same species. For instance, we described the redeployment of early signaling pathways involved in AP axis formation and symmetry breaking, the differentiation of territories at the onset of budding, which mimic the embryonic germ layers domains, and how neurogenesis and myogenesis are triggered de novo, partially rewiring embryonic gene modules in different ontogenetic contexts.

To compare independently evolved NED, we have recently described a novel budding mode in Polyandrocarpa zorritensis. We are now conducting transcriptomic and genomic studies to compare dynamics of gene expression and genomic regulation between different buds in diverse colonial species. Our goal is to identify and compare prospective GRNs repeatedly co-opted for NED in species that independently acquired asexual reproduction. The function of identified genes will then be tested using a transgenic approach recently developed in B. schlosseri.
Colonial tunicates like Botryllus schlosseri are unique among chordates in that they use two pathways, embryogenesis and blastogenesis, to produce the adult body. Despite the fact that these two developmental pathways are morphologically distinct, they both result in almost identical individuals. The ways in which organogenesis differs when comparing embryogenesis and blastogenesis, and if a convergent morphology implies convergent molecular mechanisms remains almost unknown.

By combining transcriptome sequencing with confocal, two-photons, electron microscopy and digital 3D reconstructions of major embryonic and blastogenic developmental stages, we characterized the molecular and morphological signatures along both developmental pathways. We then generated a comprehensive developmental atlas that links the gene profiles describing the entire embryogenesis and blastogenesis processes with morphological events. With tissue and cell-type specific molecular signatures, we further identified the developmental origin of the nervous system, endostyle, blood cells, and germ cells. Moreover, we followed their developmental phases including morphogenesis, cytodifferentiation, and gene expression. This study outlines the molecular and morphological landscape of the two developmental modes and demonstrates that different molecular paths can lead to the same outcomes. It also suggests that cellular trajectory is defined early in development and that the adult tissue specific stem cells and the embryonic precursor populations share the same molecular profile.
Typical solitary tunicate development is characterized by a biphasic life cycle, having a swimming larva containing 40 notochord cells that converge and elongate to form the larval tail, and a sessile filter feeding adult. After the free swim stage, tunicates absorb their larval tail and a metamorphosis into their adult stage. However, within the Molgulidae clade, several species have independently evolved an alternate “tail-less” body plan, forgoing the development tailed swimming larval form and losing the differentiation of their notochord and several other features such paraxial muscles, motor neurons, and pigmented cells associated with geotactic and light-sensing organs required for swimming and settlement. One such tail-less species, Molgula occulta, is found in close proximity to Molgula occlusa a tailed species, and in lab conditions are able to cross fertilized producing interspecific hybrids with partially rescued motile phenotypes. These animals and their hybrid have presented a system where we are able to examine the evolutionary mechanism behind alternate body plans and the rescue of the motile phenotypes. We have sequenced the genome of both parent species and key developmental transcriptome stages (gastrula, neurula, and tailbud) of the M. occulta, M. occlusa and their hybrid. This analysis has enabled us to identify key genes involved in the motile behavior/structures. One of which being ebf, an important motor neuron terminal selector gene, whose expression has been reduced in the immobile M. occulta.

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TAILLESS MOLGULID ASCIDIANS HAVE A NOTOCHORD "BALL" THAT NEVER CONVERGES OR EXTENDS

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Transcriptomic and genomic data offer exciting new approaches to examine the genetic networks underlying the origin and evolution of the chordate body plan. One system for studying chordate body plan evolution is two tunicate species with radically different larval body plans—the tailed ascidian *Molgula oculata* and the tailless *M. occulta*. Tailed *M. oculata* embryos, like most solitary ascidians, have 40 notochord cells that are converged and extended in the center of the tail of the tadpole larvae. The larvae also have tail muscle cells flanking the notochord in the tail, and, in the head, an otolith, a gravity sensory organ. The tailless *M. occulta* does not form a tail in their larval stage, and have only 20 notochord cells that do not converge and extend during larval development. We have sequenced the genomes and obtained developmental transcriptomes for both species and the hybrid embryos. We show by transcriptome analyses that the notochord gene network is intact in molgulid ascidians, and the expression of most of the downstream genes is seen in the “notoball”. Hybrid embryos made from the sperm of the tailed, *Molgula oculata* and the egg of the tailless *Molgula occulta* undergo convergence and extension of the notochord to form a short tail. We are searching for the factors that allow the notochord convergence and extension in the hybrid embryos by computational analyses. We show that the expression of the downstream notochord genes is normal in the tailless *Molgula occulta*, with a few notable exceptions. We are continuing analyses of the notochord convergence and extension in hybrid embryos in an effort to understand the genetic network necessary for convergence and extension in ascidian embryos.
TUNICATE CARDIOPHARYNGEAL GENE NETWORK

Tunicates have retained a stringently conserved program for embryonic patterning over millions of years of evolutionary divergence\(^1\). Remarkably, this conservation occurs in the context of extremely high rate of sequence polymorphism in tunicate genomes\(^2\). Thus, tunicates represent an ideal clade for examining how robust developmental networks can retain function despite changes in the architecture of underlying developmental gene regulatory networks (GRNs), a process referred to as developmental systems drift\(^3\). We employ the newly sequenced Corella \textit{inflata} genome to explore drift within the well characterized cardiopharyngeal GRN. Here we show that levels of drift are highly variable across the regulatory nodes of the cardiopharyngeal GRN. Comparative analysis of \textit{Ciona robusta} and \textit{Corella inflata} enhancers for two key cardiopharyngeal transcription factors (\textit{mesp} and \textit{notrlc/hand-like}) reveals that upstream trans factors regulating these genes have been conserved while associated binding sites have undergone extensive rearrangements. Surprisingly, comparative enhancer analysis for a third cardiopharyngeal transcription factor, \textit{foxf}, revealed stringent conservation of binding site number position and spacing. Experimental manipulation of binding site spacing compromised the function of the \textit{Ciona FoxF} enhancer. The different level of selective constraint acting on these regulatory elements may reflect discrete variations in enhancer structure/function rather the reflecting the hierarchical position of the genes they regulate in the network.

References


PROTEIN PHOSPHATASE 2A IS ESSENTIAL TO MAINTAIN MEIOTIC ARREST, AND TO PREVENT Ca²⁺ BURST AT SPAWNING AND EVENTUAL PARTHENOGENESIS IN THE LARVACEAN OIKOPLEURA DIOICA

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Unfertilized eggs of most animals are arrested at a certain point in the meiotic cell cycles. Reinitiation of meiosis and the start of embryogenesis are triggered by fertilization. This is essential for preventing parthenogenetic activation and for promoting proper initiation of development by fertilization. In the larvacean Oikopleura dioica, unfertilized eggs are arrested at metaphase-I. We show here that protein phosphatase 2A (PP2A) is essential for maintenance of meiotic arrest after spawning of oocytes. Knockdown (KD) of the maternal PP2A catalytic subunit, which was found in functional screening of maternal factors, caused unfertilized eggs to spontaneously release polar bodies after spawning, and then start pseudo-cleavages without fertilization, namely, parthenogenesis. Parthenogenetic embryos failed to undergo proper mitosis and cytokinesis because of lack of a centrosome, which is to be brought into the egg by a sperm. Activation of the KD oocytes was triggered by an ambient intracellular rise of pH upon their release from the gonad into seawater at spawning. Live recording of intracellular calcium concentration indicated that the pH rise caused an aberrant Ca²⁺ burst, which mimicked the Ca²⁺ burst that occurs at fertilization. Then, the aberrant Ca²⁺ burst triggered meiosis resumption through Calcium/calmodulin-dependent protein kinase (CaMK II). Therefore, PP2A is essential for maintenance of meiotic arrest and prevention of parthenogenesis by suppressing the aberrant Ca²⁺ burst at spawning. In addition, genomic data suggest the possibility that the molecular mechanism of meiotic arrest in O. dioica is similar to that in vertebrates rather than ascidians.
Erroneous chromosome segregation leads to aneuploidy, a condition deleterious for development. To prevent it, the Spindle Assembly Checkpoint (SAC) delays entry into anaphase until all chromosomes are properly attached to spindle microtubules. Our team has shown that whereas some embryos, like those of sea urchin, have an active SAC and delay mitotic progression in the presence of spindle defects, in chordate embryos, including C. intestinalis and P. mammillata, the SAC is inefficient, during cleavage divisions. To understand the mechanisms controlling chromosome segregation during development in chordates, we characterized further how SAC activity is regulated in embryos of the ascidian P. mammillata.

Using live imaging, we find that treatment of P. mammillata embryos with the microtubule depolymerizing drug nocodazole did not affect mitotic timing during cleavage divisions. However, mitotic progression was delayed, in a SAC dependent manner, starting from the gastrula stage. The length of the mitotic delay increased further in neurula stage embryos, and SAC efficiency varied among different populations of cells within the embryo. By interfering with embryonic patterning by egg microsurgery or ectopic expression of transcription factor, we are currently testing the contribution of cell identity to SAC efficiency in ascidian embryos. Indeed our preliminary results suggest that the difference in SAC efficiency is lost when all cells acquire the same identity.

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ANALYSES OF SPATIO-TEMPORAL INTERACTION BETWEEN MICROTUBULE AND ER OR MATERNAL mRNAs IN CIONA EGG USING NEW HIGH RESOLUTION DOUBLE STAINING METHODS

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In ascidian embryogenesis, antero-posterior axis is predetermined by the egg polarity, which is established by large movements of the myoplasm, cortical endoplasmic reticulum (cER), and maternal determinants (i.e. Type I and II postplasmic/PEM RNAs) during first cell cycle¹. We previously reported the CAMP (cortical array of microtubules in posterior vegetal region)², which resembled to cortical microtubule arrays in frog and zebrafish eggs³,⁴. Thus, there is a possibility that CAMP contributes to the transportation of cER and maternal mRNAs. However, there has not been established the appropriate techniques for the visualization of these structures and molecules, simultaneously.

In this study, we developed new double staining methods, which consisted of ER immunostaining or in situ hybridization and high-resolution microtubule immunostaining. During 2nd myoplasmic movement, we could distinguish two different compartments in cER, outermost region, which directly interact with CAMP, and inner region. Type I postplasmic RNAs were found on both inner and outermost regions of cER, while Type II localized only within inner region of cER. Moreover, Type II postplasmic mRNAs have already started to localize at about 30 min postfertilization to the vegetal pole region, where the Type I postplasmic RNAs were already colocalized with TAF (transiently accumulated microtubule fragment). These observations are consistent with the report, in which the vegetal localization pattern of both types of postplasmic RNAs overlaps with each other during 1st myoplasmic movement⁵. Our bland new double staining methods and result of this study could have great impact for understanding the axis formation in ascidian egg.

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ACTIN-DEPENDENT CONTRACTION DRIVES OOPLASMIC SEGREGATION IN ASCIDIAN OOCYTES

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Reorganization of the cytoplasm within the oocyte (ooplasm) constitutes the first step in patternning of the tunicate embryo. Ooplastic reorganization is initiated by contraction of the actomyosin cortex along the animal-vegetal axis of the oocyte, leading to the accumulation of cortical endoplasmic reticulum (cER)–associated maternal mRNAs in a region of the vegetal pole termed ‘contraction pole’ (CP). We used the species Phallusia mammillata to unravel the mechano-chemical mechanisms underlining CP formation and maternal mRNAs segregation.

We first have analyzed the dynamic distribution of actomyosin during the cortical contraction process. We found that upon fertilization a gradient of contractility along the animal-vegetal axis drives vegetal-directed flows of the actomyosin cortex leading to the formation of the CP.

To investigate how cortical actomyosin flows are linked to maternal mRNAs segregation, we extended the flow analysis to other cortical and subcortical domains involved in this process: plasma membrane, cER and a mitochondria-rich domain positioned above the actomyosin cortex termed ‘myoplasm’. The flow velocities indicate that cER and plasma membrane flow together with the actomyosin cortex. The myoplasm, in contrast, shows slower flow velocity and does not accumulate at the same degree at the vegetal pole. It also displays pronounced buckling at the CP, suggesting that a rigid myoplasm layer becomes compressed at the vegetal pole by the contracting actomyosin cortex. We are currently analyzing how these domains are coupled to each other and how their differential friction determines the distinct shape changes and, consequently, the distribution of maternal mRNAs associated with these different domains.
GAP JUNCTION-DEPENDENT COORDINATION OF INTERCELLULAR CALCIUM SIGNALLING DURING EARLY EMBRYOGENESIS OF OIKOPLEURA DIOICA

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Calcium signaling is an important feature of embryonic development. Here, we characterized spontaneous Ca2+ signals in Oikopleura dioica embryos from pre-fertilization to gastrula stages after injecting GCaMP6 into unfertilized eggs, and we addressed the potential developmental role of intercellular Ca2+ waves by dsRNA knockdown of connexin genes.

The unfertilized egg exhibited regular, transient elevations in intracellular Ca2+ concentration with an average duration of 4-6 seconds and an average frequency of about 1 every 2.5 minutes. Fertilization was accompanied by a longer Ca2+ transient that lasted several minutes. Thereafter, regular Ca2+ transients were reinstated that spread within seconds among blastomeres and gradually increased in duration and decreased in frequency by gastrulation. Each peak was preceded by about 15 seconds by a smaller Ca2+ increase (about 5% of the main peak amplitude), which we term the “minipeak”. By gastrulation, Ca2+ transients exhibited a stereotyped initiation site on either side of the embryo, likely in the nascent muscle precursor cells, and spread thereafter symmetrically in a stereotyped spatial pattern from posterior to anterior.

To assess the role of gap junctions in calcium wave spread and coordination, we first characterized the expression of two Oikopleura connexins, Od-CxA and Od-CxB, both of which are expressed during pre-gastrulation and gastrula stages, and then co-injected dsRNAs to suppress connexin expression. Connexin mRNA knockdown led to a gradual increase in Ca2+ transient peak width, a decrease of interpeak interval and a marked disruption of wave synchronization. This desynchronization was accompanied by a disruption of normal development.
Hematopoiesis is an essential process that evolved in multicellular animals. At the heart of this process are hematopoietic stem cells (HSCs), which are multipotent, self-renewing and generate the entire repertoire of blood and immune cells throughout life. Here we studied the hematopoietic system of *Botryllus schlosseri*, a colonial tunicate that has vasculature, circulating blood cells, and interesting characteristics of stem cell biology and immunity. Self-recognition between genetically compatible *B. schlosseri* colonies leads to the formation of natural parabionts with shared circulation, whereas incompatible colonies reject each other. By means of flow-cytometry in combination with screened antibodies by Cytof, lectins, and fluorescent enzymatic reagents, we isolated 34 *B. schlosseri* cell populations. Using whole-transcriptome sequencing of defined cell populations, and diverse functional assays, we identified HSCs, progenitors, immune-effector cells, and the HSC niche. Our study implies that the HSC and myeloid lineages emerged in a common ancestor of tunicates and vertebrates and suggests that hematopoietic bone marrow and the *B. schlosseri* endostyle niche evolved from the same origin. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells and demonstrated that self-recognition inhibits cytotoxic reaction.
The genus *Ciona* Fleming, 1822 constitutes an interesting “taxonomic case”. Despite the large number of studies, its evolutionary history and complex taxonomy have not been yet completely resolved. This study presents new elements on this topic, describing specimens of a non-identified *Ciona* species found along the North-eastern coasts of Sardinia (Tyrrhenian Sea), through an integrative taxonomic approach. Morphological analyses first revealed that these *Ciona* specimens showed intermediate characters compared to those of other moderate and shallow-water species belonging to the *Ciona* genus, such as a smooth tunic surface without tubercles; 6 longitudinal muscles bands; a flat branchial sac; transversal vessels of about equal sizes; and a marked preference for shady sites. Molecular characterization was based on three mitochondrial regions: cox2-cob fragment, for the first time for a phylogenetic study in *Ciona*; cox3-trnK-nad1 fragment, previously used for discriminating *Ciona* cryptic species; and cox1, a reliable DNA barcoding marker. Phylogenetic trees clearly resolved the phylogenetic position of the specimens within the genus. Altogether, our data confirmed that our *Ciona* specimens could not be assigned to any molecularly-characterized or already described species within the genus (*i.e.*, *Ciona intestinalis* (Linnaeus, 1767), *Ciona robusta* Hoshino & Tokioka, 1967, *Ciona savignyi* Herdman, 1882, *Ciona roulii* Lahille, 1887, *Ciona edwardsi* Roule, 1884, *intestinalis* spC and *intestinalis* spD) and they are closely related to *C. edwardsi*. Our findings add further complexity to the *Ciona* taxonomy, underlying the importance of integrative taxonomic approaches, and the need to gather additional knowledge regarding the evolutionary history of this still enigmatic genus.
Invasive species constitute a major threat to biodiversity and can cause high economic and ecological negative impacts. In the study of non-indigenous species, it is essential to assess genomic diversity and population structure for developing adequate management strategies. We combined whole genome amplification (WGA) and genotyping-by-sequencing (GBS) techniques to set a protocol for samples with small DNA quantities, and we applied it to single zooids of colonies of the worldwide invasive colonial ascidian Didemnum vexillum. WGA-GBS performance was tested using half zooids, providing empirical demonstration for genotyping reliability. We analysed 296 colonies from 12 localities worldwide including native and main invaded areas, generating polymorphic loci datasets by locality, region and globally. We detected some clones, always within the same locality. At the global level, we identified two groups matching Cytochrome Oxidase I clades (Stefaniak et al. 2009), confirming that only one clade spread worldwide and suggesting reproductive isolation between the two clades in the native area, Japan, and a speciation process. We identified three independent colonization events determining the presently known distribution of the species, although significant differentiation was found in all population pairwise comparisons. The lack of isolation by distance within introduced areas indicates that passive transportation would drive the distribution pattern of this species worldwide and regionally. Diverse and well differentiated populations in the introduced and native areas point to a high expansion potential of this worrisome worldwide invader.

References

**T26**  
**THE THALIACEAN SALPA FUSIFORMIS: THE RETURN OF SESSILE ASCIDIANS TO PELAGIC LIFE**

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Thaliaceans are a fascinating group of pelagic tunicates with complex life cycles, encompassing doliolids, pyrosomes and salps (Piette and Lemaire 2015). Their proximity to ascidians was noted already by Cuvier (Cuvier 1804). By an extensive phylogenomic analysis based on a large number of coding sequences we confirmed that thaliaceans are the sister group of phlebobranchs and aplousobranchs inside the polyphyletic ascidians (Delsuc et al. 2018). Moreover, our data position doliolids, some of which still possess a tadpole larva, as the sister-group of all other thaliaceans. Thaliaceans, and especially salps, are characterized by a rapid molecular evolution, even when compared to ascidians. We will present a more detailed analysis of the tempo and mode of evolution of ca. 3000 proteins, and discuss a possible role in thaliacean evolution.

We chose the salp Salpa fusiformis as a model organism to better understand thaliacean development, which is very different from the stereotyped mosaic development of their ascidian relatives. In particular, the early invasion of blastomeres by surrounding follicle cells, also called calymmocytes, led to a profound modification of salp embryogenesis when compared to that of ascidians. Calymmocytes actively participate in building the embryo, possibly by bringing to their final position the blastomeres, which will eventually form the definitive adult organs. Most intriguingly, the patterned expression of developmental regulators such as Otx in calymmocytes, suggests that these cells also exert some morphogenetic role. Finally, the striking divergence of the early embryogenesis between two salp species, Salpa fusiformis and Thalia democratica, suggests a possible relaxation of developmental constraints in salps.

We are convinced that despite the experimental challenge of studying these intriguing class of organisms, a better understanding of their development and evolution will be an essential contribution to tunicate biology.

