

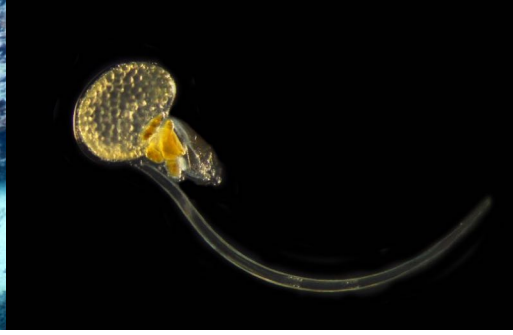

11th International Tunicate Meeting

Konan University, Kobe, Japan

July 11, 2022 – July 15, 2022

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We are excited and looking forward to meeting you all in person and online in the 11th International Tunicate Meeting which brings together scientists and students from all over the world studying this fascinating animal group.

Abstract Book

This program and abstract book are provided as PDF. Printed book is no more provided to reduce the cost to ship the books to online participants. On-site participants will receive printed books at the registration desk in the venue.

Website

https://sites.google.com/view/11th-itm/

Dates and Venue

July 11, 2022 - July 15, 2022
Konan University- Okamoto Campus
8-9-1 Okamoto, Higashinada-ku Kobe 658-8501, Japan
https://www.konan-u.ac.jp/english/location/

Time Zones

Time difference to Japan (summer time)

Japan	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Paris	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
New York	11	12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10
Los Angeles	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7

ORGANIZING COMMITTEE

Takehiro G. Kusakabe - Konan University (tgk@konan-u.ac.jp)
Takahito Nishikata - Konan University (nisikata@konan-u.ac.jp)
Hiroki Nishida - Osaka University (hnishida@bio.sci.osaka-u.ac.jp)
Yasunori Sasakura - University of Tsukuba (sasakura@shimoda.tsukuba.ac.jp)

SCIENTIFIC COMMITTEE

Kai Chen - Kunming University of Science and Technology
Bo Dong - Ocean University of China
Patrick Lemaire - CRBM - CNRS
Lucia Manni - University of Padova
Noa Shenkar - Tel-Aviv University

RESISTRATION SECRETARIAT

JTB Corp.　Western Japan MICE Branch
E-mail : westec_op1@jtb.com

Hosting Institutions

Institute for Integrative Neurobiology, Konan University
Graduate School of Natural Science, Konan University
Research Institute for Human Health Science, Konan University

Student travel awards are available! Details are given during the meeting.

Statistics of the Meeting

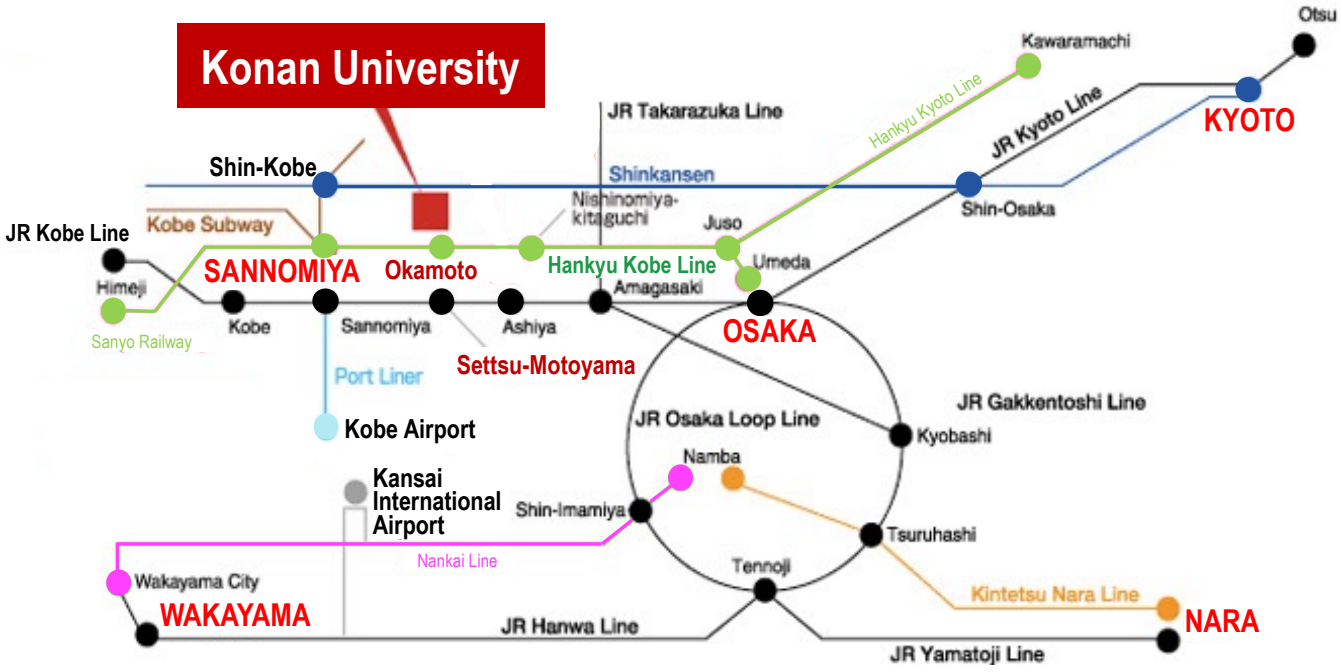
Number of participants is 151 including 12 oversea on-site attendees
(from the USA, France, Italy, Israel, Germany).
Residents in Japan : Others, 67:84
(Austria, 6;　Belgium, 1;　Brazil, 1;　Canada, 1;　China, 5;　France, 17;　Germany, 2;
India, 1;　Israel, 4;　Italy, 7;　New Zealand, 1;　Norway, 5;　Spain, 2;　Switzerland, 1;
Turkey, 1;　UK, 1;　USA, 22)
Online : On site, 58:68;　To be determined, 22
PI : Postdocs/Students, 81:70
Number of regular talks, 74