**References**


T27

PHYLOGENY OF THE BOTRYLLID ASCIDIANS

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The botryllid ascidians are a clade within the Family Styelidae (Class Aschidiacea, Order Stolidobranchia) comprising 50 species. Botryllids are used as models in several biological fields, including allorecognition, aging, development, invasion genetics, mitochondrial genome evolution, and regeneration. However, the majority of botryllid species are not well known to the ascidian research community. Lack of genetic resources and distinguishing morphological characters have made species identifications difficult, and a multi-locus phylogeny of the group has never been published. We have generated a phylogeny based on 100 nuclear loci developed through an anchored enrichment protocol. Five genomes (Botrylloides praelongus, Botrylloides violaceus, Botrylloides sp., Botryllus horridus, Botryllus schlosseri Clade E, and Symplegma brakenhielmi) were aligned with the Botryllus schlosseri Clade A genome and Botrylloides leachi transcriptome to identify phylogenetically informative loci. The current phylogeny contains 17 taxa, with additional taxa to be added in 2020. A Botryllus primigenus clade occupies the basal position in the phylogeny. The sister group to Botryllus schlosseri is Botryllus horridus. There are four major subclades within the Botrylloides clade: a B. fuscus/B. giganteum/B. violaceus clade, a B. nigrum/B. praelongus/B. diegensis clade, a Caribbean clade containing two species, and a Pacific clade containing two species. B. praelongus has been described from Japan, and B. diegensis from many temperate locations. From a phylogenetic perspective, these two taxa are likely the same species. The genus Botryllus is paraphyletic with respect to the genus Botrylloides, so the taxonomic distinction between the two genera may not be appropriate.
The subfamily Botryllinae comprises small colonial ascidians of the genera *Botryllus* and *Botrylloides*, whose morphological identification at species level is very problematic due to the high phenotypic variability and the few discriminant characters. Furthermore, the molecular phylogeny of the group is poorly resolved. Even in the model species *Botryllus schlosseri* (Pallas, 1766), the existence of cryptic species (i.e., clades from A to E) was suggested by molecular data but not yet supported by morphological analyses.

The aim of this study is to validate a molecular protocol for the discrimination of Botryllinae species, and to clarify the phylogenetic relationships within Botryllinae. Using a nested PCR strategy, we successfully amplified an elongated COI fragment of about 860 bp in more than 120 *Botryllus / Botrylloides* worldwide-sampled colonies, mainly with uncertain or unknown morphological identification or belonging to *B. schlosseri* cryptic species. The species delimitation analyses confirmed the existence of a clear barcode gap and identified 18 different OTUs (i.e., Operational Taxonomic Units, corresponding to molecularly-delineated species), each containing already-described, cryptic or putative new species. The phylogenetic reconstructions recognized as statistically significant the clades: all known species; putative new species; clades A-E; further subclades within *B. schlosseri*. Moreover, these trees effectively resolved the relationships among the *B. schlosseri* clades, but unfortunately left unresolved the basal nodes of the tree, i.e., the main relationships among Botryllinae. In conclusion, our results indicate that the Botryllinae species can be easily discriminated based on COI data but that a different molecular marker is needed for resolving the Botryllinae phylogeny.
The ascidian *Diplosoma listerianum* is distributed worldwide, and has been used as a model for a variety of biological studies. Recently, mitochondrial DNA (mtDNA) evidence suggests that the species may be a cryptic species complex of four distinct clades (Pérez-Portela et al. 2013). That study, while global in scope, included few samples from the western Atlantic, where *D. listerianum* is widespread. Here we add an additional 55 samples from coastal Brazil, Panama (both sides), Mexico (Yucatan) and the United States (Florida) to test whether those populations are part of world-wide clades or are native species hidden within this species complex. We found 33 new COI haplotypes. Phylogenetic analysis resulted in seven clades of which three were recovered before. Species delimitation analyses (ABGD, GMYC and bPTP) corroborated these clades, and divided the former Clade A into two clades: clade A1 exclusively found in the west Atlantic with one population at the Pacific side of Panama and another in South Africa, and clade A2, found worldwide. Two new clades are exclusively from Mexico and another is from Panama. In combination, the presence of exclusive clades in sympatry with widespread clades, the high diversity found in the tropical west Atlantic, and the strong morphological similarity among clades suggests that *D. listerianum* is a fast-evolving complex of cryptic species, with indications of ongoing speciation. This species complex is also easily maintained in the laboratory and thus it is an interesting candidate as a model for studies of speciation.

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3. Superior - Brazil (Capes) with scholarship to JAT.
PHYLOGENETIC ANALYSIS OF PHENOTYPIC CHARACTERS OF TUNICATA SUPPORTS BASAL APPENDICULARIA AND MONOPHYLETIC ASCIDIACEA

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With approximately 3000 marine species Tunicata represents the most disparate subtaxon of Chordata. Molecular phylogenetic studies support Tunicata as sister taxon to Craniota rendering it pivotal to understand vertebrate evolution. While successively more molecular data became available to resolve internal tunicate phylogenetic relationships, phenotypic data have not been utilized consistently. Here we address these shortcomings by cladistically analyzing 116 phenotypic characters for 49 tunicate species comprising all higher tunicate taxa, and 5 craniate and cephalochordate outgroup species. In addition, we performed a combined analysis of the phenotypic characters with 18S rDNA-sequence data in 32 OTUs. The strict consensus tree from the analysis of the phenotypic characters recovers monophyletic Appendicularia as sister taxon to the remaining tunicate taxa. “Thaliacea” is found paraphyletic with Pyrosomatida as sister taxon to Asciidiacea and the relationship between Doliolida and Salpida unresolved. Thus, the phenotypic data support the hypothesis that the last common ancestor of Tunicata was free-living, that ascidian sessility is a derived character and that the last common ancestor of Asciidiacea was colonial. The combined analysis, congruent with published purely molecular analyses, recovers Thaliacea monophyletic nested within paraphyletic “Asciidiacea”. Successively up-weighting phenotypic data indicates that phenotypic data contribute disproportionally more to the resulting phylogenetic hypothesis.
ANTERO-POSTERIOR ECTODERM PATTERNING BY CANONICAL WNT SIGNALING DURING ASCIDIAN DEVELOPMENT

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Wnt/β-catenin signaling is an ancient pathway in metazoans and controls various developmental processes, in particular the establishment and patterning of the embryonic primary axis. In vertebrates, a graded Wnt activity from posterior to anterior endows cells with positional information in the central nervous system. Recent studies in hemichordates support a conserved role for Wnt/β-catenin in ectoderm antero-posterior patterning at the base of the deuterostomes. Ascidians are marine invertebrates and the closest relatives of vertebrates. By combining gain- and loss-of-function approaches, we have determined the role of Wnt/β-catenin in patterning the three ectoderm derivatives of the ascidian Ciona intestinalis, central nervous system, peripheral nervous system and epidermis. Activating Wnt/β-catenin signaling from gastrulation led to a dramatic transformation of the ectoderm with a loss of anterior identities and a reciprocal anterior extension of posterior identities, consistent with studies in other metazoans. Surprisingly, inhibiting Wnt signaling did not produce a reciprocal anteriorization of the embryo with a loss of more posterior identities like in vertebrates and hemichordate. Epidermis patterning was overall unchanged. Only the identity of two discrete regions of the central nervous system, the anteriormost and the posteriormost regions, were under the control of Wnt. Finally, the caudal peripheral nervous system, while being initially Wnt dependent, formed normally. Our results show that the Ciona embryonic ectoderm responds to Wnt activation in a manner that is compatible with the proposed function for this pathway at the base of the deuterostomes. However, possibly because of its fast and divergent mode of development that includes extensive use of maternal determinants, the overall antero-posterior patterning of the Ciona ectoderm is Wnt independent, and Wnt/β-catenin signaling controls the formation of some sub-domains. Our results thus indicate that there has likely been a drift in the developmental systems controlling ectoderm patterning in the lineage leading to ascidians.
COORDINATION OF ASYMMETRICAL NOTOCHORD CONTRACTILITY AND EPITHELIA CELL PROLIFERATION DRIVES TAIL BENDING IN CIONA EMBRYOGENESIS

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In early embryogenesis, a bending tail surrounding the body within the chorion is evolutionally conserved feature in most of invertebrate and vertebrate. However, whether this process is genetic control and how the embryo acquires a bending shape remain completely unknown. Here, using Ciona embryogenesis, we first demonstrated that tail-bending process is a genetic control program. To reveal the cellular process during bending process, we next examined the distribution of actomyosin in Ciona embryogenesis and found that they were mainly accumulated along the ventral, but not dorsal side of the notochord during tail bending. The quantitative results revealed that dorsal-ventral asymmetry distribution of actomyosin was more prominent at the tail-bending site, indicating that asymmetrical actomyosin contractility in notochord drives the tail bending. We further characterized the cell proliferation in tail tissues during tail bending by BrdU staining. The results showed that the dorsal midline of tail epidermis divided faster than the corresponding ventral domain, suggesting that the discrepancy of the tail epidermis cell division may produce asymmetrical force to drive the tail bending. To understand further the mechanical roles of each tissue and their coordination, we develop a physical model to simulate the bending process and predict the key parameters that determine bending degree and position. Thus, we revealed that ventral actomyosin contractility in notochord coordinated with the faster dorsal epithelial cell proliferation to drive tail bending in Ciona embryo. Our results thus provide an insight on how the different tissues coordinate to determine the embryo morphogenesis.
Ascidian notochord provides a simple and highly tractable model for tubulogenesis. Combining chemical and genetic perturbations with live cell imaging, we show that 14-3-3ε a plays a crucial role for tubulogenesis. Phenotypic screening from our in-house chemical library led us to identify UTKO1, a human cancer cell migration inhibitor we previously reported, as a selective inhibitor of Ciona notochord tubulogenesis. UTKO1 directly bound to 14-3-3ε a and prevented 14-3-3ε a from interacting with ezrin/radixin/moesin (ERM), another regulator of Ciona notochord tubulogenesis. Therefore, we focused on how interactions between 14-3-3ε a and ERM could contribute to tubulogenesis in space and time. During tubulogenesis, 14-3-3ε a and ERM colocalize at the basal cortex of notochord cells. As the lumen begins to open, they undergo cycles of accumulation and disappearance. Interestingly, the disappearance of 14-3-3ε a and ERM during each cycle is tightly correlated with a transient flow of 14-3-3ε a, ERM, myosin II, and other cytoplasmic elements from the basal cortex to the lumen-facing apical domain, which is often accompanied by visible changes in lumen architecture. Pulsatile behavior, transient flow, and lumen formation were abrogated in larvae treated with UTKO1, depleted of either 14-3-3ε a or ERM, or expressed with truncated form of 14-3-3ε a that lack the ability to interact with ERM. These results suggest that 14-3-3ε a and ERM interact at the basal cortex to direct pulsatile basal-apical transport of factors that are important for lumen formation. Because many core components of this Ciona system are highly conserved, we propose that similar mechanisms may underlie tubulogenesis in other systems.
NEURULA ROTATION AND LEFT–RIGHT ASYMMETRY IN ASCIDIAN EMBRYOS: CILIARY MOVEMENTS AND THE VITELLINE MEMBRANE SIGNAL

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Tadpole larvae of the ascidian, Halocynthia roretzi, show morphological left–right asymmetry in the brain structures and the orientation of tail bending within the vitelline membrane. Neurula embryos rotate along the anterior–posterior axis in a counterclockwise direction, and then this rotation stops when the left side of the embryo is oriented downwards. Contact of the left-side epidermis with the vitelline membrane promotes nodal gene expression in the left-side epidermis. We show that epidermal monocilia, which appear at the neurula rotation stage, generate the driving force for rotation. Epidermis cilia moved in a serpentine way like sperm flagella but not in a rotational way or beating way with effective stroke and recovery stroke. They moved very slowly, at 1/7 Hertz. Similar motility was also observed in Ciona robusta embryos. Our observations suggest that the driving force of rotation is generated using the vitelline membrane as a substrate but not by making a water current around the embryo.

We also showed that a chemical signal from the vitelline membrane promotes nodal gene expression since the treatment of devitellinated neurulae with an extract of the vitelline membrane promoted nodal expression on both sides. These signal molecules are proteins but not sugars. Specific fractions in gel filtration chromatography had the nodal promoting activity. By mass spectrometry, we selected 48 candidate proteins. It is also shown that epidermal cilia drive the neurula rotation but are dispensable for sensing the signal from the vitelline membrane.

References


THE EFFECTS OF HIGH WATER TEMPERATURE ON DEVELOPMENTAL PROCESSES IN C. INTESTINALIS: LIMITS TO EMBRYONIC ROBUSTNESS.

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The normal embryogenesis of marine animals is typically confined to a species-specific range of temperatures. Within that temperature range development results in a consistent, or canalized, phenotype, whereas above and below the range abnormal phenotypes are produced. This study reveals a high temperature threshold, occurring over a 1-2°C range, for normal embryonic development in C. intestinalis. Above that threshold morphological abnormalities in the notochord and other organs are observed, beginning with cleavage and gastrula stages, and becoming more pronounced as embryogenesis proceeds. We used live imaging to study which morphogenetic processes are most susceptible to high temperature. We also examined the expression of cell type specific markers to see if cell specification is affected by high temperature. The results suggest that morphogenesis is more sensitive to high temperature than cell type specification. This working hypothesis points to further approaches to the study of the mechanisms enabling embryonic robustness in face of environmental challenges.
The central nervous system (CNS) of the Ciona intestinalis larva has only 177 neurons, making it the smallest described in any animal. The CNS compartment that has been studied in greatest detail is the Motor Ganglion (MG), a simple pattern generator that drives the swimming behaviors of the larva. Within the MG, 7 bilaterally symmetric left/right pairs of neurons from the majority of the synaptic connectivity of the MG, and can all be traced to the A7.8 pair of blastomeres of the 64-cell stage embryo. Here we focus on the comparison between two very different core MG interneuron types: the descending decussating neuron (ddN) and MG Interneuron 2 (MGIN2). As their name implies, ddNs are the only neurons whose axons cross the midline before descending towards the tail. They receive synaptic inputs from peripheral nervous system (PNS) relay neurons and in turn synapse onto other MG neurons, each in particular forming electrical synapses with their respective contralateral Motor Neuron 2 (MN2). On the other hand, MGIN2s are ipsilaterally-projecting descending interneurons that form conspicuous electrical synapses with ipsilateral MN2s, but receive synaptic inputs mainly from photoreceptor relay neurons and other interneurons of the brain, where the larval light- and gravity-sensing organs are located. Thus, these two MG neuron subtypes might modulate asymmetric swimming behaviors in response to sensory cues processed by distinct thigmotactic (ddNs) and phototactic/geotactic (MGIN2s) pathways. By analyzing and comparing the transcriptional profiles of isolated ddNs and MGIN2s, we identified and validated differentially-expressed transcripts enriched in either neuron type. This revealed candidate effectors of ddN/MGIN2 morphology, connectivity, and function. Probing the subcellular localization and functions of key ddN-enriched candidate effectors revealed a potential role for microtubule stabilization and centrosome migration in establishing the unique contralateral projection of the ddNs.
LEFT-RIGHT ASYMMETRIC DEVELOPMENT OF CELLS IN THE LARVAL BRAIN OF CIONA

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To understand development of the brain at single-cell resolution in chordates, Ciona is an ideal model organism. However, both cell lineage and developmental mechanisms of the brain vesicle of the Ciona larva remains unclear because removal of the chorion, which is a commonly used technique for manipulation of Ciona embryos, disturbs the structure and the left-right asymmetry of the brain vesicle. By improving the micro-injection technique and developing a method to label a single cell of Ciona embryos without removing the chorion, we have studied the cell lineage and the molecular mechanisms of the brain vesicle [1, 2].

Neurons of the Ciona larval brain can be classified into four subtypes by neurotransmitter phenotypes; namely, glutamatergic, GABAergic/glycinergic, cholinergic and dopaminergic. Although the left-right asymmetric distribution of neurons in the Ciona brain has been reported, how each neuron subtype is generated during development remains unknown. To elucidate the precise cell lineage of the four neuron subtypes in the brain vesicle, we chased development of each cell of the neural plate using FITC and the photo-convertible fluorescent protein Kaede. We revealed that the dopaminergic neurons (coronet cells) located at the left side of the brain vesicle are derived equally from the left and right a9.37 cells. We also found left-right asymmetric development of other neuron subtypes. We discuss the dynamic movement of brain vesicle cells during the period between the late tailbud and larval stages.

References


JAGGED ACTS AS A LATERAL INDUCER TO SPECIFY CAUDAL EPIDERMAL SENSORY NEURON FATE IN CIONA

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Notch signaling is utilized in numerous ways to specify cells during metazoan development. The Notch receptor and its ligands Delta and Jagged are transmembrane proteins that undergo specific processing events during the signal transduction process. In many cases, Notch/Delta signaling mediates the selection of one cell type from a group of cells that have the potential to develop into two different types of cells, a process referred to as lateral inhibition. Notch signaling has also been found to operate in a completely opposite manner with regards to cell type specification; rather than inhibit, Notch signaling can also laterally induce, as has been shown in the specification of sensory patches in the vertebrate inner ear from which mechanosensory hair cells form. Here we show that the Jagged ligand mediates the lateral induction of caudal epidermal sensory neurons (ESNs) in the tail of the Ciona robusta embryo. Jagged is expressed in midline stripes in early embryos and is likely activated by Msxb, one of the earliest-acting caudal midline transcription factors. Previous work from several labs has shown that ectopic epidermal expression of Delta prevents the specification of ESNs; ectopic epidermal expression of Jagged has the opposite effect and expands ESNs along the midline. Caudal ESN patterning is thus much more complex than current models suggest, requiring both inductive and inhibitory Notch signaling to specify and position these cells along the tail midline.
IN VIVO CALCIUM-IMAGING REVEALS A POSSIBLE ROLE OF THE GNRH SYSTEM IN LARVAL SWIMMING OF CIONA

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Gonadotropin-releasing hormone (GnRH) is a neuroendocrine peptide that plays a central role in reproductive control of vertebrates. Non-reproductive function of GnRH has been suggested but poorly understood. The Ciona larva, a non-reproductive stage of the simple chordate, has a prominent GnRH system spanning the entire length of the nervous system (Kusakabe et al., 2012). One of the $gnrh$ genes, $gnrh2$, is conspicuously expressed in the motor ganglion and nerve cord, which are homologous structures to the hindbrain and spinal cord of vertebrates. Correspondingly, GnRH receptor genes are expressed in the tail muscle and notochord, both of which are phylotypic axial structures along the nerve cord. The $gnrh2$ gene is also expressed in the proto-placodal sensory neurons, which are the proposed homologue of vertebrate olfactory neurons (Abitua et al., 2015). The tunicate larvae occupy a non-reproductive dispersal stage, yet the roles of their GnRH system remain elusive.

In this study, to obtain insights into physiological roles of GnRH in the Ciona larva, we identified the types of cells expressing $gnrh2$. Furthermore, we visualized activity of $gnrh2$-expressing cells in the larva by Ca$^{2+}$ imaging using a calcium sensor protein, G-CaMP8 (Ohkura et al., 2012). Some cholinergic motor neurons as well as unique cholinergic cells along the nerve cord express $gnrh2$, suggesting a role of GnRH in the control of swimming behavior. By contrast, none of the $gnrh2$-expressing cells overlap with glycinergic or GABAergic neurons. A role in the motor control is also suggested by simultaneous activation of some $gnrh2$-expressing cells with tail movements. Active Ca$^{2+}$ transients were also observed in the proto-placodal sensory neurons. Interestingly, the nerve cord ependymal cells produced Ca$^{2+}$ transients, suggesting a novel role of glial ependymal cells in the control of tail movement.

References:
Specific binding of transcription factors to a specific DNA sequence, the binding motif, is the primary step by which cells express genetic information held within their genome. Recent development of high-throughput profiling of transcription factor binding specificities has revealed that many transcription factors can recognize multiple binding motifs, rather than a single binding motif \(^1\). However, their developmental roles and mechanisms of the choice of binding motifs by transcription factors are largely unknown.

A Zic transcription factor Zic-r.a (Macho1) is a maternal muscle determinant and activates Tbx6-r.b zygotically. At the 16-cell stage, Zic-r.a dose not necessarily need to bind directly to the regulatory region to activate Tbx6-r.b \(^1\). However, in the present study, we found that Zic-r.a bound directly to a regulatory region of Tbx6-r.b to activate Tbx6-r.b in B6.4 cells of 32-cell embryos. Importantly, this region contained two Zic-r.a binding sites, which were similar to the secondary binding motif of Zic transcription factors (UniprobeUP00102) \(^2\) but not similar to the primary motif for Zic transcription factors (UniprobeUP00102) \(^2\) or a motif for Ciona Zic-r.a \(^3\). The secondary motif sites bound Zic-r.a more weakly than the primary motif sites in vivo. Our reporter assays showed the significance of secondary binding motif. Specifically, when the secondary binding motif was replaced with the primary binding motif, a reporter gene was expressed ectopically in non-muscle lineages. The data indicates that secondary binding motif sites contribute to restricting the Zic-r.a-dependent activation of Tbx6-r.b to muscle-lineage cells.

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STEREOTYPY AND VARIATION AMONG THE LARVAL MOTOR CIRCUITS

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Small nervous systems allow us to examine the entirety of a nervous system in detail, revealing both ultrastructural and network features. 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; Ryan et al. 2018).

Larval responses to environmental cues rely on the network of underlying neuronal connections that translates these cues into motion. The anatomical connectome reveals specific details of motor networks at a snapshot during development, which are involved in central pattern generation and a putative escape response. To examine the consistency of these networks, we have undertaken comparative cell-by-cell analysis of the morphology and synaptic connections between cells of the motor networks of two individual sibling larvae at the same stage. The validation of cell types, and their synaptic properties and connections are revealed through these analyses, as well as some putative distinctions in important network components. These include individual differences in some motor neurons and the ACINs. These comparisons also emphasize the location of synaptic contacts and reveal the variable stereotypy of axonal bundling. Comparing network properties helps to identify robustness of substrates for more complex larval behaviours based on interactions between pathways that converge on the motor generating network of the Ciona intestinalis larva.

References
te towards the establishment of the tunicate *Ciona intestinalis* as a neuroethology model. We are trying to match the growing insights into the structure\textsuperscript{1–3} and development of the larval nervous system with a detailed characterization of its neural circuits and behavioural output.

As a starting point, we have been developing open-source hardware and software in order to perform quantitative behavioural analysis of swimming larvae and functional imaging of the tadpole nervous system.

Previously, using our behavioural setups, we collected large datasets of free-swimming *C. intestinalis* larvae and employed machine-learning methods to create an objective ethogram of the larvae\textsuperscript{4}. In addition, we went on to identify behavioural modes using agglomerative clustering. Here we will be presenting new data on the role of biogenic amines in modulating *C. intestinalis* larval behaviour.

Furthermore, we will be presenting our on-going efforts to perform functional imaging of the larval nervous system. Our dataset includes, both spontaneous neuronal activity at a brain-wide level as well as sensory stimulus evoked responses from a small set of sensory receptor cells and downstream neurons.

Finally, we will be discussing some of the challenges and possible solutions towards a systems level mapping of neuronal function onto the wiring diagram of *C. intestinalis*.

References:

It is likely that the nervous systems of all bilaterally symmetric animals are left-right asymmetric with respect to processing of information and control of behaviour. However, we know very little about how asymmetries arise in development, how they are encoded in circuits and what their importance is for nervous system function. We are using developmental, genetic, imaging and behavioural approaches to study habenular asymmetry in zebrafish to address these issues. One focus is to determine the mechanisms that lead to neurons on the left and the right acquiring different character and establishing different circuit connectivity between left and right sides of the brain. Through a forward genetic screen, we identified the *rorschach* mutant in which neurons on both left and right sides adopt left-sided character independent of signals from the parapineal nucleus (which are normally required to impart left-sided character). The *rorschach* mutation is in a gene which encodes an orphan transmembrane receptor that we find interacts with Wnt pathway receptors. To explore the behavioural consequences of disrupting habenular asymmetry, we are assessing how habenular circuitry impacts an innate exploration strategy. We find that exploration strategy is modulated by levels of light and the ability to switch strategy upon changes in illumination is lost in embryos in which both habenulae develop with right-sided character.

The people who have done this work and our many collaborators will be acknowledged in the presentation. This research is supported by the Wellcome Trust.
Cell-cell junctions respond to mechanical forces by changing their organization and function. Tension-dependent conformational changes of junctional proteins are thought to underlie this junctional mechanosensitivity. Here we show that in the gastrulating zebrafish embryo, tight junctions (TJ) mechanosensitivity is mediated by actomyosin-driven flow of phase separated Zonula occludens-1 (ZO-1) clusters. We found that ZO-1 junctional accumulation at the contact between the Enveloping Layer (EVL) and the Yolk Syncytial Layer (YSL) closely scales with actomyosin tension. Actomyosin tension triggers ZO-1 junctional accumulation by driving retrograde actomyosin flow within the YSL that transport non-junctional ZO-1 clusters towards the TJ. Non-junctional ZO-1 clusters form by phase separation, and their effective formation is dependent on the actin binding region (ABR) within the C-terminus of ZO-1. If the non-junctional ZO-1 pool is absent, TJ lose their mechanosensitivity, and, consequently, EVL-YSL movement is impaired. Thus, phase separation and flow of nonjunctional ZO-1 confer mechanosensitivity to TJ.
Membrane transporters play essential roles in development, including protection of the embryo and control of cell differentiation and motility. ATP-Binding Cassette (ABC) transporters are a major family of small molecule transporters that include proteins that handle xenobiotics, signaling molecules and metabolites. These transporters are already well-appreciated for their roles in drug disposition of adults, however less appreciated for their diverse functions in the embryo. Here I will summarize our recent results elucidating how these proteins are integrated into the program of early embryogenesis. These include conserved developmental roles in the formation of primordial germ cells, and in differentiation of the gut. To place ABC transporter genes within the regulatory networks of development, we have systematically identified the major ABC transporters of the sea urchin embryo, which include \textit{ABCB1}, \textit{ABCB4}, \textit{ABCC1}, \textit{ABCC4}, \textit{ABCC5}, \textit{ABCC9}, and \textit{ABCG2}, and aligned their patterns of activity and expression, using \textit{in-situ} hybridization, live-cell nanobodies, and \textit{in-vivo} membrane transport assays. The results have revealed protective and homeostatic genes such as \textit{ABCB1}, \textit{ABCC1} and \textit{ABCC9} which are expressed ubiquitously, as well as developmental transporters such as \textit{ABCC4} and \textit{ABCC5} which are expressed in distinct territories within the embryo. \textit{ABCC4}, a lipid-derived signal molecule transporter, is expressed in ring of mesodermal cells of the early blastula, and later becomes restricted to mesoderm and germ-line-fated tissues. In addition, we have identified two genes, including \textit{ABCB4} and \textit{ABCG2}, that are expressed exclusively in the hindgut at the onset of gut development, and eventually expand to the midgut region. Both proteins are further activated in larval guts post-feeding. These are the most comprehensive systems-level descriptions of transporter expression during embryogenesis of any embryo, and lay the groundwork for defining the regulatory programs that specialize membrane function.
Corals are world-class ecosystem engineers that rely on dissolved inorganic carbon (DIC) to perform important ecological roles: CO$_2$ for photosynthesis, and CO$_3^{2-}$ for calcification. Low pH promotes formation of CO$_2$ from other forms of DIC, whereas higher pH favours CO$_3^{2-}$, but at normal intracellular pH most DIC exists as HCO$_3^-$. As such, corals are presented with an internal pH regulatory challenge in order to support photosynthesis and calcification. One objective of our work has been to characterise the extent to which internal pH varies in corals at sites of photosynthesis and calcification. Coral photosynthesis is carried out by intracellular algal symbionts and we have investigated how corals decrease pH in the immediate vicinity of the symbionts to maximise CO$_2$ uptake. Coral calcification occurs in an extracellular calcifying medium under the calcifying cells, where pH is elevated. This pH “up-regulation” potentially promotes both the flux of DIC to the calcification site and the formation of calcium carbonate. Additionally, we have investigated if corals can sustain pH upregulation against changes in seawater pH expected to occur under ocean acidification. Our findings suggest that pH regulation may be a key physiological trait shaping the response of these organisms to a rapidly changing ocean environment.
Regenerated body parts are similar to the ones that were developed during embryonic development. This observation is at the origin of a century old hypothesis proposing that regeneration reutilizes developmental processes. If this is true, the genetic interactions driving these two processes are predicted to be largely overlapping. One organism that is perfectly suited to compare the gene regulatory networks (GRNs) underlying embryogenesis and regeneration is the anthozoan cnidarian Nematostella vectensis. Not only a global GRN underlying embryogenesis has established for this cnidarian but also, this sea anemone is able to undergo whole body regeneration and reform missing body part in only five days after amputation. After characterizing in detail the regenerative capacity and the morphological and cellular events underlying the regeneration process of Nematostella, we performed a large scale and high-resolution temporal RNA-seq time-course for oral regeneration. Latter was compared to available and novel embryonic RNAseq data sets, enabling us to determine genes that are shared between embryogenesis and regeneration but importantly also, to highlight genes whose dynamic expression is specific to the regeneration process. Our global comparison revealed temporal modules corresponding to core biological processes that are highly conserved between both developmental trajectories. Remarkably, this analysis also unveiled several ‘regeneration specific’ modules that govern distinct cellular processes, such as apoptosis. Taken together with the molecular analysis of pathway specific perturbation experiments, we show that regeneration is a partial and rewired re-deployment of the embryonic GRN rather than a complete recapitulation of the embryonic program.
Comparative gene regulatory network (GRN) approaches have been proven to be very useful in studying evolution of specification processes. Using the sea urchin as main model system, we are studying the GRNs that control the formation of feeding related cell types and organs: in particular, the circum-esophageal muscles, the pancreatic cell type and the posterior gut, the latter differentiating into stomach, pyloric sphincter and intestine. The comparison of these different GRNs with their putative homologs in other echinoderm (sea star), vertebrate and also protostome animals highlighted striking commonalities: except for the use of some recurrent sub-circuits (such as the *hnfl-ptflα* sub-circuit controlling exocrine pancreatic-like cell type formation), these developmental GRNs appear to be subject of considerable rewiring even when they share very similar transcription factor toolkits.

We are currently using an approach integrating multiple NGS applications, including Assay for transposase-accessible chromatin sequencing (ATAC-Seq) and single cell RNA-Seq, for the prediction and validation of gene interactions operating in these GRNs. We expect to gain insight in the development and evolution of such a crucial innovation for the evolution of multicellular organisms: the emergence of a specialized system for food digestion and nutrient absorption.
EVOLUTIONARY NOVELTIES IN TUNICATES: PAX37B IS ESSENTIAL FOR THE DEVELOPMENT OF GIANT FOL CELLS IN THE HOUSE SECRETING EPITHELIUM OF OIKOPLEURA DIOICA

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Considering their phylogenetic position among chordates, tunicates may be the outcome of anatomical simplification. While only a few gene regulatory pathways have been uncovered for tunicates, they have been shown to have lost a number of developmental genes, including transcription factors present in the ancestor of chordates. Gene loss appears more pronounced in larvaceans than in ascidians. However, evolutionary novelties have also appeared such as the important house of larvaceans, a complex extracellular structure for filtering seawater and concentrating food particles. It is secreted by multiple territories of a highly modified epithelium of the trunk, whose development is of high interest. Sequencing of the O. dioica genome revealed many duplicated homeobox transcription factor genes compared to other tunicates [1], many of which are expressed in the developing epithelium soon after hatching, indicating importance for its patterning [2,3].

Functional studies using RNA interference showed that two of these genes, propA and propB, are involved in the development of a region situated around the dorsal midline of the epithelium and ultimately in the production of Oikosin41a (a protein of the house) [3]. Here the function of the pax37B homeobox gene, strongly expressed in the putative precursors of the Fol cell territory [3] (producing the food concentrating filter of the house in adults), were examined by introducing a 4 bp deletion using CRISPR-Cas9. When examined during development, it was clear that the mutants lack all giant Fol cells. Thus, they most likely fail to produce a functioning food concentration filter and probably die due to starvation. This work shows an important co-option of multiple ancient transcription factors for the genesis of a novelty that is essential for the planktonic life of tunicate larvaceans.

References


We study the initial step of ascidian neural induction to address how a cell interprets a graded signal to generate a threshold response. During this process, four ectoderm cells among sixteen are selected as neural precursors. Mesendoderm-derived FGF9/16/20 acts as a neural inducer and directly activates $Otx$ expression though the RTK-ERK-Ets pathway (Bertrand et al, 2003, Cell). Each ectoderm cell is in direct contact with FGF-expressing cells, with neural precursors having the largest surface contact (Tassy et al, 2006, Curr. Biol).

Our quantitative analyses have revealed that each ectoderm cell exhibits a level of ERK activation in proportion to its area of surface contact with FGF-expressing cells. In contrast, the transcriptional response of $Otx$ is restricted to only the four neural precursors and operates in a bimodal manner. During neural induction, ephrin signals, acting between the ectoderm cells themselves, antagonise FGF signalling. In embryos inhibited for ephrin/Eph signals, ERK activation levels increase in all ectoderm cells. Under these conditions, the spatial precision of $Otx$ expression is lost with additional ectoderm cells exhibiting the ‘ON’ status of $Otx$ expression. This suggests that ephrin/Eph signals act to reduce the overall levels of ERK activation, such that the non-neural ectoderm cells remain below the threshold required for $Otx$ gene activation. We have confirmed this by re-establishing the normal $Otx$ expression profile in ephrin-blocked embryos with low doses of a MEK inhibitor. Our study has thus uncovered a mechanism whereby signal damping underlies the spatial precision of threshold response to graded signal inputs.
During temperature stress, Shp2, a protein tyrosine phosphatase, is highly upregulated in the ovaries of *C. intestinalis*. Shp2 has been implicated in cell proliferation, migration, stem cell renewal, and differentiation and can also act as an adaptor protein. In order to investigate the role of Shp2 in embryogenesis we used an inhibitor to block the dephosphorylation site of Shp2. This treatment results in embryos that mimic the phenotypic abnormalities seen in embryos grown at high temperatures. It has been shown that Shp2 is an upstream activator of the MapK pathway in zebrafish, dephosphorylating MapK to allow for the recycling of the protein. The goal of this study is to use CRISPR/Cas9 knockouts and transgene overexpression to determine if Shp2 in *C. intestinalis* also functions in the MapK pathway to mediate the animal’s ability to develop normally at high temperatures.
The response of embryonic cells to inductive signals is strongly influenced by endosomal trafficking of receptors and associated proteins. Despite this, the regulation of receptor trafficking in dividing cells remains poorly characterized. We study inductive signal processing in the cardiopharyngeal founder cells of the model chordate *Ciona robusta*. In dividing founder cells, biased mitotic redistribution of Fibroblast Growth Factor Receptors (FGFRs) drives differential induction of cardiopharyngeal progenitor cell (Trunk Ventral Cell, TVC) fate. Here we delineate the role of the mitotic kinases Cyclin Dependent Kinase 1 (CDK1) and Aurora Kinase (AurK) in FGFR receptor trafficking during asymmetric founder cell division. In vivo perturbation of kinase activity revealed that CDK1 promotes mitotic storage of FGFRs by suppressing endosomal degradation of these receptors. CDK1 and AurK further promote receptor storage by additionally suppressing short and long-loop recycling respectively. Through perturbation of a conserved CDK1 phosphorylation site, we also show that CDK1 suppresses short loop recycling through direct phosphorylation of a key regulator of short-loop recycling, RAB4. As cells progress through metaphase, loss of kinase activity permits differential degradation and targeted recycling of stored receptors, leading to asymmetric induction. Mitotic FGFR receptor storage, as delineated in this study, may facilitate rapid reestablishment of signaling competence in nascent daughter cells. However, mutations that limit or enhance the release of mitotically stored signaling components could alter daughter cell fate or behavior, disrupting development or tissue homeostasis.

References


T53
BRAVEHEART, OIKOPLEURA DIOICA A CARDIOGENIC LOSER, BUT NOT A HEARTLESS CHORDATE

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Gene loss has been a significant source of genetic variation during animal evolution, and among chordates, appendicularians such as Oikopleura dioica are probably the most successful losers. As a case study, we investigate the impact of gene loss on the evolution of the mechanisms of heart development in O. dioica. The heart of O. dioica is likely the simplest of all chordates with a bi-dimensional chamber-less structure made of only two layers, the pericardium and myocardium, the latter beating against the stomach wall. Our work provides the first modern developmental atlas of the heart of O. dioica and describes the cell lineage fate map of cardiac progenitors up to tailbud stage. Our data results are consistent with the idea that the cardiac precursors derive from the most anterior muscular cells and migrate from the anterior part of the tail into the trunk, in a similar way as described in ascidians. Despite the cardio-ontogenic similarities between O. dioica and ascidians, our exhaustive in silico survey for cardiogenic factors conserved in other chordates reveals striking differences in O. dioica regarding its early signaling pathways as well as cardiac transcription factors involved in migration and differentiation. Thus, our work unveils that the braveheart story of O. dioica has been shaped by a process of deconstruction of the cardiac genetic toolkit including prominent gene losses, loss of cardiac expression domains, and the abolishment of developmental signaling pathways that are fundamental to make a heart in other chordates.
Ascidian papillae (palps) constitute a transient sensory adhesive organ that assures larval settlement and the onset of metamorphosis. For Ciona, cell numbers and discriminative molecular markers for the different cell types were missing. Most attention was given to neural cell types only. We converge serial-section electron microscopy and confocal imaging with various marker combinations to document the 3D organization of the Ciona papillae. We show the papillar development with 4 axial columnar cells (ACCs), 4 lateral primary sensory neurons (PSNs) and 12 central collocytes (CCs). We propose molecular markers for each cell type including novel ones for collocytes. Interestingly, we detect two different types of collocyte granules, one of them containing fibrous material and larger quantities of polysaccharides. We further propose CCs to derive from an evolutionary ancient neurosecretory cell type. We have further explored the components of adhesive secretions and screened for additional specificities of initial larval adhesion [2]. Our findings contribute to understanding the development of the anterior (‘new head’) region of the Ciona larva and notably the adhesive secreting cells important for developmental biology, evolution and bioadhesion.

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GABA-MEDIATED GNRH RELEASE TRIGGERS METAMORPHOSIS OF CIONA

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The larva of ascidians performs perhaps the most dramatic metamorphosis of any animal, changing from a swimming tadpole to a vase-like, sessile adult. The mechanisms of metamorphosis remain mysterious, despite many attempts to characterize them. Here we report the neuronal signaling essential for metamorphosis of Ciona. Pharmacological analyses and gene knockout/knockdown experiments indicated that GABA is the neurotransmitter required for starting all metamorphic events. GABA uses its metabotropic receptor to relay metamorphic signals to the post-synaptic cells. The neuropeptide Gonadotropin-releasing hormone (GnRH) is the factor inducing tail regression as the downstream factor of GABA. Our epistasis analysis suggests that GABA positively regulates secretion of GnRH for inducing metamorphosis, although GABA is a well-known inhibitory neurotransmitter that generally relaxes postsynaptic neurons. GABA is an important regulator of GnRH neurons in vertebrate hypothalamus for promoting maturation during puberty. In the hypothalamus, both inhibitory and excitatory activities of GABA on GnRH have been reported. Our study suggests an unexpected similarity between metamorphosis of ascidians and reproductive maturation of vertebrates, both of which are the major events pushing animals toward adulthood.
MOLECULAR PROFILE AND FUNCTION OF THE AXIAL COLUMNAR CELLS OF CIONA PAPILLAE

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Recent scRNAseq data from our laboratory (Sharma et al. 2018) revealed the transcriptome profile of the axial columnar cells (ACCs) of the Ciona robusta papilla. Unexpectedly, various genes encoding smooth muscle-like function were identified as enriched in ACCs, including Myosin heavy chain 10/11/14, Myosin light chain kinase, Calponin, IP3 receptor, Atp2a (SERCA), and various calmodulins. Given this profile, we are probing the morphological and physiological functions of the ACCs. A putative contractile function would suggest the ACCs may have a role in retracting the papillae once a suitable location to settle is established. This retraction could also be a signal for metamorphosis. Calcium signaling, important for sensory and contractile processes, was visualized in the ACCs using CryBG>Gcamp6s, which revealed waves of Ca2+ flux prior to settlement and metamorphosis. These waves of Ca2+ could be resulting from a chemo- or mechanosensory mechanism, which could in turn be triggering papilla retraction and metamorphosis. We have begun to use tissue-specific CRISPR/Cas9-mediated mutagenesis to disrupt the function of candidate effector genes in the papillae to see if they play any role in Ca2+ signaling, papilla retraction, and/or metamorphosis.
MIR-4055 REGULATES Ciona Sensory Organ Morphogenesis Through AKT-MAPK Signaling Pathway

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The sensory organs such as ocellus and otolith of ascidian larva localize within a sensory vesicle, which is homologous with the vertebrate’s forebrain. Formation and the regulatory mechanisms for ascidian sensory vesicle are largely unknown. In this study, we aim to screen small miRNA that regulate sensory morphogenesis through the construction of three small RNA libraries in Ciona savignyi. After screening, miR-4055 was identified and validated to highly express in ascidian sensory organ in 28, 32 and 36 hpf’s larvae. Loss of miR-4055 through CRISPR-Cas9 knocking out (KO) induced abnormal morphology of ocellus, and the increased distance between ocellus and otoliths than that in wild type. Behavioral studies conducted on those mutants showed that the photo-response to dim light stimuli was abolished in miR-4055 KO larvae. The results demonstrated that miR-4055 were involved in the regulation of ocellus formation and development. Furthermore, we screened and verified the RAC-alpha serine/threonine-protein kinase (AKT) as one target of miR-4055 through bioinformatics and luciferase reporter gene analysis. Immunostaining results showed that AKT and its downstream gene mitogen-activated protein kinase (MAPK1) localized in photoreceptor cells ( Arrestin-positive cells). The expression level of AKT was significantly higher in miR-4055 KO cells than that in the wild-type ones. MAPK1 was down-regulated in sensory vesicle in those AKT overexpressed larvae. Overexpression of AKT or inhibition of MAPK1 produced the consistent phenotypes in sensory vesicles as observed in miR-4055 KO larvae. These results indicated that miR-4055 regulated the development and function of ocellus through AKT-MAPK signaling pathway.
Calcium ion (Ca$^{2+}$) is one of the major second messengers and involved in many important biological phenomena. We have reported the dynamics of Ca$^{2+}$ in the Ciona embryo from gastrula to tailbud stages (Akahoshi et al., 2017). However, Ca$^{2+}$ dynamics in later developmental stages have never been reported. In the metamorphosis meta-period, the larvae start metamorphosis with settlement by the adhesive papillae (palps), but its mechanisms are still unknown. We don’t know even the detailed role of ascidian palps, which is necessary to induce tail absorption.