How to Reach the Meeting Venue

Konan University is located on the east edge of Kobe City, between Osaka and Sannomiya (central district of Kobe). The closest stations are Hankyu-Railway Okamoto Station or JR (Japan Railway) Settsu-Motoyama Station. It will take 10-12 min from these stations to Konan University on foot. You can take a taxi from these stations. Taxi fare will be about ¥700. If you stay in Sannomiya area, taking Hankyu Railway is strongly recommended, as Okamoto Station is one stop away from Sannomiya Station by using Limited Express.

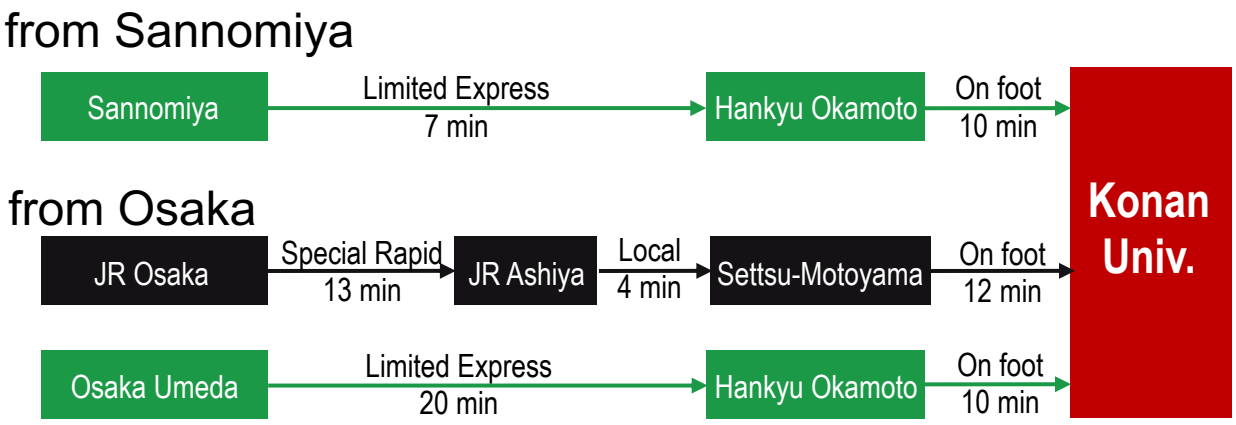
Meeting venues are Koyu-Hall and iCommons, which are situated in the west of the main campus of Konan University. Please find the venues on the following maps.