To answer these questions, we first developed a novel experimental system to observe individual larva from swimming to metamorphosis stages including the moment of adhesion. This system enables us to artificially induce metamorphosis and we successfully observed Ca$^{2+}$ dynamics of the larva through pre- and post-metamorphosis meta-period with a Ca$^{2+}$ sensor, GCaMP6s. The timing of metamorphosis was precisely determined by a newly observed initiation point of metamorphosis. As a result, it was observed that Ca$^{2+}$ concentration at specific organs transiently increased before metamorphosis. In adhesion period, Ca$^{2+}$ transients in palps were discovered just after their mechanical stimulation. In addition, the Ca$^{2+}$ dynamics in palps consisted of several temporally distinct components. These findings indicated that Ca$^{2+}$ signaling in palps contributes to the beginning of metamorphosis.

This is first report showing direct evidence that mechanical stimulation induces metamorphosis. We also discovered the role of palps as a mechano-sensory organ. Our study will help further understanding of mechanisms of mechanical sensor and metamorphosis.
Canalization of developmental processes ensures the reproducibility and robustness of embryogenesis within each species. In its extreme form, found in ascidians, early embryonic cell lineages are invariant between embryos within and between species, despite rapid genomic divergence. To resolve this paradox, we used live light-sheet imaging and developed automated single-cell segmentation and tracking procedures to quantify individual embryonic cell behaviors. This quantitative approach revealed that individual cell lineages, cell geometries, positions and even contacts are highly reproducible between embryos. This extreme reproducibility may be linked to the control of fate specification by local cell inductions. While in vertebrates the outcome of cell inductions is usually controlled by the concentration of diffusible extracellular ligands, ascidian cell inductions appear to be controlled by the area of contacts between signalling and responding cells, rather than by differential concentrations of ligands. We propose that the duality between genetic and geometric control of inductions contributes to the counter-intuitive inverse correlation between geometric and genetic variability during embryogenesis.
Enhancers are elements within the genome that act as switches to control the location and timing of gene expression. Thus, enhancers are fundamental for successful development. The functional features of an enhancer are “binding sites”, sequences that recruit specific transcription factors to allow activation of gene expression. The order, orientation, and spacing of these sites — collectively known as “enhancer grammar” — is thought to be important for the activity of an enhancer. We bioinformatically identified an enhancer that has conserved its grammar for over 500 million years across all deuterostomes and some protostomes. We tested this enhancer from 4 protostomes, including scorpion, and 6 deuterostomes for activity in Ciona intestinalis embryos. The conserved enhancer grammar encodes a conserved expression pattern in the developing nervous system. Further studies of this rare conservation could uncover important principles governing enhancer grammar and help identify how grammar confers fitness during evolution.
Although ascidians are the sister group of Vertebrates, they have much faster evolving coding genes (Delsuc et al., 2018). We have sequenced, assembled and annotated 4 solitary ascidians genomes (2 Phallusia and 2 Halocynthia) and performed a comparative analysis of our data along with 4 other solitary ascidians (2 Ciona and 2 Molgula) and a colonial ascidian (Botrylloides leachi, Blanchoud et al., 2018).

New genes are considered to significantly contribute to adaptive evolutionary innovation. Our analysis identified approximately twenty percent of tunicate-specific genes with no detectable orthologs outside the tunicate group, of which about a quarter are completely novel genes showing no protein sequence similarity with other bilaterians. We found that the majority of these novel genes are not genus- or family-specific but emerged at the root of the tunicata subphylum. The majority of these genes showed no evidence for horizontal transfer, suggesting they originated de novo. We will report the results of our global analysis of the evolution of the tunicate gene repertoire and compare the regulation and chromosome environment of novel and ancient genes.

References


The gene regulatory network for Ciona notochord fate is thought to resemble an hourglass, with upstream regulators such as FoxA, ZicL and FGF converging to induce Brachyury (Bra) expression in the notochord founder cells, and Bra directly or indirectly inducing the expression of downstream notochord-enriched genes (1-4). It is unclear, however, if the current GRN models include all the key players, and many of the potential network relationships have not been directly tested. To build a comprehensive and quantitative notochord GRN model, we are both transcriptionally profiling embryos in which important notochord transcription factors have been perturbed, and also dissecting selected cis-regulatory regions. We have optimized a protocol for somatic CRISPR gene disruption by egg injection of CRISPR/Cas ribonucleoprotein complexes. We have identified effective guide RNAs for both Bra and FoxA and analyzed multiple replicates with RNAseq on crispant embryos. Major effects are seen on the expression of notochord-enriched genes, but the results are not consistent with a simple model in which Bra is strictly downstream of FoxA and other notochord genes are strictly downstream of Bra. In parallel, we are also systematically mutating putative transcription factor binding sites in the main Bra enhancer and shadow Bra enhancer to test the dependence of these cis-regulatory modules on predicted regulators. Results from our ongoing experiments will be integrated into a model of early notochord gene regulation.

References
In embryogenesis, chromatin accessibility plays a fundamental role in the establishment of cell types controlling gene expression and modulating their transcription. The interaction between genes and the multiple distal and accessible regulatory elements impacts multipotent progenitor states and subsequent fate choices. Subsets of cardiac and pharyngeal/head muscles share a common origin in the cardiopharyngeal mesoderm, but the chromatin landscapes that govern multipotent progenitors’ competence and early fate choices remain largely elusive.

Here, we used the simple chordate model *Ciona robusta* to profile chromatin accessibility through stereotyped transitions from naive Mesp+ mesoderm to distinct fate-restricted heart and pharyngeal muscle precursors. To characterize chromatin dynamics in cardiopharyngeal development, we induced targeted molecular perturbations that altered heart vs. pharyngeal muscle fate specification and then profiled chromatin accessibility on FACS-purified cells using ATAC-seq.

We built an atlas of accessible regions to analyze differential accessibility and integrated single cell and bulk RNA-seq data to compare the chromatin states and gene expression in cardiopharyngeal precursors. We revealed an FGF-Foxf pathway acting in multipotent progenitors to establish cardiopharyngeal-specific chromatin accessibility. This pathway governs later heart vs. pharyngeal muscle-specific expression profiles, demonstrating spatiotemporal decoupling between early enhancer accessibility and late cell-type-specific activity. We further show that combinations of accessible cis-regulatory elements with distinct chromatin accessibility profiles are required to activate key determinants of cardiopharyngeal fate choices, such as *Tbx1/10* and *Ebf/COE*.

We propose that this combinatorial logic increases the repertoire of regulatory inputs that control gene expression, thus fostering spatially and temporally accurate fate choices.
FOXD ACTS AS AN ACTIVATOR AND A REPRESSOR FOR PATTERNING ALONG THE ANIMAL-VEGETAL AXIS IN EARLY EMBRYOS

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In many animal embryos, localized maternal factors induce specific gene expression along the animal-vegetal axis at early developmental stages(1-3). In Ciona embryos, soon after the animal and vegetal hemispheres divide to different blastomeres, two maternal factors Gata.a and β-catenin activate specific genes in the animal and vegetal hemispheres, respectively(4-8). Foxd is one of β-catenin targets and activated in the vegetal hemisphere at the 16-cell stage(9). In the vegetal hemisphere of 32-cell embryos, Foxd represses animal-hemisphere specific genes including Dmrt1 and Dlx.b, and activates vegetal-hemisphere specific genes including Neurogenin and Lhx3/4(10-11). Thus, Foxd transcription factors acts as an activator and repressor simultaneously, and stabilizes the first patterning along the animal-vegetal axis established by β-catenin and Gata.a.

In the present study, to understand how Foxd acts as an activator and repressor, we analyzed the upstream regulatory regions of the target genes. We examined a possibility that cis-regulatory sequences for Foxd binding are different between genes activated and repressed by Foxd. We also examined a possibility that transcription factors acting with Foxd are different between these two groups of genes.

References
FOXG IS REQUIRED FOR THE PALP FORMATION IN ASCIDIAN EMBRYOS

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The ascidian larva has three palps, which are adhesive structures required for attaching to substrates before metamorphosis. Previous studies have shown that the anterior region of the neural plate contributes to the palps under the control of Foxc and Islet[1-4]. In the present study, we found that Foxg was expressed in the presumptive palp and oral siphon primordium region at the early neurula stage. Knockdown of Foxg resulted in loss of expression of Islet and loss of the palp protrusions, but we found no obvious effects in the formation of oral siphon and sensory vesicles. We also found that Foxg expression at the early neurula stage was controlled by Foxc and the MAPK signaling pathway. Ephrinα.d was expressed in the entire presumptive palp and oral siphon primordium region, and negatively regulated the MAPK signaling pathway to restrict Foxg expression domain. Because Foxg is important for specification of cranial placodes in vertebrate embryos[5], our results provide additional evidence suggesting that the ascidian palps and vertebrate cranial placodes share an evolutionary origin[1-4,6,7]. Specifically, Foxg may have played an important role in specification of placode-like structures in the last common ancestor of vertebrates and ascidians.

References

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EVOLUTION OF DEVELOPMENTAL PROGRAMS FOR THE MIDLINE STRUCTURES IN CHORDATES: INSIGHTS FROM GENE REGULATION IN THE FLOOR PLATE AND HYPOCHORD HOMOLOGUES OF CIONA EMBRYOS

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In vertebrate embryos, Sonic hedgehog is produced in midline tissues, including the notochord, prechordal plate, and floor plate, and plays important roles in patterning of the central nervous system and somites. The Ciona genome contains 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 the ventral row of embryonic nerve cord cells, which is the homolog of the vertebrate floor plate, at the tailbud stage (Takatori et al., 2002; Islam et al., 2010). Neither hedgehog.a nor hedgehog.b is expressed in the notochord. We have identified a cis-regulatory region that were sufficient to drive a reporter gene expression in the ventral nerve cord (floor plate). Interestingly, the hedgehog.b cis-regulatory region 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. The endodermal strand occupies the same topographic position of the embryo as does the vertebrate hypochord. In vertebrate embryos, the hypochord shares expression of several genes with the floor plate, including sonic hedgehog (Yan et al., 1995; Peyrot et al., 2011). A number of genes specifically expressed in both the floor plate and the endodermal strand in Ciona tailbud embryos were predicted by whole-embryo single-cell transcriptomics and confirmed by whole-mount in situ hybridization. These genes and their transcriptional regulation suggest an ancient evolutionary origin of a common developmental program for the midline structures in Olfactores.

References

In vertebrate embryos, both head muscles and the chambered heart arise from a common pool of progenitor cells in the cardiopharyngeal field. Similarly, in the simple chordate model *Ciona robusta*, multipotent cardiopharyngeal progenitors go through subsequent asymmetric divisions giving rise to early Atrial Siphon Muscle (ASM) and heart precursors. These multipotent progenitors display multi-lineage transcriptional priming: they express a combination of early ASM- and heart-specific regulators that become restricted to their corresponding precursors following oriented asymmetric divisions. This suggests that heart progenitors need to clear gene products that pertain to the ASM program rapidly after the asymmetric divisions, and vice versa. Consistent with the hypothesis that post-transcriptional regulation controls fate specification, single-cell RNA-seq identified ubiquitination-related and RNA-binding proteins among ASM and heart specific markers. We sought to observe post-transcriptional activity in these progenitors by using proteomic approaches, however benchmarked proteomic using tandem mass spectrometry workflows require >100 times the number of cells we can obtain from one experiment. Therefore I have developed a low-input workflow that allows robust protein identifications using only 1,000 cells with high reproducibility. Using this new approach, we are investigating the post-transcriptional regulation of cell specification in heart and ASM by profiling their proteomes. In addition, we performed a small-scale CRISPR screen on post-transcriptional regulation candidates, assaying cardiopharyngeal fate specification phenotypes. Knock-out of the candidate *Rnf149* resulted in early ASM specification and cell behavior defects. I will present progress in understanding post-transcriptional regulation of heart specification and Rnf149’s role in regulating pharyngeal muscle specification.
POSTERS
ABSTRACTS
P1-P65
Tunicata is a group of marine invertebrate chordates with both solitary and colonial species. Transcriptomic phylogenies and embryological evidence suggests that colonial species that reproduce asexually in the Styelidae evolved from a solitary ancestor that reproduced only sexually. Progenitor Germ Stem Cells (PGSC) are located in the gonads and cells outside the gonads of extant colonial styelids. Vasa, Piwi, PL10 and CiYB1 are protein markers that have been previously been used in the identification of PGSCs of ascidians. Our objective is to compare the occurrence of PGCs in colonial and solitary styelids to understand how the evolution of coloniality occurred. We cut specimens of *Styela plicata* and *Symplegma rubra* (solitary and colonial Styelidae ascidians, respectively) in order to characterize histological sections and carry out both immunocytochemistry and western blot using the germ line markers mentioned above. According to expectations, was observed expression of Vasa and Piwi in ovocytes and strong expression of PL10 and CiBY1 in follicular cells; however, we documented several cells outside the gonads of colonial and solitary ascidians that expressed these proteins, including at least one type of blood cell and expression in epithelia surrounding primordial gonads and mantle. If Piwi+, Vasa+, PL10+ and CiBY1 cells are confirmed outside the gonads of solitary ascidians, we can hypothesize that these genes may have previously unknown somatic roles in solitary ascidians, or that PGSCs are present in the blood and other tissues of solitary ascidians for yet unknown reasons.
The spatio-temporal variation patterns of species diversity reflect how environmental, biological, anthropic and stochastic processes influence ecosystems (Legendre et al., 2005). It has been described that the processes that contribute most to the variation in the distribution patterns of marine benthic species operate at smaller scales of a few meters and hundreds of kilometers (Fraschetti et al., 2005, Guerra-Castro et al., 2016) and that the availability of habitat and temporal variations in recruits allow explaining different types of succession in substrates separated by just a few meters, and such interaction of small spatio-temporal processes is independent of regional processes (Guerra-Castro et al., 2018). In this study, models that could explain spatial variation at a few meters scale are evaluated, considering the effect of the regions on species of ascidians in the south GMx of Mexico and the Mexican Caribbean Sea on autonomous structures of reef monitoring (ARM) through sampling trimesters over 18 months. Preliminary results after 3 months of settlement, indicate the presence of at least 15 species of ascidians, corresponding to six families and 15 genera, where species of the family Styelidae and Dimenidae had a greater domain in terms of coverage per plate and presenting differences between the orentation of each of the plates.

References


Currently, ascidians are recognized as the main hosts of many marine organisms, either as a reproduction or food refuge. A total of 169 solitary ascidians were collected corresponding to four families in seven genera and 15 species where only shrimp corresponding to the species of *Ascidonia miserabilis* (Holthuis, 1951) were found in cryptic ascidians of the genus Ascidia in the reef systems of Alacranes, Cayo Arenas and Cayo Arcas in the southeast of the Gulf of Mexico between 2015-2018. A total of twenty-one shrimps living alone or in pairs specifically in the pharynx of the ascidians; It is suggested that the preference for the genus is due to the habitat type of the cryptic ascidians (hidden from predators), to which other ascidians already had symbiosis with other organisms (availability of the host) and for the morphology of the tunic and pharynx proper to the order. On the other hand, the incidence in the male / female relationship within the same ascidia is assumed to be directly related to reproductive purposes.
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:

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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/gbrowse/kh/
INVESTIGATING THE ROLE OF FLRT IN NEURAL TUBE CLOSURE

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The chordate central nervous system starts out as a flat neural plate. Through a morphogenetic process known as neural tube closure (NTC) this flat plate will transform into a hollow neural tube which gives rise to the brain and spinal cord. Defects in NTC are some of the most prevalent human birth defects and affect as many as 1 out of every 1000 births. We have discovered a novel role for a T-Type Calcium Channel (CAV3) in this process that is conserved among chordates. In the basal chordate *Ciona*, loss of CAV3 leads to defective NTC characterized by an open brain. RNA-Seq analysis of a CAV3 mutant, *bug-eye* (*bug*), has revealed misregulation in a number of transcripts including ones that are involved in cell-cell recognition and adhesion. Of particular interest is the aberrant upregulation of the transcript for FLRT. In wildtype *Ciona*, FLRT is expressed during NTC and then declines precipitously. However, in *bug* embryos FLRT does not appear to be properly downregulated following this process. FLRT is best known for its role in neurite outgrowth and can act to encourage either adhesion or repulsion depending on its interaction partners. One of those partners is Latrophilin. We show that Latrophilin has a dynamic expression pattern around the closing neural folds and opposes the FLRT expression domain. Taken together, we speculate that FLRT plays a transient role in tissue separation and adhesion during neural tube closure and that failure to downregulate FLRT leads to an open neural tube.
PARALLEL FOLD-CHANGE DETECTION VISUAL CIRCUITS IN CIONA LARVA

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The Ciona tadpole larval central nervous system (CNS) has a common structure with the CNS of its close chordate relatives, the vertebrates. An advantage to using Ciona as a model organism is that it has one of the simplest CNS and has a complete connectome available. Beginning with visual circuits predicted from its connectome in combination with past studies, we used behavioral assays, pharmacology, and mutant lines to further assess their visual circuits. Ciona have two groups of photoreceptors: group 1 responsible for detecting directional light and group 2 responsible for detecting ambient light. We find that both circuits appear to form overlapping but different fold-change detection networks: group 1 showing an incoherent type-1 feedforward loop (I1-FFL) and group 2 showing a nonlinear integral feedback loop (NLIFL). Group 1, composed of glutamatergic photoreceptors, projects to two groups of relay neurons: cholinergic relay neurons to signal the motor ganglion for navigational swimming and GABAergic relay neurons that also projects to the cholinergic neurons to modulate the signal. Group 2, composed of GABAergic photoreceptors, appears to project to GABAergic relay neurons to signal, through a likely disinhibitory pathway, shadow response swimming. This is then modulated by the same cholinergic relay neurons as group 1. Tadpoles exhibit a shadow response and adaptation time that is dependent on the fold-change of light as opposed to absolute change. The fold-change detection processing is shown to be in the relay neurons themselves as pharmacologically increasing or decreasing their inputs lead to an altered or extinguished response.

References
The complement system is one of the most ancient immune modulator mechanism of bilateral metazoans. In vertebrates, three complement-activation pathways are known: the classical, the alternative and the lectin pathways: all of them converge on the cleavage of C3.

The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. As an invertebrate, *B. schlosseri* relies only on innate immunity for its defense and immunocytes. We already demonstrated the presence, in *Botryllus*, of homologues of mammalian C3, Bf, MBL and MASP1, referred to as BsC3, BsBf, BsMBL and BsMASP, respectively. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes.

In mammals, once the complement system is activated, a cascade of reactions that involves proteolysis and polymerization occurs resulting in the cleavage of the third complement component (C3) to C3a and C3b, the former exerting a chemotactic activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the microbial surfaces.

Here, we describe, in *B. schlosseri*, two new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. In addition, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of *bsc3* knockdown on immune responses.

Results indicate that only morula cells, and no other immunocytes type, are labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells, known as hemoblasts, are the cells stained by the probe for BsCR1. This suggests the presence of an important cross-talk between these two immunocytes types.

Both the *bsc3ar* and *bscr1* genes are constitutively transcribed as almost all morula cells and phagocytes, respectively, resulted labelled by the antisense probe in the ISH assay, independently of their previous challenge with zymosan, a known activator of *B. schlosseri* hemocytes. However, a modulation in the extent of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at TO, whereas no differences were observed when EC and MC were compared. This is probably related to the renewing of circulating cells at TO, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.
In the early ascidian embryo, a series of unequal cleavages shape the posterior cleavage pattern and segregate the germ line. These asymmetric cell divisions are regulated by a defined cortical structure termed the centrosome attracting body (CAB). The CAB is formed in the zygote, when sperm aster microtubules concentrate a domain rich in endoplasmic reticulum and mRNA determinants. We have found that the microtubule de-polymerase Kinesin 13 (Kif2/MCAK) is localized to the cortical endoplasmic reticulum domain and released during each mitosis, causing the proximal aster to shrink in size and the spindle to shift off-center (Costache et al 2017). At the 16 cell stage, one centrosome migrates toward the midline cortex adjacent to the CAB, suggesting that a cortical pulling force may be localized to this posterior midline site. In order to visualize force generators, we used cytochalasin to weaken the actin cortex and observed strong membrane invaginations at the cortical site toward which the centrosome migrates. We analyzed the localization of the dynein partners NuMa and LGN and found both proteins to be enriched at the midline site during anaphase. Inhibition of LGN leads to defective spindle positioning; the CAB-containing cells continue to divide but in the wrong direction and such embryos fail to form swimming tadpole larvae. We propose a mechanism whereby the spindle is both pushed and pulled during sequential phases of the cell cycle to generate unequal cleavage in the ascidian embryo.
We have found that T-type Calcium channels (Cav3) play a crucial role in neural tube closure (NTC) in chordates. This requirement is seen in the Ciona mutant line bugeye which carries a mutation in Cav3 and which has incomplete anterior NTC. We hypothesize that Cav3 serves a regulatory role in NTC, monitoring cell fusion and controlling a suite of genes necessary for NTC. We have performed RNAseq to identify genes differentially expressed (DE) in Cav3 mutants. We have found upregulated genes belonging to three signaling pathways; neurite outgrowth (e.g., Flrt), wound healing (e.g., Selectin) and neural crest development (e.g., SoxE). NTC involves a sequence of tissue separation and adhesion events as neural plate cells must first detach from epidermis, and then adhere to each other. Flrt, Selectin and neural crest cells all have adherent and repulsive properties and we are interested in how these common attributes are contributing to NTC. We have found that these genes (as well as others identified as DE in the RNAseq analysis) are expressed in the right tissues (neural) and the right time (during neural stages) to facilitate NTC. Crispr/Cas9 knock out of Flrt or its binding partner Latrophilin (but not another interacting protein, Unc5) results in a failure of NTC, as does CRISPR of Selectin. We are looking at other members of these pathways (such as Graineyhead-like which has a role in wound healing), DE transcription factors, SoxE and EGR-1, and the role of a long non-coding RNA.
P10
ROLE OF MIDBODY REMNANT IN MEIOSIS II CREATING TETHERED POLAR BODIES

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Polar body (PB) formation is an extreme form of unequal cell division that occurs in oocytes due to the eccentric position of the small meiotic spindle near the oocyte cortex. Prior to PB formation, a chromatin-centered process causes the cortex overlying the meiotic chromosomes to become polarized. This polarized cortical subdomain marks the site where a cortical protrusion or outpocket forms at the oocyte surface creating the future PBs. We observed that PB1 becomes tethered to the egg via PB2, indicating that the site of PB1 cytokinesis directed the precise site for PB2 emission. We therefore studied whether the midbody remnant left behind following PB1 emission was involved, together with the egg chromatin, in defining the precise cortical site for PB2 emission. During outpocketing of PB2 in ascidians, we discovered that a small corps around 1µm in diameter protruded from the center of the cortical outpocket that will form the future PB2, which we call the “polar corps”. During emission of PB2, this small polar corps became localized between PB2 and PB1 and appeared to link PB2 to PB1. We tested the hypothesis that this small polar corps on the surface of the forming PB2 was the midbody remnant from the previous round of PB1 cytokinesis. We had previously discovered that Plk1::Ven labeled midbody remnants in ascidian embryos. We therefore used Plk1::Ven to follow the dynamics of the PB1 midbody remnant during meiosis II. Plk1::Ven strongly labeled the small polar corps that formed on the surface of the cortical outpocket that created PB2. Following emission of PB2, this polar corps was rich in Plk1::Ven and linked PB2 to PB1. By labelling actin (with LifeAct::mCherry/GFP or TRITC-Phalloidin) we also demonstrated that actin accumulates at the midbody remnant and also forms a cortical cap around the midbody remnant in meiosis II that prefigured the precise site of cortical outpocketing during PB2 emission. Phalloidin staining of actin and immunolabelling of anti-phospho aPKC during meiosis II in eggs that had PB1 removed showed that the midbody remnant remained within the egg following emission of PB1. Dynamic imaging of microtubules labelled with Ens::3GFP, MAP7::GFP or EB3::3GFP showed that one pole of the second meiotic spindle was located near the midbody remnant while the other pole rotated away from the cortex during outpocketing. Finally, we report that failure of the second meiotic spindle to rotate can lead to the formation of two cortical outpockets at anaphase II, one above each set of chromatids. It is not known whether the midbody remnant of PB1 is involved in directing the precise location of PB2 in other species as in ascidians.
A CHORDATE SPECIES LACKING NODAL UTILIZES CALCIUM OSCILLATION AND BMP4 FOR LEFT-RIGHT PATTERNING

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Larvacean is a chordate that has tadpole morphology. By contrast to most chordates of which early embryonic morphology is bilaterally symmetric, invariant left-right (L-R) asymmetry becomes morphologically visible in the arrangement of blastomeres as early as four-cell stage. Eventually, the tail rotates in 90˚ counter-clockwise direction relative to the trunk, and thus the neural tube locates on the left side in the tail. Here we unraveled three types of nonconventional L-R asymmetries in regard to L- and R-blastomeres of the two-cell embryo. First, tracing of descendant cells of the L- and R-blastomeres demonstrated that their distribution was largely asymmetrical in the functional body. For instance, bilateral giant cell layers in the endostyle are exclusively derived from the L-blastomere. Second, repetitive Ca\textsuperscript{2+} waves were observed in asymmetric direction along the L-R axis through embryogenesis. Third, Nodal, an evolutionary conserved gene that shows left-sided expression in all chordates studied to date, was absent in the genome. Survey of TGF\textbeta superfamily genes uncovered that Bmp4 shows right-sided expression in tailbud larva. The right-sided Bmp4 expression was occurred in descendants of the R-blastomere. The right-side Bmp4 expression was abrogated by disruption of the Ca\textsuperscript{2+} oscillation using pharmacological inhibitors of inositol triphosphate and ryanodine receptors. Blockage of BMP signaling by Dorsomorphin induced ectopic expression of a brain marker gene, supporting BMP’s suppressive function in nervous system differentiation on the right side.

These results raised an atypical L-R patterning mechanism that Ca\textsuperscript{2+} oscillation generates embryonic L-R asymmetry. The right-side Bmp4 expression may have arisen via co-option of conventional role of BMP signaling in order to restrict neural tube formation to the left side.
CELLS AND TISSUES OF DORMANCY IN ASCIDIANS: UNDERLYING DEVELOPMENTAL MECHANISMS AND EVOLUTIONARY ORIGINS

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Many colonial species survive adverse conditions by going dormant, where modules or whole colonies enter a state of physiological and morphological inactivity. Coloniality arose independently many times in the metazoans, and dormancy has evolved in all classes of colonial animals. Yet the link between coloniality and dormancy remains unexplored. Colonial species rely on populations of circulatory stem cells that drive asexual development. It is possible that the origins of dormancy in colonial species involved a cytological recruitment of these stem cells (or visa versa). However, the cellular mechanisms underlying dormancy in colonial species are poorly known. Ascidians provide a fitting model for understanding the origins/mechanisms of dormancy because both coloniality and dormancy evolved multiple times independently. I am examining the environmental, morphological, and molecular characteristics of dormancy in two ascidian species: the stolidobranch Polyandrocarpa zorritensis and the aplousobranch Clavelina lepadiformis. These species have evolved budding and dormancy modes independently. To understand the dynamics of budding and dormancy, I determined which temperatures cause induction and release of dormancy in the lab and the proportions of colonies in dormant states in the field over the year. I have examined dormant tissues and cells using histology and microscopy. Finally, I am comparing transcriptome profiles of different stages of dormant tissues to determine which genes are expressed in the induction and release of dormancy. This project will provide insight into the mechanisms underlying dormancy in tunicates, and may provide clues to the links between dormancy and coloniality.
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NEUROTRANSMITTER USE AT CELL RESOLUTION IN THE CIONA CNS: INTEGRATING IN SITU LABELING WITH THE CONNECTOME FOR BETTER MODELS OF LARVAL BEHAVIOR

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A complete larval connectome for Ciona intestinalis has opened new areas of inquiry linking neural anatomy to behavior. We are building a cellular-level atlas of neurotransmitter and neurotransmitter receptor use to complement the resolution of the connectome. The coordination of modalities, light-microscopic for neurotransmitter use with e.m.-derived for the connectome, should in turn refine our efforts to describe the circuit logic which underlies larval behaviors. The hybridization chain reaction (HCR), a fluorescent in situ protocol, reveals defined boundaries of expression for markers of neurotransmitters and their receptors. In the photoreceptor complex, for example, we characterize glutamate and GABA use within Group I and Group II photoreceptors using HCR probes for vglut and vgat, respectively. An isolated pair of cells within Group I, otherwise surrounded by a field of vglut expression, labels with vgat. A single cell in this pair also co-expresses vglut. We can predict with high confidence the cell identities of this pair, and thus infer their connectivity, by applying a registration algorithm, aligning centroid positions between multiple HCR-labeled larvae - each with the vglut/vgat pair - to centroid positions of photoreceptor neurons from the larva which provided the connectome. This registration technique is generalizable, but HCR labeling of many larvae reveals that natural variation makes assignment of identities difficult for some cell populations. So, while HCR data help to flesh out the connectome, providing a better picture of neurotransmitter use, these data additionally highlight which aspects of Ciona neuroanatomy are stereotyped and which are variable through sampling of multiple individuals.
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MORE THAN ONE-TO-FOUR VIA 2R: EVIDENCE OF AN INDEPENDENT AMPHIOXUS EXPANSION AND TWO-GENE ANCESTRAL STATE IN VERTEBRATES FOR CHORDATE MYOD-RELATED MRFs

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MyoD (Myogenic differentiation) has long been recognized as a master developmental control gene and a key element of the bilaterian developmental toolkit. The MyoD family of bHLH transcription factors (Myogenic regulatory factors, MRFs) drives myogenesis across the bilaterians, though these comparisons are complicated by multiple instances of gene duplication and loss in several lineages. Following duplications, for instance the two rounds of whole genome duplication (2R WGD) at the origin of the vertebrates, duplicate regulatory genes like the MRFs often subfunctionalise, whereby the function of the ancestral gene is partitioned amongst the daughter genes, a process which is frequently focused on the complex regulatory regions characteristic of developmental transcription factors. Subfunctionalisation has been well-documented for MRFs in the vertebrates, where MyoD and Myf5 act early in myogenic determination while Myog and Myf6 are expressed later, in differentiating myoblasts. Comparing chordate MRFs, we find an independent expansion of MRFs in the invertebrate chordate amphioxus, with evidence for a parallel instance of subfunctionalisation relative to that of vertebrates. Conserved synteny amongst chordate MRF loci supports the 2R WGD events as a major force in shaping the evolution of vertebrate MRFs. We also resolve vertebrate MRF complements and organization and infer an ancestral two-gene state in the vertebrates which corresponds to the creation of early- and late-acting types of MRFs. This necessitates a revision of previous conclusions about the simple one-to-four origin of vertebrate MRFs.
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A REDESCRIPTION OF SYNCARPA COMPOSITA (ASCIIDIACEA, STOLIDOBRANCHIA) WITH AN INFERENCE OF ITS PHYLOGENETIC POSITION WITHIN STYELIDAE

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Two species of styelid colonial ascidians in the genus *Syncarpa* Redikorzev, 1913 are known from the Northwest Pacific. The species status of one species, *Syncarpa composita* (Tokioka, 1951) (type locality: Akkeshi, Japan), has been doubted in relation to another, *S. oviformis* Redikorzev, 1913 (type locality: Ul’banskij Bay, Russia). To elucidate the taxonomic identity of *S. composita*, we examined the morphology of one of the syntypes and freshly collected topotypes of *S. composita*, in comparison to a syntype of *S. oviformis*. In this paper, we redescribe *S. composita* and infer its phylogenetic position within Styelidae based on the 18S rRNA and cytochrome *c* oxidase subunit I gene sequences. The morphological differences between *S. composita* and *S. oviformis* may be due to intraspecific variation. *Syncarpa composita* is possibly a junior synonym of *S. oviformis*. In our phylogenetic tree, *Syncarpa* formed a well-supported clade together with *Dendrodoa*. In these two genera, one gonad is present on the right side of the body wall, a character state unique among other styelids and thus can be regarded as a synapomorphy for this clade.
ANOCTAMIN IS ESSENTIAL FOR NOTOCHORD DEVELOPMENT IN CIONA INTESTINALIS

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Anoctamin/TMEM16 is a recently identified group of proteins, which are highly conserved across the metazoans. They have been shown to function either as Ca\(^{2+}\)-activated Cl\(^{-}\) channels (CaCCs) or phospholipid scramblases[1-5]. Few reports have explored the function of Anoctamins in animal development[6]. We examined the function of Ci-Ano10 during early development in Ciona intestinalis. WMISH of Ci-Ano10 showed that during early phases of development Ci-Ano10 gene is broadly expressed, while during the tailbud stages its expression is restricted to the notochord, a hallmark structure of the chordate body plan. Subcellular localization studies of Ci-Ano10 translational fusions show that the protein is localized mostly in the ER and possibly to the plasma membrane of notochord cells. Combining transient transgenesis with genetic perturbations and confocal imaging, we demonstrate that the Ci-Ano10 is required for the proper migration and intercalation of notochord cells. We are currently characterizing the biophysical properties of Ci-Ano10 using heterologous expression systems combined with functional imaging and electrophysiology. Our study thus reveals the Anoctamin is essential for embryo notochord development in Ciona intestinalis.

3. TMEM16A, A Membrane Protein Associated with Calcium-Dependent Chloride Channel Activity. scinece, 2008
In multicellular organisms, a simple fertilized egg develops along divergent embryonic lineages to produce distinct cell types. What governs these processes is central to the understanding of cell fate specification and stem cell engineering. Recent advances of single-cell technologies enabled systematic characterization of cell types as well as the molecular programs that define them. The fixed embryonic lineage and low cell number of *Ciona* early embryo presents a perfect opportunity to test the utility of single cell transcriptomics in identifying embryonic developmental program. In this study, we generated single cell transcriptomes of essentially every cell from *Ciona savignyi* embryos staged from the zygote to the 110-cell stage when major tissue types appear using the Smart2-seq approach. In total, we obtained 745 single cell expression profiles with two to eight embryos for each stage. We conducted de novo reconstruction of the trajectories based on the gene expression profiles and successfully recovered 78% of the progenitor-progeny relationships across stages. 293 genes were identified as differentially expressed genes (DEGs) and the success rate was about 70%. Based on the success rate and the number of detected DEGs, we projected that there are about 400 DEGs for early lineage differentiation in *Ciona*. To explore the requirement of FGF signaling for embryonic cell types, we sequenced single cells from U0106 treated embryo and when analyzed together with wt cells, we readily detected all the known FGF-dependent cell type transformations. In addition, we also identified several novel fate transformation events, such as the TVC to muscle conversion. Finally, we compared the single cell expression profiles of *Ciona* early embryo to those of the mouse. Between these two species, we showed a substantial drift of gene expression over chordate evolution, with similarity between homologous cell types largely limited to key regulators of cell fates. We also provided further evidence that rudimentary programs for neural crest cells, an innovation in vertebrates, exist in the form of overlapping transcriptional programs between neural and mesodermal fates in *Ciona*. This study demonstrates a general approach to study embryonic development and gene network regulation across a broad spectrum of non-traditional model organisms at an unprecedented single cell resolution.
Bisphenol A (BPA) is an organic compound used in the manufacturing of polycarbonate plastic and epoxy resins, employed for food and beverage containers, dental materials and water supply pipes. After degradation of these products, BPA is released into the environment, where it can act both as an endocrine disruptor and as a teratogenic molecule.

Alteration of otolith and eye dysplasia induced by exposure to BPA were reported in zebrafish and amphibian respectively. In zebrafish, the malformations were due to BPA interaction with Estrogen-Related Receptor (ERR).

We analyzed the effects of BPA exposure on the development of pigmented organs in Ciona robusta and Phallusia mammilata. A co-exposure with an ERR antagonist (4-OHT) was performed to elucidate the role of these receptors in the teratogenic mechanisms.

In both species, exposure to low BPA concentrations caused altered pigment deposition. Absence of one or both pigmented organs or extranumerary pigmented organs were observed after exposure to high BPA concentrations, either two otoliths or two ocelli can differentiate in treated larvae.

Moreover, BPA alters the expression of some transcription factors localized in the sensory vesicle. These genes are under the regulation of FGF and Wnt signaling, suggesting that BPA acts at some point of the cascade activated by these pathways.

The co-exposure to BPA and 4-OHT resulted in a partial rescue of the normal phenotype, suggesting that BPA could act through binding to ERR also in ascidians.

These results highlight that ascidians are valuable invertebrate models for testing pollutants and investigating their mode of action.
Tissue culture is an important tool in biochemistry, cell biology, and physiology research. There are currently few cell culture methods in marine invertebrates for in vitro study. We describe generation of 3D primary tissue culture organoids from dissociated ovarian cells of *Ciona intestinalis*. We used the “3D Petri Dish” system (Microtissues, Inc.) which is a device to mold microwells in agarose in which dissociated cells are seeded. Comparison of cell proliferation of the 3D culture and traditional cell culture on culture treated plastic showed that the 3D culture system results in 185% more cell proliferation over a 22-day period. Culture medium was optimized using cell counts over time for different combinations of salinity, antibiotics, and growth additives such as fetal bovine serum and *Ciona* hemolymph in L-15 medium. Of note, we found that *Ciona* hemolymph was the best growth additive for optimal cell proliferation, and protects the cell culture from protist contamination. Histology revealed that the organoids develop morphology similar to that of ovaries. These findings suggest that 3D culture is a viable technique for tissue and organoid culture in *C. intestinalis*, opening up new avenues for in vitro research.
Embryonic shell development of *Mytilus* spp. has long been used as a model to test the effect of stressors and toxicants because of the similar and consistent shell phenotypes readily identifiable in the D-Veligers. However, the absence of earlier time-points of analysis or comparison between different stressors prevent the discrimination of specific phenotypes or the identification of common targets. The present study investigates the effect on shell development of *Mytilus galloprovincialis* of two environmental stressors, Bisphenol-A and pH, known to induce shell malformations. We focused on the time course of shell biogenesis during trochophore stages in physiological conditions and after exposure to both stressors using the double staining Calcofluor and Calcein to identify and compare the first occurrence of shell phenotypes. As molecular target, we selected an embryonic Tyrosinase gene (Mg-TYR), a phenol oxidase enzyme, key effector gene for the secretion of embryonic organic matrix whose expression level in adults of *Mytilus edulis* is significantly altered by exposure to low pH. We first inhibited TYR enzymatic activity with N-Phenylthiourea (PTU) and found that it altered matrix shape and calcification patterns thus confirming its pivotal role in shell formation. The effect of exposure to BPA or to acidified SW (pH=7.4) on shell morphogenesis and on the spatio-temporal expression of Mg-TYR was then studied. In trochophores, both stressors induced hinge indentations, delay in matrix expansion and shell deposition; BPA also caused asymmetric expansion of the organic matrix. In D-Veligers, the stressors induced similar shell malformations, although, their intensity and frequency were clearly different. Mg-TYR expression levels were higher in trochophore than in D-Veligers and the pattern of expression blueprinted the expansion of the organic shell matrix. Exposure to BPA altered Mg-TYR transcripts level and pattern of expression as the signal of Mg-TYR matched shell malformations. We are now investigating the expression of TYR in low pH exposed embryos. This work shows that both stressors induced similar but not equal shell malformations from the trochophore stage and that their occurrence might be caused by a modulation of Tyrosinase expression and activity.
Ascidian larva shows swimming behavior until metamorphosis. The motor neurons located in the motor ganglion control rhythmic tail muscle contractions, and these are only five pairs of cholinergic motor neurons (Horie et al., 2009). These motor neurons and their projection to tail muscles have been investigated by immunohistochemistry or electron microscopy in larva. However, it is still unknown how these neurons regulate the tail beating during development. In the previous study, we identified that the Ca\textsuperscript{2+} oscillation was observed in motor ganglion at the mid-tailbud stage, with a duration of 22 ± 4 s (Akahoshi et al., 2017). In this study, we investigated the relationship between the Ca\textsuperscript{2+} oscillation and tail beating until larval stage. To identify the cell lineage and the number of cells showing Ca\textsuperscript{2+} oscillation, H2B-GCaMP6s mRNA was injected into eggs prior to fertilization. We found the Ca\textsuperscript{2+} oscillation is derived from a single pair of posterior motor neurons, A10.64. In the mid-tailbud (stage 23), the Ca\textsuperscript{2+} oscillations showed no synchronization in a pair of motor neurons and no correlation with the excitation of tail muscles. In the late tailbud (stage 24), the Ca\textsuperscript{2+} oscillations of single neuron were correlated with the excitation of ipsilateral tail muscle. After stage 24, the timing of the Ca\textsuperscript{2+} oscillation synchronized with each other motor neuron. Furthermore, the interval of Ca\textsuperscript{2+} oscillation decreased gradually from approximately 80 seconds (stage 23) to approximately 15 seconds, approached to the interval of tail beating of swimming-larva (stage 27). These results allowed us to understand how motor neurons wire the network for tail beating.