Railway network of Kansai area

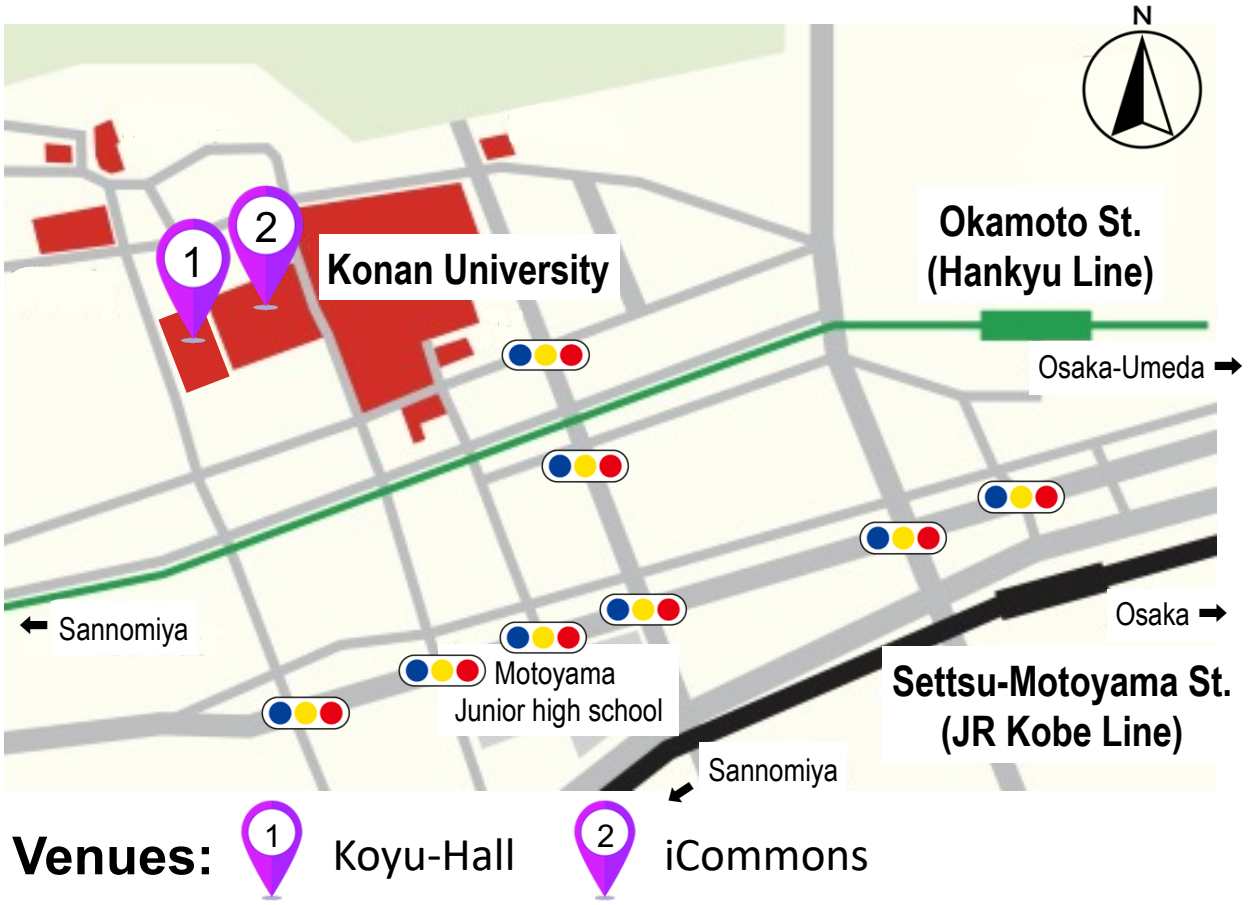


Transportations to Konan University

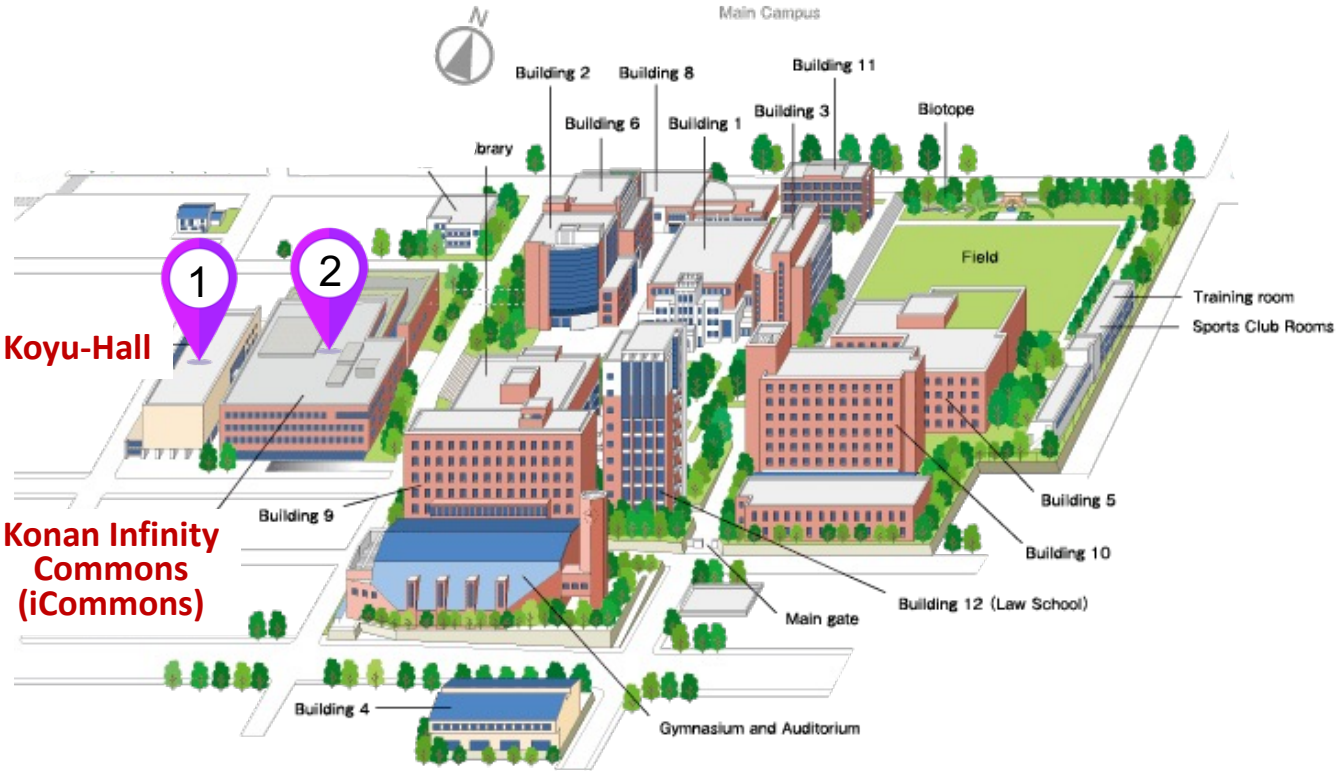
We recommend to use Hankyu Railway (indicated in green).