References


In the colonial ascidian *Botryllus schlosseri* three generations of zooids coexist (adults, buds, and budlets). These generations develop synchronously in relation to each other in a blastogenetic cycle that lasts one week at 18°C. A radial vessel forms in each individual when passing from budlet to bud life phase, and serves for the connection to the common colonial vasculature that synchronize colony development. Budlets, meantime, appear and develop from the body wall in a process called palleal budding mediated by stem cells. A second type of budding, called vascular budding, can be induced when all the individuals are removed from a colony, and circulating stem cells aggregate and organize a new budlet, able to restore the colony. Thanks to the relatively fast blastogenetic cycle and its high regenerative capacities, this species has been recognized and adopted since 1950 as model for the study of a variety of biological processes. Here we present the experiments based on the removal of buds and adult zooids from colonies, which have been performed in the last decades to assess the cross talk between different blastogenetic generations. In particular, we show in detail the surgical manipulations to induce a different array of responses by removing one or more generations of zooids. We highlight colony responses in terms of variation in blastogenetic potential, bilateral asymmetry, and growth of the remaining zooids. We discuss the results in terms of homeostatic capabilities, regenerative potential, methods used, and future perspective offered by this tunicate model.
Neurons are the primary signaling units of the nervous system. However, in order for them to function correctly, they rely on the surrounding non-neuronal cells. These cells play important roles during neurogenesis, synapse formation and pruning in early development. Moreover, they supply essential nutrients to neurons and participate in neurotransmitter reuptake. Furthermore, it is now clear that non-neuronal cells can also actively participate in neuronal signaling. Given their importance in the development and function of the nervous system, these cells represent a very interesting cell type class to study. Non-neuronal cells have been described in several taxa among bilateria, but the presence of these cells in many groups, including invertebrate chordates remains an open question (Hartline, 2011). Tunicates possess ependymal cells, which line the cavity of the sensory vesicle (Nishitsuji et al., 2012), while morphological studies in the cephalochordate amphioxus demonstrated the presence of supportive cells in the anterior neural tissue of the larvae (Lacalli and Kelly 2002). We are performing a more in depth characterization of these cell types using the tunicates Ciona intestinalis, Oikopleura dioica and the cephalochordate Branchiostoma lanceolatum. We will present the results of a candidate gene screen based on vertebrate markers across these three organisms. In addition, through functional imaging and behavioural analysis, we will provide further insight on their contribution to nervous system development and function. Our results highlight the importance of studying non-neuronal cells to elucidate their role in the nervous system evolution.

References

DNA BARCOSING STRATEGIES FOR FUNCTIONAL GENOMICS BY MULTIPLEXED SINGLE CELL RNA-SEQ ANALYSES

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In the past few years, single cell genomic technologies have revolutionized many fields in the life sciences, including developmental genetics and systems biology. Providing single cell suspensions can be obtained, most biological systems are amenable to high-throughput single cell genomics, especially RNA-seq. Single cell RNA-seq (scRNA-seq) has provided unprecedented insights into the cellular diversity of complex tissues and organs, as well as a high-resolution view of the dynamics of developing systems, owing to such emerging concepts as pseudotime\(^1\). Concomitant to the rapid development of technologies, there has been an explosion of systematic efforts to build comprehensive atlases for various species, stages and tissues of interest\(^2\). Work in tunicates has not escaped the single cell frenzy, with reports of single cell transcriptomes in the cardiopharyngeal lineage\(^3\), followed by nervous system\(^4\) and whole embryo\(^5\) profiling in Ciona. Indeed, ascidians, with their fixed lineages, limited cell numbers and easy to dissociate embryos are particularly well suited for single cell analyses.

Comparatively limited efforts have attempted to go beyond systematic descriptions of control animals and the building of catalogs. To harness the power of single cell methods for functional genomics and combine systematic CRISPR/Cas9-mediated mutagenesis with scRNA-seq, we are developing DNA barcoding strategies to mix samples from different sources in multiplexed assays, followed by in silico deconvolution using both user-defined sample barcodes and random cell barcodes. We will present progress in harnessing novel experimental and computational approaches for functional genomics and analysis of systems dynamics, with a special focus on the cardiopharyngeal mesoderm and surrounding tissues.

References

Spatio-temporal expression of zygotic genes is regulated by transcription factors, which promotes cell fate decision and morphogenesis. Investigation of the transcriptional regulatory relationships would be one of the crucial ways to understand embryonic development. In our previous research, staged RNA-seq of the ascidian, *Halocynthia roretzi*, has shown that ten transcription factors are transiently expressed at the blastula stage, which is the stage that cell fates are specified and differentiation starts. Six of these transcription factors have already been shown to play important roles during early development, while the remaining transcription factors, PRDM1-r, SP8, FoxJ-r and SoxF were still unknown.

Investigation of the spatial and temporal expression patterns showed that all of the four genes are expressed in the animal hemisphere from the 16-cell stage, which is probably because most of transcription factor genes expressed in the vegetal hemisphere have been comprehensively analyzed previously. Functional analyses suggested that SP8 doesn’t regulate any developmental process, although SP8 is expressed during blastula stages. Knockdown embryos of FoxJ-r showed disruption in laterality and the absence of mono-cilia, suggesting its function in cilia formation and left-right asymmetry. Knockdown of SoxF resulted in two phenotypes, some developed into disorganized cell mass while the others had protruded cells presented outside at the tailbud stage. However, function of PRDM1-r is still unknown due to non-specific effect of the morpholino, which caused failure in gastrulation. These findings provide information for further experiments to investigate the regulatory functions of these transcription factors, and improve our understanding about ascidian embryonic development.
A GENOME-WIDE SURVEY OF MUSCLE STRUCTURAL GENES IN MOLGULA TECTIFORMIS SUGGESTS AN ANCIENT ORIGIN OF ITS ANURAL MODE OF DEVELOPMENT

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Some ascidian species in the families Molgulidae and Styelidae do not develop into a conventional urodele larva. Instead, they form an anural (tailless) larva. An anural larva does not have tail muscle, notochord or sensory organs. Previous studies revealed several molecular aspects underlying the anural mode of development. For example, a) mRNA for a putative Zn finger transcription factor, Manx, is stored in urodele Molgula oculata oocytes but not in those of its anural sister species, M. occulta. Manx seems to have an essential role in tail formation1. b) Larval muscle actin genes have become pseudogenes in the anural M. occulta2 and M. bleizi. c) The expression of muscle structural genes, such as muscle actin, myosin, tropomyosin, and troponin, is largely suppressed in anural M. tectiformis embryos and larvae, which was revealed from a large-scale EST analysis3. Recently, genomes of three Molgulid species, namely M. oculata (urodele), M. occulta (anural), and M. occidentalis (urodele), were decoded. A genomic survey has revealed that tyrosinase and its related genes have become pseudogenes in the anural species M. occulta4. Because it was shown that anural development occurred several times independently in ascidian evolution, it is desirable to decode genomes of more Molgulid species, especially anural ones, to obtain deeper insights into the emergence of anural development.

In this study, we newly decoded two Molgulid ascidian genomes, the anural species M. tectiformis, and the urodele species M. manhattensis. Genomic DNA was sequenced using Illumina and nanopore sequencers, and then assembled and scaffolded. Scaffold N50 values are 864 kbp in M. tectiformis and 1624 kbp in M. manhattensis. Genes were predicted by Braker/Augustus. BUSCO analysis suggested approximately 85-90% genes were predicted with high accuracy.

To characterize larval muscle loss in M. tectiformis, we surveyed muscle structural genes in both genomes. M. manhattensis has many larval muscle actin copies in the genome. M. tectiformis has at least three muscle actin pseudogenes. Those pseudogenes have much more substitutions, insertions and deletions compared to those in M. occulta, suggesting their ancient origin. We speculate that acquisition of anural development in M. tectiformis may have occurred earlier than that in M. occulta in the course of Molgulid evolution.

We also surveyed several other muscle structural genes including myosin, tropomyosin, and troponin genes. For each muscle structural gene, one or more copy for both larval and adult form was obtained in the M. tectiformis genome. In contrast, no seemingly-functional larval muscle structural genes were obtained in M. tectiformis. Furthermore, few pseudogene candidates for those genes were obtained. We speculate that these genes have become pseudogenes in anural evolution leading to M. tectiformis, but their DNA sequences may have accumulated too many mutations to be detected.

References
Maternally expressed Gata.a, which is known to direct animal hemisphere-specific expression, was required for activating genes in various regions of early ascidian embryos, with priming being the most likely mechanism.

In ascidian embryos, the earliest transcription from the zygotic genome begins between the 8-cell and 16-cell stages. Gata.a, a maternally expressed Gata transcription factor, activates target genes specifically in the animal hemisphere, whereas the complex of β-catenin and Tcf7 antagonizes the activity of Gata.a and activates target genes specifically in the vegetal hemisphere. Here, we show, through knockdown experiments for Gata.a and reporter assays, that Gata.a is required for the expression of not only animal hemisphere-specific genes, but also vegetal hemisphere-specific genes. On the basis of this finding, we performed a global analysis, and found that genes expressed in early embryos have significantly more Gata motifs in their upstream regions. These data suggest that Gata.a function is not limited to the genes expressed specifically in the animal or vegetal hemispheres, and that Gata.a plays an important role in the earliest transcription of the zygotic genome. Because Gata.a is present even in the nuclei of 2-cell embryos, Gata.a binding may precede and facilitate the binding of Tcf7 or other factors in the upstream regions of genes expressed in early embryos.
The formation of chimeric entities through colony fusion has been hypothesized to favour colonisation success and resilience to multiple stressors in modular organisms. We tested the prevalence of chimerism in the ascidian *Didemnum vexillum* at the Ebro Delta population and the capacity of chimera formation through colony fusion experiments in the Venetian lagoon. For the prevalence study, we sampled five different fragments from 9 large colonies on oyster cultures. For each fragment, we analysed a single zooid by whole genome amplification and genotyping-by-sequencing. The percentage of shared genotypes allowed us to clearly identify pairs of zooids with the same genotype (96.8±0.14 identical loci) and with different genotypes (48.07±0.07). Our results showed that 44% of the colonies were in fact chimeras. For the fusion experiment 45 pairs, corresponding to 15 intra-colony and 30 intercolony pairs, were fixed on a slide using cotton threads, with contacting edges, and monitored over 4 weeks. Single zooids per colony were analysed as mentioned above. In about 50% of the intra and intercolony pairs one or both of the fragments regressed and died. In the remaining pairs all the isogeneic pairs fused, while only 31% resulted in fusion in allogeneic contacts. We found no significant differences in overall genetic distances between fused and non-fused allogeneic pairs. We conclude that colony fusion occurs frequently in Mediterranean populations of *D. vexillum* and seems to be unlinked to genetic relatedness among fusing genotypes. However, we cannot discard that a few loci are responsible for increasing compatibility among them.

References

Rimbp (RIM-binding proteins) is a poorly studied gene family involved in the proper functioning of presynaptic machinery calcium-dependent. Starting from an ascidian Rimbp gene, we shed light on the evolution of Rimbp family in metazoans, defining how duplications modelled its evolutionary scenario, mainly in vertebrates. The unique Rimbp2/3 of tunicates, is orthologous to vertebrate Rimbp2 and Rimbp3. In sea squirt Ciona robusta, Rimbp2/3 is expressed in the pigment cell precursors (that will give rise to otolith and ocellus) and in cells belonging to both central and peripheral nervous system (CNS and PNS). In situ hybridizations indicated expression, at early tailbud stage, in bipolar tail neurons (BTNs), prior of the expression in otolith and ocellus precursors. In the aim to study the regulation of this gene we identified a cis-regulatory region of 0.3 kb conserved with sibling species C. savignyi harboured inside its seventh intron (intR7), which guides a strong GFP expression in BTNs and Ascending Motor Ganglion neurons (AMGNs) at larva stage. Moreover, we unravelled the intR7>eGFP presence also in two glutamatergic neurons of palps (papillae) belonging to PNS, documenting, by double electroporation experiments, that these neurons originated from FoxC+ territory of palps.

In sum, we produced first data regarding the expression of unique family member Rimbp2/3 in tunicates. Our findings speak in favour of a conserved involvement in the functions of distinct cells belonging to the nervous system.
Laboratory Cultivation of Ciona and Other Tunicates

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Tunicates have long been the subject of scientific research due to their close phylogenetic relationship with vertebrates. Interest increased in the 2000s with publication of the full genome sequence of Ciona robusta. In order to accompany the development of model species by the scientific community, the Marine Biological Ressource Center of the Roscoff Biological Station started a breeding program on several ascidian species in 2008 in the context of the EMBRC infrastructure and associated projects. Zootechnical parameters for the laboratory cultivation of Ciona intestinalis have been defined and the full life cycle completed ex situ, allowing the establishment of laboratory strains (wild-type/inbred), and, since 2016, of a semi-intensive “farming” mode of production. These techniques have been fully or partially transferred to the cultivation of other species of tunicate, including Ciona robusta, Phallusia mammillata, Asciidiella aspersa, and Styela clava.
The evolution of the notochord and paraxial muscle linked to locomotion powered by tail beating is likely one of the key innovations that facilitated the origin and radiation of chordates. This innovation was accompanied by gene duplications that gave rise to muscular actins from cytoplasmic ancestral forms, which acquired contractile capability thanks to the recruitment of the myosin motor-machinery. In our work, we have characterized the complete actin catalogue of the appendicularian *Oikopleura dioica*, an urochordate that maintains a chordate body plan throughout their life, including the notochord in a muscled tail that confers an active free-living pelagic style. Our genomic survey, phylogenetic analyses and Diagnostic-Actin-Values (DAVs) reveal that *O. dioica* has four muscular actins (*ActnM1-4*) and three cytoplasmic actins (*ActnC1-3*), most of which originated by appendicularian-specific gene duplications. Our results reveal differences in the temporal-regulation and tissue-specificity of different actin paralogs, suggesting complex processes of subfunctionalization during the evolution of urochordates. Our results suggest the presence of a “cardio-paraxial” muscular actin in the last common ancestor of vertebrates and urochordates. Cytoplasmic actins show highly dynamic tissue-specific expression domains, which include the notochord, ciliated cells and neurons with axonal projections, which challenge the classic housekeeping notion ascribed to these genes. Considering that previous work had demonstrated the existence of notochord-specific actins in cephalochordates, the tissue-specific expression of two cytoplasmic actins in the notochord of *O. dioica* suggests that this pattern plausibly reflects the ancestral condition of chordates, and provides new insights to better understand the evolutionary origin of the notochord.

Reference
DIVING INTO THE UNKNOWN OF ASCIDIAN DIVERSITY IN ECUADOR

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To date, only few species of sessile tunicates (i.e. ascidians) have been recorded for the Tropical Eastern Pacific. To fill this gap, we collected specimens along a 600 km stretch of coastline in central and southern Ecuador (3°11’40.28” S - 0°34’54.6” W). In several expeditions that lasted over three years, we collected by scuba-diving (5 – 20 meters in depth) at sites suggested by local divers or fishermen to contain a high diversity of marine sessile invertebrates on hard substrata, i.e. rocks and corals. We also collected in the intertidal zones during low tides, as well as from artificial substrates and docks. Samples were relaxed in menthol, fixed in formaldehyde (4%), preserved in ethanol (80%), and species were identified. We collected approximately 393 specimens in total, and in this manuscript we report the identification of 155 samples, which represents about 40% of the total collection. We report 27 species of three ascidian orders (6 species of Phlebobranchia, 11 species of Aplousobranchia, and 10 species of Stolidobranchia), including nine new species: Aplidium lambertae sp. nov., Ascidia valdiviensis sp. nov., Ascidia vulgaris sp. nov., Ascidia huancavilca sp. nov., Eudistoma caras sp. nov., Polyandrocarpa teticas sp. nov., Polyandrocarpa santaclarensis sp. nov., Pyura machallila sp. nov., and Trididemnum azureus sp. nov. We report four new geographic records in the Pacific Ocean for Eudistoma platense, Eudistoma clarum, Eudistoma obscuratum and Perophora carpenteria. The highest number of ascidian species was collected in Isla Santa Clara close to the border with Peru. The cold Humboldt Current converges with the warm Equatorial Current at this site allowing both cold and warm water species to co-inhabit here, which could explain the high diversity of ascidians reported for this area. Species that were found across distant sites and presumably with broad distribution include Aplidium lambertae sp. nov., Ascidia vulgaris, Ascidia huancavilca sp. nov., Ascidia sydneiensis, Cystodytes dellechiajei, and Eudistoma clarum.
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PROVISION OF COMMUNITY TOOLS FOR THE TUNICATE COMMUNITY

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Biocurators are involved in the development of tools that allow the community to share and comment data. Resources such as repositories, portals, and websites that specify to a community which data are available and where, as well as shared documents that facilitate interaction between researchers, developers, annotators, and biocurators in a specific community are necessary. Biocurators may also be involved in the management of social networks. Social networks and media are improving the connectivity between biocurators, journals, researchers, engineers, PhD candidates, postdocs, and students.

The multitude of platforms allows for choosing the optimum networking tool: Twitter to maintain a professional network, Facebook to reach a mainstream audience whereas scientific networks like ResearchGate or MyScienceWork offer the possibility of developing a more specialized and more professional network.

I will present here some tools I set up for the tunicate community.

Reference

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P34
EXPLORING THE ROLE OF CDK5 IN ASCIDIAN DEVELOPMENT

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Cyclin dependent kinase 5 (Cdk5) is a serine-threonine kinase predominantly expressed in neural tissues and involved in both brain development and degeneration. During vertebrate neurogenesis, Cdk5 is implicated in cell cycle regulation, neural migration as well as synapse formation (1, 2). Cdk5 gets activated by its neuron-specific activator p35 (3). Stressed conditions induce cleavage of p35 to p25 by calpain which forms a more stable yet hyperactive Cdk5/p25 complex (4), leading to neural disruption. Although Cdk5/p35 is a key player of neural homeostasis, no study on its involvement in ascidians neural development has been performed yet.

In Ciona intestinalis and C. robusta genomes homologs of Cdk5 and its main regulators are present, and, based on our analysis, their expression patterns are comparable with those reported in vertebrates. Thus, we started exploring Cdk5 involvement in larval neural development, specifically inhibiting Cdk5 activity by drug treatments. Larval central nervous system was highly affected by Cdk5/p35 inhibition: sensory vesicle appeared reduced in size and fused with the motor ganglion and neurites outgrowth was impaired along the tail in a dose dependent manner.

Overall, our results suggested that Cdk5/p35 functions are highly conserved between ascidians and vertebrates, setting the stage for further research related to its involvement in ascidian development.

References
Microscopic plastics, 1 μm-1 mm diameter, are widespread in marine environment and can be ingested by a variety of marine organisms. They derive from the fragmentation of larger plastic debris, such as plastic bottles and bags. The impact of these particles can depend on their concentration and size. Filter-feeders like ascidians are more sensitive to microplastics pollution than predators due to their less selective strategy of feeding. Once ingested microplastics can exert different effects, from damaging and blocking the feeding appendages and digestive system, to limiting the food intake. Only few studies have investigated the translocation of microplastics from the gut cavity to the circulatory system and body tissues.

We analyzed the effects of 1 μm polystyrene particles on the larval and juvenile development in the ascidian Ciona intestinalis. At the highest concentration tested, they caused a delay in the juvenile growth, probably due to inadequate food intake. Instead, larval development was not affected by the presence of microplastics.

A histological analysis of juveniles revealed that 1 μm particles can translocate from the gut to the internal extracellular compartment in just 4 days and they can be phagocytized by specific blood cells identified as granular amebocytes with phagocyte activity.

Therefore, small microplastics can exert also an immune response in ascidians with consequences that are still to be clarified thus raising the concern regarding the presence of these pollutants in marine environment.

References
ELUCIDATING THE MECHANISMS DRIVING SECONDARY NOTOCHORD-ENRICHED GENE EXPRESSION

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The primary (A-line) and secondary (B-line) Ciona notochord lineages have distinct cell behaviors, but most notochord-enriched genes are thought to be uniformly expressed across the two lineages. We recently identified three notochord genes, *C11.331*, *C12.115*, and *C8.891*, with expression that is specifically enriched in only the secondary notochord. The tail tip expresses both Wnt and FGF ligands, so we tested pharmacological modulators of these pathways and found that both *C11.331* and *C12.115* are positively regulated by FGF and Wnt signaling. We developed a dual reporter strategy to quantify regionalized expression while accounting for transgene mosaicism. Extensive analysis of the *C11.331* upstream regions revealed multiple non-overlapping regions that drive enriched expression in the secondary notochord. Dissection of one minimal cis-regulatory module revealed evidence for distinct positive elements that activate notochord expression and an important silencer element that shuts expression off in the primary notochord. This minimal CRM contains predicted binding sites for ETS, GATA, LMX, and Myb in or near the silencer region. Mutation of each of these sites individually reduces expression of the minimal reporter in the secondary notochord, but these mutations neither eliminate expression altogether nor release the primary notochord from silencing. These results suggest multiple inputs into the regulation of the *C11.331* secondary notochord-enriched expression pattern, including Wnt and FGF ligands from the tail tip, notochord-specific regulators, and a yet to be identified silencer in the primary notochord.
Oikopleura dioica (Appendicularia) is a model organism to understand the evolution of chordates. Although its nuclear genome has been sequenced, its mitochondrial (mt) genome has not been determined, probably because of their fast evolutionary rate and extensive RNA editing. We sequenced cDNA of the cox1 gene and nuclear genomes (using Illumina) from O. dioica and Fritillaria pellucida. Mapping the resulting DNA reads onto the cox1 cDNA sequences, we confirmed the previously-published observation that, at DNA level, long poly-T stretches interrupt the mt ORFs, hampering their identification. In addition, we noticed that mitogenomic poly-T correspond at RNA level to TTTTTT (6T) regions in O. dioica but to shorter TTTT (4T) regions in F. pellucida, suggesting species-specific RNA editings by deletion. Remarkably, different editing sites were observed between Mediterranean and Japanese populations of O. dioica suggesting a rapid evolution of these sites.

While tRNAs usually punctuate the protein-coding genes in chordate mt genome, only one tRNA, tRNAmet, was identified among O. dioica mt contigs assembled from published cDNA reads. To test if mt tRNAs could have been lost, we searched the genomic assembly for the presence of mt aminoacyl tRNA synthetase (aaRS) genes, which encode the proteins responsible for the aminoacylation of its cognate tRNA. No specific mt aaRS genes were found in the nuclear genome assembly of O. dioica, except for mt-MetRS, which catalyzes the mt-tRNAMet aminoacylation. This suggests that the mt genome of O. dioica encodes only the single tRNAMet.
The use of model organisms in research has been paramount in understanding animal development, as well as evolution of developmental diversity. Many powerful developmental models have benefited from genetic screens to identify regulators of different developmental processes.

Our lab studies the specification and development of the cardiopharyngeal cell lineage using the ascidian Ciona robusta, a model organism highly amenable to Cell and Developmental Systems Biology. Our lab has extensively profiled the cardiopharyngeal transcriptomes, and we are thus poised to systematically interrogate the function of zygotically expressed and lineage-specific genes in cardiopharyngeal development. To this aim, we have adapted the CRISPR/Cas9 system to be used in Ciona and have described a protocol to design efficient single guide RNAs for lineage-specific CRISPR/Cas9-mediated mutagenesis.

Here, we report the development of a library of single guide RNA constructs. We describe optimized strategies for sgRNA design and validation, as well as a high-throughput cloning method. Further, we identified an optimal Cas9 variant for gene knockout. We also report the development of imaging chambers designed to accommodate Ciona embryonic development. We are now developing a pipeline for high-content microscopy and image analysis to screen for cell migration and division phenotypes in the cardiopharyngeal lineage. This approach will permit the reconstruction of biomolecular network models for cardiopharyngeal cell behavior in Ciona robusta, by analogy with previous work in C. elegans, and empower the community with a resource for functional genomics analyses of a variety of developmental systems.

References
We previously used FACS-RNAseq and in situ hybridization to quantify and validate an extensive set of genes enriched in the Ciona notochord. This newly comprehensive notochord-enriched gene set allows us to test the long-held model that Brachyury is a master regulator of the notochord gene regulatory network. Specifically, is Brachyury function necessary for expression of the full notochord transcriptome, and is ectopic expression of Brachyury sufficient to induce notochord fate in other tissues?

To test Brachyury’s ability to induce the notochord-specific transcriptional program in other cell types, we ectopically expressed Brachyury using three different tissue-specific enhancers. Whole embryo RNAseq showed that the majority of notochord-enriched genes were neither significantly nor strongly induced by ectopic Brachyury expression and that targeted tissues also continued to express many of their own unique markers. This suggests that Brachyury is not a true master regulator, as it can only partially transform other cell types to notochord fate.

To test whether Brachyury is necessary for the expression of all notochord-enriched genes, we disrupted it by somatic CRISPR ribonucleoprotein injection followed by RNAseq transcriptional profiling. Surprisingly, we found that many notochord enriched genes were not strongly affected by loss of Brachyury function. Enhancer TFBS analysis and perturbation of notochord fate induction identified other transcription factors that may be acting in parallel to Brachyury. We are currently testing the function of these candidate transcription factors in the notochord GRN. Instead of being a straightforward regulatory cascade entirely downstream of Brachyury, the notochord GRN appears to be more complex.
Consisting of only 40 cells in a small embryo well suited for quantitative in toto microscopy, the *Ciona* notochord is a tractable system for studying the cellular behaviors giving rise to organ shape. It forms a single-file column at tailbud stages, which has a tapered shape that involves differences in cell volume along the anterior-posterior axis. We have quantified sibling cell volume asymmetry throughout the developing notochord and find there are distinctive, stereotyped patterns of unequal cleavage in all 4 bilateral pairs of A-line primary notochord founder cells and their descendants, and also in the B-line derived secondary notochord lineage. A quantitative model confirms that the observed patterns of unequal cleavage are sufficient to explain all the anterior-posterior variation in notochord cell volume. Many examples are known of cells that divide asymmetrically to give daughter cells of different size and fate. Here, by contrast, a series of subtle but iterative and finely patterned asymmetric divisions controls the shape of an entire organ. We have quantified how these asymmetries are driven by distinct cellular mechanisms including mitotic spindle displacement, mother cell shape, and effects occurring post-anaphase that potentially involve unequal cortical contractility. We find that different combinations of these mechanisms are used in each blastomere. Inhibition of Nodal signaling reverses the direction of asymmetric division in the A8.5 and A8.6 blastomeres, largely via changes in spindle displacement. These results demonstrate a new role for asymmetric division in directly shaping a developing organ and point towards complex underlying mechanisms.
Regeneration is widespread in the animal kingdom, and a variety of model systems are employed to better understand the principles and genetic programs underlying this process. Ascidians are remarkable for their regenerative abilities; and while the majority of regenerative studies focused on well-known models such as *Ciona intestinalis*, our previous work suggested a new model system: the solitary ascidian *Polycarpa mytiligera* (order Stolidobranchia).

In *vivo* experimental observations revealed this species extraordinary ability to regenerate all major body parts following their removal, including the digestive system, nervous system, and heart.

Our current study further describes *P. mytiligera*’s impressive regenerative potential and provides a cellular description of its regeneration processes over time. We focus on the central nervous system (CNS) regeneration and characterize the cellular process and time-course of its regeneration using immuno- and histochemical methods. One week following neural complex removal, we observed wound healing process and 21 days following its amputation the neural complex was completely regenerated.

*P. mytiligera* transcriptomic profiling, performed at five different time points of CNS regeneration, revealed many genes with dynamic expression over time. This includes genes involved in the regulation of cell cycle and in Wnt and Hedgehog signaling pathways.

Our new findings provide an in-depth characterization of *P. mytiligera*’s regeneration, presenting insights into the cellular and molecular aspects of CNS regeneration, further emphasizing this ascidian’s potential to serve as a new model system for studying regeneration and its evolution within the tunicates.
Ascidians such as *Ciona robusta* are classic developmental models for deterministic or “mosaic” development. Mosaic/determinative development is typified by the embryo’s strict adherence to invariant cleavage patterns and the inability to recover lost lineages after ablation. Despite being unable to replace lost cells as embryos, adult ascidians have remarkable regenerative abilities ranging from the replacement of specific organs in solitary ascidians, to the remarkable whole-body regeneration found in colonial ascidians. An open question is how they can acquire such regenerative capacity as adults after having a highly deterministic embryonic development. To answer this question, we are developing a genetic system to ablate specific lineages in a temporally controlled manner to identify the transition from deterministic development to a more regulative mode of development that allows for the ability to amount a regenerative response. As a complement, we have designed an experimental strategy to harness the power of single cell transcriptome profiling and characterize this “regulative transition” in whole metamorphosing larvae. In addition to identifying the mosaic to regulative transition, a genetic ablation system will empower studies of the potential regenerative ability of internal organs that are traditionally difficult to access surgically, such as the heart and body wall muscles.
MOLECULAR SIGNATURES OF CHORDATE DEVELOPMENT:
TWO DISPARATE PATHWAYS, ONE TUNICATE

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Sexual development in chordates is well-described by embryogenesis. Other developmental pathways including asexual reproduction and whole body or tissue regeneration share some essential processes such as establishment of the body axes, morphogenetic patterning and organ formation but differ in origin. Although studies have identified conserved aspects of embryogenesis across and within phyla, this pathway has not been linked to the other developmental pathways. In particular, it is unknown if convergent morphology implies convergent molecular mechanisms. Colonial tunicates provide a key to answering these questions, they are unique amongst chordates in possessing two disparate developmental pathways that produce the adult body, either sexually through embryogenesis, or through a stem cell mediated asexual renewal termed blastogenesis.

Using the model organism Botryllus schlosseri we have combined transcriptome sequencing of major embryonic and blastogenic stages with confocal, two-photon and electron microscopy to characterize the molecular and morphological signatures along both developmental pathways. We identify de novo, periods of transcriptional transition and shared molecular characteristics including stem cell associated transcription factors. Phylotypic conservation is investigated by comparing developmental gene signatures from other chordates. This study generated a complete gene profile database on the entire embryogenesis process and is the first to similarly describe asexual development. By combining microscopy with transcriptome sequencing, it demonstrates the extent to which convergent morphology implies different molecular mechanisms and reveals the basic principles and evolutionary conserved elements of chordate development.
During development, multipotent progenitors divide and generate a diverse range of cell types. This often requires precise spatiotemporal coordination between cell-intrinsic properties and extracellular cues. Here we focus on mechanisms of fate specification in the cardiopharyngeal mesoderm of the simple chordate, Ciona robusta. Ciona has two pairs of cardiopharyngeal precursors called trunk ventral cells (TVCs). They divide asymmetrically and are specified as either cardiac or pharyngeal muscle precursors. As multipotent progenitors, TVCs are transcriptionally primed for both developmental programs. We focus on the cell biology, with a specific emphasis on cell behaviors that orchestrate asymmetric contact with an extrinsic source of Fgf ligand(s). Towards this aim, we implicate the RhoGAP protein Depdc1.a in the regulation of oriented, asymmetric TVC divisions. We propose a model whereby TVCs divide stereotypically along the medio-lateral axis of the embryo to position pharyngeal muscle precursors directly adjacent to a mesenchyme-derived source of Fgf ligand, thus restricting pharyngeal muscle fate to laterally positioned cells. These observations provide the foundation for an ongoing project aimed at understanding the mechanisms by which oriented cell divisions instruct fate specification in cardiopharyngeal progenitors.
The central nervous system of ascidian larvae is formed through vertebrate-like neurulation processes in the dorsal midline. The transcription factor Cdx is important for neural tube formation, since suppression of its function leads to incomplete closure of the neural tube. Cdx is expressed in A9.15, A9.29, and A9.31 cells in the neural plate of the Ciona robusta gastrula. The expression of Cdx in these cells requires the growth factor Nodal. A9.29 and A9.31 receive Nodal, while A9.15 does not. How Nodal activates Cdx in both of these cells is unclear. We cloned a 6.1-kb 5’ flanking region of the Cdx locus and carried out reporter analyses. Deletion analyses revealed that the region between -4469 and -2250 contained enhancer elements activated in A9.15, A9.29, and A9.31 cells at the late gastrula stage. Although further deletion diminished the enhancer activity, the region between -3319 and -2249 appeared to contain a minimal essential element. We are now trying to identify transcription factors and their binding sites within this region.
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COMPARATIVE ANATOMY OF ASCIDIAN MINIATURE TAILBUD

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The tailbud stage belongs to the evolutionary conserved period (organogenesis period) among chordates and is essential for deciding the characteristics of chordate body plan. Although the ratio of egg volume between Halocynthia roretzi and Ciona sp. is about 8 times, their shape of tailbud embryos are similar and basic structure is maintained. Halocynthia can produce normal-looking miniature (dwarf) tailbud embryos when eggs are artificially cut in a plane. Although cell lineages during ascidian embryogenesis are known to be invariant, it was reported that the number of cell divisions in the dwarf embryo is changed by a distinct mechanism in each tissue [1]. In this study, to elucidate the size regulation of each tissue in the dwarf tailbud embryo, we compared anatomical structure, the developmental speed, the cell number of each tissue, cell volume between dwarf tailbuds and normal one, by constructing dwarf 3D virtual mid-tailbud embryo (3DVMTE [2]). As a result, the ratio of volume per tissue is maintained in the dwarf tailbud embryo. To compare each cell volume quantitatively, receiver operating characteristic (ROC) curve are drew and calculated area under curve (AUC). Epidermis, nervous system and mesenchyme reduced the number of cells but maintained cell volumes in the dwarf. On the other hand, notochord, muscle and endoderm maintained the number of cells but relatively reduced their cell volumes in the dwarf. Germline precursor doesn’t belong to both groups. These results showed there are the distinct strategies of size regulation in each tissue of the dwarf tailbud embryo.

References:
Nanoplastics (<100 nm) represent an emerging threat for marine ecosystem and can have a significant impact on aquatic organisms due to dimensions and higher surface area. The aim of this study is to evaluate the effect of polystyrene nanoparticles (PS NPs) on the embryogenesis of the tunicate Ciona robusta. Ascidians, are recognized as valuable biological models for ecotoxicity studies thanks to their rapid embryonic and larval development and resemblance to vertebrates. Based on recent findings on embryo and larval disruption upon nanoplastics exposure in marine invertebrates species, ascidians also might represent a target which deserves further investigation. PS NPs, owing negative and positive surface charges, respectively as carboxylated (PS-COOH) and amino-modified (PS-NH\textsubscript{2}) were tested for 22 h in the range of 0-100 µg/mL. PS NPs were characterized in natural seawater (NSW) exposure media by DLS, while embriotoxicity was evaluated looking at % of larval development and morphology. PS-COOH formed micro-aggregates (Z-Average >1 µm) and no sign of embryotoxicity were recorded up to 100 µg/mL. On the contrary amino-modified PS NP (PS-NH\textsubscript{2}) still in nano-scale form (Z-Average in NSW ≈60 nm) compromise hatching and the normal development of the trunk of the larvae even at the lowest concentration tested with an EC50 of 6.81 µg/mL. Our results suggest that differences in PS NP surface charges and aggregation in seawater affect their behavior and embriotoxicity in ascidians therefore further investigation on mechanism of toxicity of amino-modified PS NPs (PS-NH\textsubscript{2}) are strongly required due to their ecological role in marine coastal environments.
PROBABLE RECORD OF *PHALLUSIA DEPRESSICULA* AND *P. PHILIPPINENSIS* ALONG WITH MORPHOMETRIC COMPARISON WITH *P. NIGRA* FROM ANDAMAN AND NICOBAR ISLANDS, INDIA

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Altogether 70 species of ascidians under 25 genera, 11 families and 3 orders including 2 new species were documented during the study period (September 2013- November, 2016) from Andaman and Nicobar Islands. Among the three orders of Class Asciidacea most of the species were identified under the order Stolidobranchia i.e. 28 species (40%) followed by 25 species (35.71%) from Aplousobranchia and 17 species (24.29%) under Phlebobranchia. Genus *Phallusia* is belonging to the family Asciidiidae currently comprise of 20 valid extant species world-wide (Asciidacea World Database). Till date seven species under the genus *Phallusia* are recorded from Andaman and Nicobar Islands. However, *Phallusia depressicula* (Heller, 1878), *P. philippinensis* Millar, 1975 and *P. nigra* Savigny, 1816 are closely resembled in anatomical characteristics. Morphologically *P. nigra* is with jet black colour along with its non-papillated pre-pharyngeal area which is easily distinguishable from other two species. However, other two species is quite similar in external morphology as well as in anatomy i.e. in both the species 12 lobes found on branchial aperture and 8 on atrial aperture along with massive cauliflower shaped ovary. Besides, in *P. depressicula* has about 45 branchial tentacles in three different orders whereas it is 60 branchial tentacles in four different orders for *P. philippinensis*. The anal border of *P. depressicula* is plicated without definite roll and it is bi-lobed anus with rolled rim for *P. philippinensis*. The morphological characteristics features of these three species are compared.

**Key words:** *Phallusia*, Taxonomy, Pre-pharyngeal band, India.

**References**

Asciidacea World Database:
as viewed on 30/03/2019
PHYLOGENETIC ANALYSIS OF THE CELLULOSE SYNTHASE GENE AND IDENTIFICATION OF AN INDEPENDENT GLYCOSYL HYDROLASE FAMILY 6-DOMAIN GENE OF CIONA

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Horizontal gene transfer is one of the important force that shapes animal genomic architectures and contributes to biological diversity. The tunicate cellulose synthase gene CesA [1], a gene transferred from bacteria, potentiates the structural innovation of the tunic and the house. The tunicate CesA gene contains not only a CesA domain of the glycosyl transferase family 2 (GT2) but also a glycosyl hydrolase family 6 (GH6) domain, which is similar to bacterial cellulases [1]. During our phylogenetic analysis on CesA using the ORTHOSCOPE tool [2], we identified a separate GH6-containing gene (tentatively called GH6-1) in Ciona intestinalis type A. We found that CesA and GH6-1 homologs were present in seven tunicate genomes but not in any other non-tunicate metazoans. In this study, we also examined the spatial and temporal expression of the GH6-1 gene in Ciona by in situ hybridization and quantitative PCR. The GH6-1 gene was expressed at epidermis of tailbud embryos, and its expression level was increased around ten hours after fertilization (mid-tailbud stage). Although a mutant, swimming juvenile, had been generated, in which the CesA expression was disrupted [3], the mechanisms underlying its phenotypic changes were still enigmatic. To investigate the function of the CesA and GH6-1 genes further, we are performing functional studies to clarify how these genes are incorporated into the host genetic networks. Our results support the uniqueness of tunicate CesA genes in the animal kingdom and provide knowledge of horizontally transferred genes acquiring expression control in the animal host.