Map of Okamoto area



Campus Map of Konan University



INFORMATION ON MEETING

General Information

The meeting is organized in a hybrid style because of Covid-19.

Every paper will be given by sharing the presentation slides in zoom meeting, irrespective of whether speakers are on site or not. When talks are given in Zoom by online speakers, the talks are also projected on the screen in the meeting room. No poster sessions. 29 min (24 min talk and 5 min discussion) is assigned for PI, and 14 min (11 min talk and 3 min discussion) for others (students and postdocs). Zoom movies of talks will be recorded in real-time by organizers during the meeting days and will be uploaded for on-demand viewing as soon as we can. However, one or two-day lag will happen after the talk.

International attendees have to give talks in Japanese daytime. To increase comfortableness of every speaker from different time zones, each session will be organized taking account of the speaker's time zone. Thus, each session is a mixture of topics from several research fields. This is a big difference from the previous ITMs, but we hope this will facilitate all attendees to contribute various topics on tunicate biology as in the previous on-site ITMs.

Plenary lecture nor talk on animals other than tunicates is not planned in this meeting because of tight schedule of the meeting.

Welcome Party and on-site Reception

Monday, July 11

A welcome party will take place at Bar Salu Okamoto (1 minute walk from Hankyu Okamoto station, 3 minute walk from JR Settsu-Motoyama station; Phone 078-855-4136) from 17:00 to 20:00 for all the registered participants on site. A reception desk will be open there.
https://barusalu-okamoto.owst.jp/
For those not attending the welcome party, a reception desk will be available at the site of the meeting (Koyu Hall, No. 1 in the map) during meeting days. Please receive your name tag and a printed abstract book.

Technical Session

Takeo Horie and Alberto Stolfi will organize a technical session on "Single Cell Transcriptional Analysis".
It is scheduled at 10:30-12:30 in Japan time on 12th. Do not miss the session at the beginning of the meeting. The time is not comfortable for European researchers. So the talks will be also available on demand. In order to facilitate asking questions to the speakers, a "Question Session" relevant to

the technical session is scheduled at 23:00- 24:00 in Japan time on the third day of the meeting, Thursday, 14th.

Round Table Discussion

Round table discussion is scheduled just after the Question Session of the technical session at 24:00- 25:00 on 14th. This would be a time slot at which most of people on the globe is able to attend the session (see time zone table). The subject is “Towards a tunicate community data management plan” led by the ANISEED Team.

Group Photo

We are planning to take Group Photos at a brief break between the technical session and the round table discussion at 24:00 on 14th. We guess most people will attend the sessions using Zoom because it is midnight in Japan, so we will take snapshots of Zoom screen. Please turn on the video of Zoom for the group photo at that time.

11th ITM-KONAN Multidisciplinary Poster Session

The host institute Konan University and 11th ITM jointly organize a multidisciplinary poster session by students and postdocs of Konan University. Graduate and undergraduate students of Faculty of Science and Engineering, Faculty of Intelligence and Informatics, and Faculty of Frontiers of Innovative Research in Science and Technology will present posters at lunch time (13:30-15:00) on Wednesday July 13th. The session will have a diverse repertoire of their research fields including biology, astronomy, physics, chemistry, informatics, nanoscience, and so on. We would appreciate your visit to the posters and discussions with students in the main lobby of Koyu Hall (No. 1 in the campus map) and Agora West (2nd floor of iCommons, No. 2 in the map).

Wireless Internet Access

The Meeting venue is provided with free wireless internet service. Passwords will be provided at the reception desk.

Oral Presentations

All talks will be presented by sharing the presentation slides in zoom meeting, irrespective of whether speakers are on site or not. 29 min (24 min talk and 5 min discussion) is assigned for PI, and 14 min (11 min talk and 3 min discussion) for students, postdocs, and those who chose the 15-

min option. No poster session is planned in this meeting. It was hard to plan poster session online because of time differences on the globe.

Lunches and Coffee Breaks

Lunch is NOT included in the registration fee. Lunch boxes will be available at a reasonable price at the reception desk of the meeting. Coffee and snacks are included in the registration fee and will be served in afternoon coffee breaks. A cafeteria and a convenience store are also available in the meeting venue (iCommons, No. 2 in the campus map). Eating and drinking will not be permitted in the meeting room.

Excursion

A short excursion around Kobe will be set on 14th at 12:30-15:30. Details of the excursion will be announced during the meeting. Tentatively, visiting Kobe Animal Kingdom is under planning.
https://en.kobe-oukoku.com/

Farewell party

A closing dinner will be held on July 15 at 18:00 at the typical Japanese restaurant "SAKABAYASHI" (Kobe Shushin-Kan: https://www.enjoyfukuju.com/en/visitus/) located about 10 min drive from the meeting venue. A chartered bus will be arranged from the meeting venue to "SAKABAYASHI." SAKABAYASHI is owned by one of the famous SAKE breweries of Nadagogou. Its flag-ship bland SAKE "FUKUJU(福寿)" was served at the Nobel Prize award ceremonies and banquets. Reservation is necessary. Please contact the organizing committee if you have not booked it but would like to join. Details will be announced during the meeting.

General Cautions

Please do not spread the information on Zoom and on demand viewing to those who have not registered. Recording of the streamed presentations is prohibited.

On Site Participation

Do not connect your computers to Wi-Fi in the meeting room. It will exert pressure on Wi-Fi capacity in the room. When talks are given in Zoom by online speakers, the talks are also projected on the screen in the meeting room. So just behave as in ordinary on-site meeting. You may ask

questions using microphones in the room. All talks are live-streamed on Zoom and recorded for on demand viewing.

Zoom Real Time Participation

Information on access to Zoom meeting will be provided by email before the meeting starts. Please turn off the microphone and video of Zoom. To ask questions, please click “rise hand button” or write down your questions in chat of Zoom.

Speakers: Please access the Zoom before the session starts. You may check your internet access speed using the following website. At least 100 Mbps is required. More than 200 Mbps is desired. https://fast.com/ja/

On Demand Viewing

Zoom movies of talks will be recorded in real time during the meeting days and will be uploaded to Vimeo website for on-demand viewing as soon as we can. However, one or two-day lag will happen after the talk. The movies will be available for a month after the meeting.

How to start on demand viewing:

Access to the ITM website (https://sites.google.com/view/11th-itm/). Click “On-demand viewing” tab on upper right. This is necessary. One cannot find the meeting movies by searching at Vimeo website. The talks are ordered in two ways, in chronological order (D1-01 to D4-17) and based on topic categories (Genomics, Gene regulation, Evolution and taxonomy, Asexual reproduction and regeneration, Developmental and cell biology, Post-embryonic development, Nervous system, Imaging and modelling). Click a talk that you want to look at. Then, type the password. The password will be announced by email before the meeting starts.

Questions, comments, and answers in Vimeo

You may make comments and ask questions to speakers using comment window in Vimeo. To do this, you have to first register to Vimeo as shown in the prompt window when you click comment window in Vimeo. To answer comments, it is the same. We would like the speakers to access your Vimeo-movie site and check questions several days after the meeting.

Efforts to Prevent the Spread of COVID-19

When you have fever or feel sick, stay in the hotel room. Please call meeting organizers (Takehiro Kusakabe, phone 090-5052-8030). Then, we will arrange and instruct how you should do.

The event will be held after sufficient efforts have been taken to prevent infection, including body temperature checks, disinfection, wearing of masks, and reducing close contact between people. In Japan, wearing of masks is not an obligation in outdoor, but most of people are still wearing masks even in outdoor at the moment.

Guests who should refrain from attending on site

We request that those who fall under any of the following categories refrain from participating on site. We especially ask that those at high risk of serious illness (the elderly and those with a history of illness) take a cautious approach.

- Has a fever that is higher than normal.
- Has symptoms of cough, dyspnea, general malaise, sore throat, nasal discharge/nasal obstruction, taste or smell disorder, eye pain or conjunctival redness, headache, joint or muscle pain, diarrhea, nausea, or vomiting.
- Has had close contact with a person who has tested positive for novel coronavirus infection within the past one week.