References


Understanding the regenerative mechanisms of tissues in model species helps advance our knowledge for how to treat limb, tissue, and organ damage in humans. The present study focuses on regeneration in the colonial ascidian *Botryllus schlosseri*, which under normal conditions grows via a regenerative process called blastogenesis where entire bodies, including all somatic and germline tissues, are generated de novo on a weekly basis. In addition, *B. schlosseri* has also been reported to undergo a process called whole body regeneration (WBR), during which regeneration is stimulated by injury, and occurs ectopically compared to blastogenesis. Blastogenesis and WBR have been studied in several related botryllid ascidians, and both processes show species-specific characteristics. Here we utilized time-lapse microscopy to characterize WBR in *B. schlosseri*. Our data suggests that, unlike other botryllid species, WBR in *B. schlosseri* is not due to a novel ectopic budding process, but that successful regeneration always was initiated in specific niches, which we found were remnants of the blastogenic process itself.
The appendicularian *Oikopleura dioica* possesses a simplified chordate body plan, which is retained after the metamorphosis. In order to understand how this simplification proceeded at the molecular level, we study conserved key developmental genes, such as Hox genes. Hox genes are homeobox-containing transcription factors known to pattern the antero-posterior (AP) axis in many bilaterians. Previously, *O. dioica* Hox genes complement and cluster organisation have been described (Seo et al., 2004), showing the presence of nine Hox genes, and highlighting the disintegration of the cluster and alteration of the spatial collinearity. These features lead us to comprehend the function of Hox genes in *O. dioica* development, and to elucidate if the function in AP axis patterning has been conserved or if some Hox genes have acquired lineage specific function.

To address this issue, we studied the temporal and spatial expression of Hox1, the most anterior gene. The results showed a high expression level at the tailshift stage, and a strong expression at the trunk-tail transition and in the central nervous system. To go further, we have generated a mutant line using CRISPR/Cas9 system (Deng et al., 2018) to unravel the biological function of Hox1. The Hox1 knock out was confirmed by *in situ* hybridization and immunostaining, and the mutant phenotype appeared to be lethal. We will present morphological and physiological characterizations of the homozygotes, focusing more particularly on the neuroanatomy.

References:


Based on nuclear and mitochondrial genes, in 2012 Bock et al. demonstrated the existence of five highly divergent clades (A-E), corresponding to morphologically-indistinguishable cryptic species, in the cosmopolitan model ascidian Botryllus schlosseri (Pallas, 1766). While clade A is globally widespread and its nuclear genome was already sequenced, clade E was so far identified only in European waters, both Atlantic and Mediterranean. Here we present combined molecular and morphological data definitely demonstrating that clade E is a new species, with unique characters distinguishing it from B. schlosseri sensu Brunetti et al. 2017 (belonging to clade A) and other botryllids. Samplings in the Venice Lagoon and in Southern Italy were carried out so that molecular and morphological analyses were feasible on the same specimens. Based on cox1 analyses, clade E was identified in all investigated localities, always in sympathy with clade A. Sequencing of the whole mitochondrial genome of clade E showed no gene order differences compared to clade A. Remarkably, intra-species and congeneric comparisons between public ascidian mitogenomes revealed that the clade A-clade E divergence is incompatible with intra-species divergences but very similar to congeneric distances. Moreover, the clade A-clade E divergence is almost identical to that observed in the congeneric pairs Ciona intestinalis-Ciona robusta, and Botryloides niger-Botryloides leachi i.e., in species considered for long time morphologically indistinguishable and recently reconsidered as true species thanks to genetic data. These molecular results were supported by the identification of morphological discriminant traits. The description of a new species is therefore presented and discussed.

References


Invertebrate chordates, such as ascidians and amphioxus, possess a peripheral nervous system (PNS) while they are lacking neurogenic placodes and neural crest. These dorsal structures are known to be vertebrate-specific and lead to the formation of vertebrates PNS. In ascidians and amphioxus, the PNS is mostly ventral\(^1,2\). This ventral peripheral nervous system (vPNS) likely correspond to an ancient feature present in the ancestral chordate that may have been lost in vertebrates or shifted dorsally to form placodes and neural crest.

To test these hypotheses and better understand the evolution of PNS formation in chordates, our project aims at performing a side-by-side functional comparative analysis of the vPNS formation in invertebrate chordates, using the amphioxus Branchiostoma lanceolatum and the ascidian Phallusia mammillata. To do so, we have done a differential gene expression analysis by RNA-seq, on both species, after disruption of two signaling pathways known to be implicated in vPNS formation: BMP and Delta/Notch\(^1,2\). Analysis of these large datasets allowed us to identify vPNS candidate genes, for whom expression profiles will be determined by in situ hybridization, throughout development, in order to validate them as vPNS markers. Alternatively, we have look at the conservation of the regulatory logic controlling vPNS formation between ascidians and amphioxus. Altogether, these are first steps to reconstruct a vPNS gene regulatory network (GRN) for each organism but also for the ancestral chordate.

References
PATTERNS OF DISTRIBUTION AND DIVERSITY OF ASCIDIANS (TUNICATA: ASCIDIACEA) IN THE CORAL REEFS OF THE SOUTH OF THE GULF OF MEXICO.


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The ascidians are one of the most diverse taxonomic groups in the reefs of the Gulf of Mexico and the Caribbean Sea. Actually, 76 species have been registered in 5 studies only in Gulf of México. However, the patterns of distribution and diversity of species in these regions have not been described, using standardized methodologies that allow postulating theories about the possible processes that sustain such a level of diversity. The objective of this study is to describe the diversity components of ascidian species in coral reefs in the Gulf of Mexico and the Mexican Caribbean Sea through a standardized sampling protocol and a hierarchical spatial sampling that allows the identification of spatial scales of variation. A total of 930 quadrants have been made, 28 sampling sites in 5 reefs of two reef systems of the Gulf of Mexico. 52 species of ascidians corresponding to 12 families and 21 genera have been identified. In the Gulf of Mexico, the diversity of ascidians decreases in the East-West direction. The greatest number of species was recorded in the Reefs Bajos de Campeche (S = 44 n reefs = 2), decreasing progressively towards the coral reefs of the Veracruz Reef System, 13 species were recorded (n reefs = 3).

References

ENHANCER ACTIVITIES OF AMPHIOXUS BRACHYURY GENES IN THE ASCIDIAN Ciona EMBRYOS

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The notochord is a prominent organ of chordates. A T-box transcription factor gene Brachyury (Bra) is expressed there and play pivotal role in the formation. In the cephalochordate Branchiostoma floridae, Bra is duplicated into two genes (BfBra1 and BfBra2), which are expressed in somite-formation region as well. As a series of experiments to elucidate chordate Bra enhancer activity, we here carried out lacZ reporter assay of BfBra in embryos of the ascidian Ciona intestinalis. Vista analyses suggest the presence of conserved non-coding sequences (CNSs) not only in the 5'-upstream but also in the 3'-downstream and introns of BfBra. The experiment showed that (1) 5'-upstream sequences of both genes promote lacZ reporter expression in muscle cells, (2) 3'-downstream sequences have enhancer activity to promote lacZ expression in notochord cells, and (3) introns of BfBra2 and BfBra1 exhibit lacZ expression primarily in muscle and notochord cells, respectively. These results suggest shared Bra enhancer machinery between cephalochordates and urochordates, which was discussed in relation to the mode of formation of chordate-specific organs in the two lineages.

References

First described more than a century ago, ascidian embryonic cleavage has a stereotypical pattern. Development however allows for some variability, which we need to quantify in space and time as a prerequisite to the study of the mechanisms constraining ascidian development. With less than 3000 cells in the hatched larva, the solitary ascidian embryo is an ideal system to systematically investigate individual cell properties such as cell shape, cell position, cell division and cell/cell contacts throughout embryogenesis. We have started to paint a picture of the intra- and inter-species variability of embryogenesis using live single-cell high-throughput 4D embryo imaging (MuViSPIM technology) (1).

A central part of this research program was the development of ASTEC (Adaptative Segmentation and Tracking of Embryonic Cells) (1), a high-throughput computational pipeline for the digitalization (systematic whole-cell segmentation and tracking) of massive high-throughput light-sheet datasets and for the extraction of geometric and cell division parameters.

In this presentation, we will introduce a recent overhaul and extension of ASTEC, focusing on two new modules that decrease imaging quality requirements, and a refactoring effort to reduce the time of the segmentation. We will also present how Morphonet, has been adapted to manually curate the few remaining segmentation and tracking errors made by ASTEC. This enhanced set to tools allowed us to carry out a deeper statistical analysis of embryonic variability based on a set of ten high-quality fully-segmented and tracked WT Phallusia embryos, which also necessitated the development of methods to spatially and temporally align independently imaged digital embryos. They also open the way to the comparison of the embryogenesis of the embryos of different ascidian species, in particular Phallusia mammillata and Ascidiella aspersa.

References

ROLE OF PUMILIO DURING GERM CELL FORMATION IN COLONIAL ASCIDIAN, BOTRYLLUS PRIMIGENUS

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Colonial ascidians generate new individuals from somatic tissues asexually. In breeding season, each of the asexual individuals produces hermaphrodite gonads. In order to examine how germ cells differentiate in those animals, we proceed with the isolation of the genes involved in the germ cell differentiation. In this study, we focused on PUF protein Pumilio. We examined the structure, expression, and possible function of a Botryllus primigenus Pumilio gene (BpPum) during gametogenesis. The deduced amino acid sequence of BpPum contained PUM Homology Domain (PUM-HD) in the C-terminal region. It has been established that PUM-HD functions as RNA-binding domain. In B. primigenus, the gametes and gonadal primordia originate from cell aggregates of germline precursor. BpPum was expressed strongly in the germline cells such juvenile oocytes in the ovary. Oogonia and primordial testis expressed it weakly. No signals were emitted from germline stem cells and well-developed gonad. On the other hand, some of the somatic tissues in developing buds expressed BpPum. To identify the target mRNA of PUM-HD in BpPum, we screened the 3'-UTR sequences of the expected germline genes. The 3'-UTR of Vasa (BpVas) mRNA contained NRE-like sequence. In vitro analyses demonstrated that recombinant PUM-HD protein directly bound to the NRE-like sequence and that recombinant BpPum protein bound to recombinant Nanos (BpNos) protein. When BpPum was knocked down using siRNAs, the amount of BpVas protein was increased in the oocytes. These results suggest that BpPum fine-tunes the protein expression level of BpVas by inactivating translation during gametogenesis.
The Bipolar Tail Neurons (BTNs) are two bilaterally symmetric pairs of neurons that delaminate from the dorsal midline of the tail tip ectoderm and migrate anteriorly along the developing tail muscle cells until they reach a position in the middle of the tail. During this process, they first extend an anterior, or proximal process, then invert their anterior-posterior polarity and extend a posterior, or distal process. These processes are subdivisions of a single axon, and this bipolar subdivision allows the BTNs to form a synaptic relay between epidermal neurons of the tail and the central nervous system (Motor Ganglion and Brain). We have previously shown that the bHLH factor Neurogenin is necessary and sufficient to specify BTNs from the tail tip ectodermal midline, and ectopic BTN precursors all migrate and differentiate as an expanded chain of cells. Thus, Neurogenin is a key regulator of BTN morphogenesis, including processes that specifically interest us like delamination, migration, polarity inversion, and axon extension. We have used fluorescence-activated cell sorting (FACS) to isolate BTN precursors from wildtype embryos as well as embryos subjected to Neurogenin overexpression or expression of a dominant repressor form of Neurogenin (Neurogenin::WRPW). RNAseq of sorted cell populations from these three conditions was used to identify putative targets of Neurogenin in the BTNs. Top candidates have been validated by in situ hybridization and we have begun to test the localization and function of potentially interesting effectors of BTN development.
Successful embryogenesis requires the differentiation of the correct cell types, in defined numbers and in appropriate positions. In most cases, decisions taken by individual cells are instructed by signals emitted by their neighbors. A surprisingly small number of signaling pathways act repeatedly in widely different cellular contexts to produce diverse output. The Ras/ERK (Extracellular Regulated Kinase) pathway is one of these important embryonic signaling avenues for which the cascade of activations from the transmembrane receptor to the ultimately phosphorylated ERK nuclear targets has been well described over the years.

However, our current knowledge of this pathway is mostly static and we lack an integrated understanding of its spatio-temporal dynamics. Indeed, recent studies reveal additional levels of complexity showing that, although the core components of this pathway are the same, depending on the cellular context they can specify different cell fates in function of their dynamic features such as amplitude, duration or frequency of activation. The emergence of such qualitatively and quantitatively diverse outputs from a relatively simple pathway is a fascinating basic biology issue.

Studies of the vertebrate Ras/ERK pathway are complexified by the presence of multiple paralogues for each component, with slightly divergent activities. During my PhD project, I study it in ascidians that have single FGFR, SOS, Ras, Raf, MEK and ERK genes and make extensive use of the FGF/Ras/ERK pathway to pattern their early embryos. The embryos of the ascidian *Phallosia mammillata* have two additional favorable properties: they are fully transparent and easy to image and they develop with invariant cell lineages, providing a rigid framework allowing single cell analysis.

Thus, to address this issue, I have started to quantify in real time the dynamics of activation of ERK, the most downstream component of the cascade, using a live sensor. To study the dynamics and robustness of the pathway output, I now want to combine these measurements with spatio-temporal optogenetic modulations of upstream components of the cascade including the FGF receptor (opto-FGFR1) and SOS protein (opto-SOS) and ultimately computational modeling. My project benefits of our MuViSPIM light-sheet microscope and our unique ability developed over the past years to identify/segment/track individual cells during development.
In the tunicate model *Ciona robusta*, a neural plate consisting of six rows of cells gives rise to the central nervous system (CNS), the oral siphon placode (OSP), and the adhesive papillae. Despite the interest fueled by their comparisons with the head development in vertebrates, current cell fate descriptions of the most anterior rows (IV-VI) of the neural plate remain partial and contradictory.

Here, we document the fate of neural (row IV) and placodal ectoderm (row V and VI) from the early embryo through metamorphosis to the juvenile stage using several fluorescent reporters expressed in distinct cell populations.

We show that a subpopulation of cells descending from row IV separates from the CNS and surrounds the OSP, which derives from a combination of cells from rows IV and V. During metamorphosis, these cells continue proliferating to form a layer of tissue fully detached from the CNS and positioned between the more rostral OSP-derived cells and the endoderm derived periphraryngeal band. We name these peri-oral cells the “Kano cells”, after Shungo Kano who first described a folded structure protruding from the CNS in the *Ciona* larva. Even though the function(s) and cell type(s) of the Kano cells remain to be identified, their morphology evokes connective tissue rather than a neural fate, hinting at an ectomesenchyme-like cell population.

Using cell fate conversion induced by misexpression of transcriptional factors and signaling molecules, we are now investigating the roles of the Kano cells and other cell populations originating from the anterior neural plate in *Ciona*.

Altogether, our results lead us to compare the interactions between the Kano cells and the OSP in tunicates to those between the extreme anterior domain and adjacent neural crest cells during primary mouth morphogenesis in vertebrates.
ANISEED (Brozovic et al. 2017) (Ascidian Network for In Situ Expression and Embryological Data) is a major database system for the genetic control and anatomy of ascidian embryos. Since 2017, the system has been enriched in several aspects and its robustness improved. The ANISEED database, the genomic browser WashU (Zhou et al. 2011) and the synteny genomicus browser (Nguyen et al. 2018) were entirely rebuilt, the gene model set for Ciona robusta was improved by the inclusion of about 1000 NCBI models of previously missed genes. In addition, all gene annotations data and files were updated using the latest version of InterPro, blast similarity ontologies (Gene Ontology, ...).

Moreover, new annotations have been added such as GOSlim annotation for each gene and gene models versioning. Genome annotations have also been added for 2 new species: Botrylloides leachi (styelid stolidobranchians) and Molgula occulta (molgulid stolidobranchians), raising the number of covered species to eleven.

For these eleven species and vertebrates species, improved orthology relationships have been computed.

We will present the novelties of ANISEED and our strategy for the future.

References


In ascidians most cell fates are restricted by the onset of gastrulation. Advances in microscopy, image analysis and sequencing technologies are allowing to quantitatively measure cell morphological and transcriptional dynamics paving the ground for rendering ascidians into an in silico modelling system for developmental studies. In this work we capture the individual cellular developmental trajectories during ascidian early embryonic development (zygote to gastrula) using a combination of single-cell RNA sequencing (scRNAseq) and digital representations of developing embryos derived from light-sheet microscopy imaging. Using high coverage scRNAseq, we devised a computational framework that stratified single cells of individual embryos into cell types without prior knowledge. The analysis identifies 18 different cell types at 64-cell stage. We also developed methods identifying mother-daughter cell relationships for every cell directly from the transcriptome data and allowing to infer some degree of spatial information from transcriptional data. We additionally generated digital representations of developing early ascidian embryos allowing to extract the temporally resolved lineage trees and cell and embryo morphological properties. We created an atlas for whole embryo cellular gene expression profiles from zygote to gastrula (64-cells stage) where gene expression pattern formation and cellular morphology and organisation can be explored during early ascidian development.
Colonial ascidians are the only known class of chordates capable of undergoing whole-body regeneration (WBR) throughout their adult life. In *Botryllus schlosseri* and *Botrylloides leachii*, WBR starts after the removal of all adult zooids, and develops exclusively within the remaining vasculature. In ten days, fully functional adult zooids are regenerated from as little as 200 cells.

Stem cells in tunicates appear to play a role in a myriad of biological scenarios, including regeneration. In *B. schlosseri*, cells with stem-like morphology gather at the site of regeneration. In *B. leachii*, cells expressing the conserved stemness marker *Piwi* have been observed in the vasculature during the first 48h of WBR. Overall, populations of circulating stem-like cells are believed to be required for WBR in colonial ascidians.

To assess whether these are actual stem cells, we have set out to isolate them. Developing a new intravascular micro-transfusion technique enabled us to collect pure and healthy haemocytes. We thus sampled the early onset of WBR when the stem-like cells have been reported to appear in both species.

By comparing haemolymph composition in each species, we searched for cell populations with common stem-like features using flow cytometry and immunolabeling. Using FACS, candidate cell populations could be isolated for characterization. Cell labeling and re-injection will assess their contribution during regeneration and transcriptome sequencing will establish whether they express known stemness markers.

This novel approach is a first step towards characterizing the stem-like cells during regeneration in colonial ascidians.
Horizontal gene transfer is one of the important forces that shapes animal genomic architectures and contributes to biological diversity. The tunicate cellulose synthase gene CesA [1], a gene likely transferred from bacteria, potentiates the structural innovation of the tunic. The tunicate CesA gene contains not only a CesA domain of the glycosyl transferase family 2 (GT2) but also a glycosyl hydrolase family 6 (GH6) domain [1]. During our phylogenetic analysis on CesA using the ORTHOSCOPE tool [2], we identified a separate GH6-containing gene (tentatively called GH6-1) in Ciona intestinalis type A. We found that CesA and GH6-1 homologs were present in seven tunicate genomes but not in any other non-tunicate metazoans. We then examined the spatial and temporal expression of the GH6-1 gene in Ciona by in situ hybridization and quantitative PCR. The GH6-1 gene was expressed at epidermis of tailbud embryos, and its expression level was increased around ten hours after fertilization (mid-tailbud stage).

We are trying to use morpholino antisense oligos or TALEN-mediated genome editing to knock down or knock out the GH6-1 gene. Also, we will prepare reporter constructs using the genomic DNA neighboring to GH6-1 to find its driving enhancers. We may show how this likely horizontally transferred GH6-1 gene is retained in tunicate genomes.

References
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LEATHERY SEA SQUIRT GENOME PROVIDES INSIGHTS INTO MECHANISMS OF ENVIRONMENTAL ADAPTATION AND LARVAL METAMORPHOSIS IN INVASIVE TUNICATES

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The invertebrate sea squirt, which undergoes a unique vertebrate-like larval stage, occupies a unique evolutionary position between invertebrates and vertebrates. It exhibits adaptation to broad environmental conditions and is distributed globally. Despite hundreds of years of embryogenesis studies, the genetic basis of ascidian invasive habits remains largely unknown. We utilized a whole genome and transcriptome of leathery sea squirt, *Styela clava*, the first fossil tunicate species identified from the Early Cambrian, to explore genomic and molecular network-based mechanisms of adaptation to environments. Compared to other tunicate genomes, the size of *S. clava* genome expanded two-fold although the gene number was comparable. Increase in transposon numbers and transition types were identified as potential expansion mechanisms. In the expanded *Styela* genome, heat shock protein 70 family and the complement system genes expanded significantly, and cold shock protein genes were horizontally transferred into the *Styela* genome from bacteria. The expanded gene families potentially play roles in the adaptation of *S. clava* to its environments. While the gene loss may affect the galactan synthesis pathway and lead to different tunic structure and hardness compared to *Ciona*. We demonstrated further that the integrated thyroid hormone synthesis pathway regulates sea squirt larval metamorphosis. A thyroglobulin-like precursor gene, the core of the thyroid hormone synthesis system, was identified for the first time in a urochordate. The results indicated that *Styela* has genetic mechanisms of regulating physiological and developmental processes to facilitate adaptation to the marine environment.
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