Infection control in the venue

- Body temperature has to be checked prior to the visit; those with a temperature of 37.5 degrees Celsius or higher will not be allowed to enter the venue.
- Please disinfect your hands upon entering the venue.
- Please wear a mask and practice good cough etiquette in the venue. Please do not enter the venue if you are not wearing a mask. If you do not have a mask, please inform the staff. We ask that you wear a non-woven or other mask of reliable effectiveness.
- In the event of an outbreak of infection, we may provide your name and contact information to public health centers as necessary.

Check List

(1) I accept that if I become a close contact person, I may be asked to stay in hotel room for up to one week after the contact.

(2) In the event that a cluster occurs among the participants, I will consent to the disclosure of the contact information to health centers, and I will cooperate in follow-up investigations.

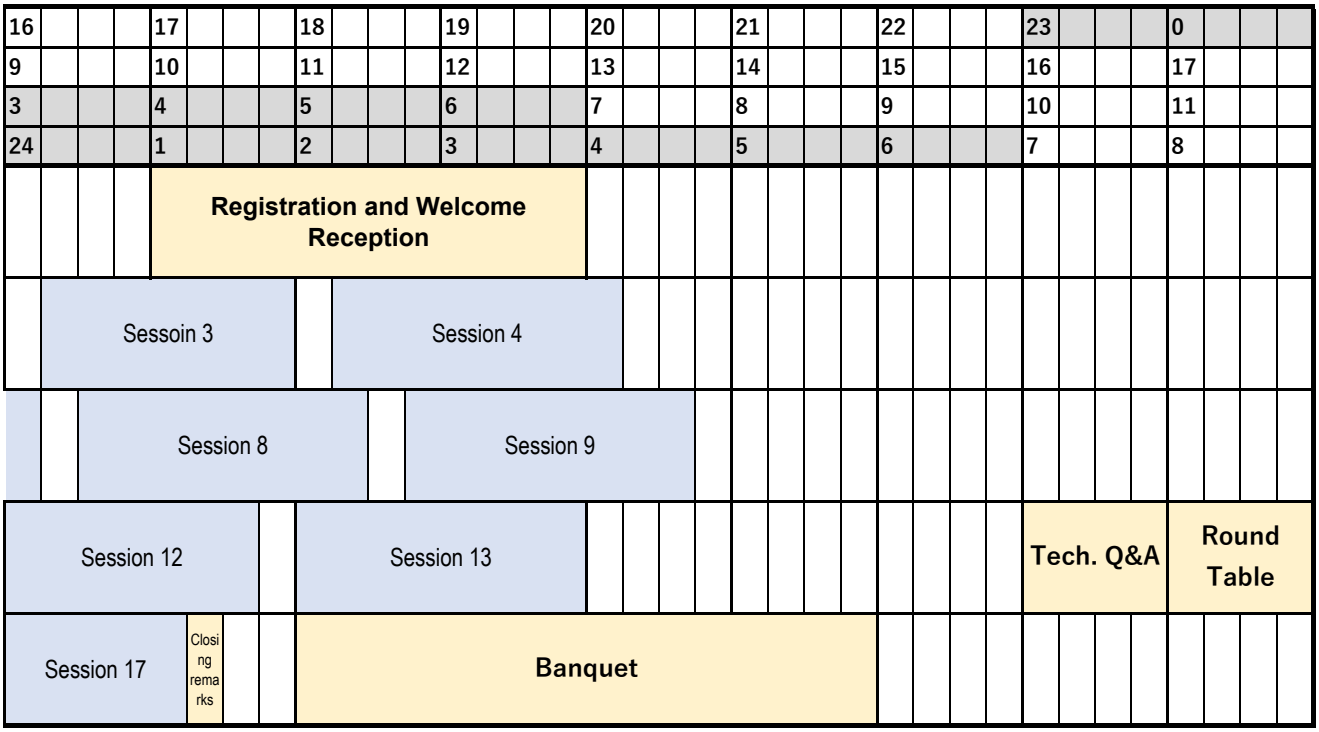
(3)I accept that there is a possibility that admission to the venue may be restricted, or changes may be made to the program, participation, etc., in the event of congestion. In such cases, I will follow the instructions and guidance of the organizers, venue managers and venue staffs.

Program

2022 11th ITM Time Table

Time Zone	Kobe	8				9				10				11				12				13				14				15			
	Paris	1				2				3				4				5				6				7				8			
	NY	19				20				21				22				23				24				1				2			
	LA	16				17				18				19				20				21				22				23			
Date	Monday July 11																																
	Tuesday July 12					Opening Remarks			Session 1					Technical Session												Session 2							
	Wednesday July 13					Session 5								Session 6										Konan Poster Session				Session 7			
	Thursday July 14					Session 10								Session 11						Short Excursion												
	Friday July 15					Session 14								Session 15												Session 16							

Public Events
Scientific Sessions
Bedtime


16 17 18 19 20 21 22 23 0
9 10 11 12 13 14 15 16 17
3 4 5 6 7 8 9 10 11
24 1 2 3 4 5 6 7 8
Registration and Welcome Reception
Sessoin 3
Session 4
Session 8
Session 9
Session 12
Session 13
Tech. Q&A
Round Table
Session 17
Closing remarks
Banquet

PROGRAM

Monday, July 11, 2022

17:00-20:00 **Reception and Welcome Party**

Tuesday, July 12, 2022

9:00-9:30 **Opening Remarks**

9:30-10:30	**Session 1**	(Chair: Lionel Christiaen)
9:30-10:00	D1-01	**Bradley Davidson** (Online): *Synergistic modulation of mitotic receptor trafficking through polarized degradation and recycling*
10:00-10:30	D1-02	**Alberto Stolfi** (Online): *Evolution of a chordate-specific mechanism for myoblast fusion*

10:30-12:30	**Technical Session** (Chairs: Takeo Horie and Alberto Stolfi)	
10:30-10:35	Tech-01	**Alberto Stolfi** (Online): *Opening remarks*
10:35-12:05	Tech-02	**Chen Cao** (Online): *Single-Cell Transcriptional Analysis*
12:05-12:25	Tech-03	**Takeo Horie** (Onsite): *Regulatory cocktail for dopaminergic neurons in ascidian identified by single cell transcriptomics*
12:25-12:30	Tech-04	**Takeo Horie** (Onsite): *Closing remarks*

12:30-14:00 LUNCH

14:00-16:00	**Session 2**	(Chair: Atsuko Sato)
14:00-14:30	D1-03	**Hitoshi Sawada** (Onsite): *Sperm astacin-like metalloproteases are involved in sperm penetration through the vitelline coat during ascidian fertilization*
14:30-14:45	D1-04	**Takako Saito** (Onsite): *Insights into self-incompatibility by structural modelling*
14:45-15:15	D1-05	**Manabu Yoshida** (Onsite): *Plasma membrane-type Ca2+/ATPase and CatSper contribute to ascidian sperm chemotaxis*
15:15-15:45	D1-06	**Serina Lee** (Online): *Fertilization and embryogenesis of two tropical ascidians in varied salinities and temperatures*
15:45-16:00	D1-07	**Daisuke Shimizu** (Onsite): *Photoreceptive tissues and photoresponse in the adult of Ciona intestinalis type A*

16:00-16:15 BREAK

16:15-18:00	**Session 3**	(Chair: Manabu Yoshida)
16:15-16:45	D1-08	**Hiroki Nishida** (Onsite): *Ascidian gastrulation and blebbing activity of isolated endoderm blastomeres*
16:45-17:00	D1-09	**Yuki Kogure** (Onsite): *Admp controls the pMLC localization to the apical side of the ventral epidermal cells and leads the ventral tail bending in ascidian tailbud embryo*
17:00-17:15	D1-10	**Lisa Thomann** (Online): *Role of Pi3K signaling during Phallusia mammillata endoderm invagination*
17:15-17:30	D1-11	**Ayana Maruo** (Onsite): *Molecular and functional diversity of the nerve cord ependymal cells in Ciona larvae*
17:30-17:45	D1-12	**Nanako Okawa** (Onsite): *Investigation of neuron-glia interactions using a combination of optogenetics and calcium imaging in Ciona swimming larvae*
17:45-18:00	D1-13	**Athira Athira** (Online): *Comprehensive analysis of behavioral dynamics in the protochordate Ciona intestinalis*
18:00-18:15	BREAK	
18:15-20:15	**Session 4**	(Chair: Charles Plessy)
18:15-18:45	D1-14	**Joel Glover** (Online): *Dopaminergic neurons in Oikopleura dioica: Connectivity and regulation of movement states*
18:45-19:15	D1-15	**Takeshi Onuma** (Onsite): *Transcriptomes of the fast-evolving chordate, Oikopleura dioica, uncover drastic difference in transcription factor and post-plasmic RNA composition that is expressed in early embryos from those of ascidians*
19:15-19:45	D1-16	**Thomas Stach** (Online): *Beyond Oikopleura dioica - evolution of appendicularian diversity*
19:45-20:15	D1-17	**Cristian Canestro** (Online): *Deconstruction of the cardiopharyngeal gene regulatory network in appendicularians and the evolution of their complete free-living lifestyle from an ascidian-like biphasic ancestral tunicate*

Wednesday, July 13, 2022

9:00-10:15	**Session 5**	(Chair: Loriano Ballarin)
9:00-9:30	D2-01	**Ayelet Voskoboynik** (Online): *The ticking clock, an aging study of a colonial chordate linking stem cell aging to molecular decline of circadian regulation*
9:30-9:45	D2-02	**Chiara Anselmi** (Online): *Botryllus schlosseri, an emerging evo-devo model for the study of neurogenesis, neurodegeneration, and aging*
9:45-10:00	D2-03	**Henry Rodriguez-Valbuena** (Online): *Evolutionary origin and genomic dynamics of Botryllus schlosseri allorecognition genes*
10:00-10:15	D2-04	**Laurel Hiebert** (Online): *Comparing dormancy in two distantly related tunicates*
10:15-10:30	BREAK	
10:30-12:30	**Session 6**	(Chair: Noa Shenkar)
10:30-10:45	D2-05	**Marcel Velasquez Sayago** (Online): *Oceanographic drivers of ascidian biogeographic patterns (Tunicata: Ascidiacea) along the Western Atlantic coast*
10:45-11:15	D2-06	**Atsuko Sato** (Onsite): *How developmental buffering stabilizes development in the face of thermal stress?*
11:15-11:45	D2-07	**Shuichi Wada** (Onsite): *Towards an understanding of detoxification mechanisms induced by polycyclic aromatic hydrocarbons in the ascidian Ciona intestinalis.*
11:45-12:15	D2-08	**Euichi Hirose** (Onsite): *Nipple array of tunic cuticle: a review of the multifunctional nanostructure under water*
12:15-12:30	D2-09	**Naohiro Hasegawa** (Onsite): *Graveyards of Giant Pandas at the Bottom of the Sea? A Strange-Looking Undescribed Species of Colonial Ascidians in the Genus Clavelina (Tunicata: Ascidiacea)*
12:30-13:30	LUNCH	
13:30-15:00	**11th ITM-KONAN Multidisciplinary Poster Session**	
15:00-16:15	**Session 7**	(Chair: Honoo Satake)
15:00-15:30	D2-10	**Yasunori Sasakura** (Onsite): *G-protein signaling relay promotes Ciona metamorphosis*
15:30-15:45	D2-11	**Xueping Sun** (Online): *A microRNA Cluster-Lefty Pathway is Required for Cellulose Synthesis During Ascidian Larval Metamorphosis*
15:45-16:00	D2-12	**Nozomu Totsuka** (Onsite): *Timing of replacement of the non-self-test cells and the self-tunic cells during Ciona metamorphosis*
16:00-16:15	D2-13	**Gabriel Krasovec** (Online): *D-serine controls epidermal vesicle release via NMDA receptor allowing tissue migration during the metamorphosis of Ciona*

16:15-16:30	BREAK	
16:30-18:30	**Session 8**	(Chair: Roberta Pennati)
16:30-17:00	D2-14	**Simon Blanchoud** (Online): *In-lab breeding of Botrylloides diegensis requires a suitable marine microbiome*
17:00-17:15	D2-15	**Berivan Temiz** (Online): *Characterization of Botrylloides diegensis whole body regeneration through single-cell RNA-sequencing*
17:15-17:45	D2-16	**Lucia Manni** (Onsite): *Stem cell niche characterization in Botryllus schlosseri*
17:45-18:15	D2-17	**Maria Sirakov** (Online): *Ciona robusta as model system for Intestinal Stem Cells biology*
18:15-18:30	D2-18	**Tal Gordon** (Online): *Insights into the cellular and molecular mechanisms of Polycarpa mytiligera's central nervous system regeneration*
18:30-18:45	BREAK	
18:45-20:45	**Session 9**	(Chair: Lucia Manni)
18:45-19:15	D2-19	**Loriano Ballarin** (Onsite): *The complement system of ascidians: clues from the colonial ascidian Botryllus schlosseri*
19:15-19:45	D2-20	**Marta Pascual** (Online): *Lessons from the invasive species Styela plicata. From the genome to the holobiome*
19:45-20:00	D2-21	**Laura Drago** (Onsite): S*tress granules in ascidians: an overview*
20:00-20:30	D2-22	**Noa Shenkar** (Onsite): *Ascidians as model organisms for assessing the extent and impact of anthropogenic pollutants in marine environments*
20:30-20:45	D2-23	**Raz Platin** (Online): *Styela on the edge: life cycle and reproductive patterns of the solitary ascidian Styela plicata along the Mediterranean coasts of Israel*

Thursday, July 14, 2022

9:00-10:15	**Session 10**	(Chair: Kohji Hotta)
9:00-9:30	D3-01	**William Smith** (Online): *The mutation frimousse uncovers a cryptic oscillator that drives spontaneous orienting behavior*
9:30-9:45	D3-02	**Jeneva Chung** (Online): *Previously uncharacterized inhibitory autonomous oscillator revealed by frimousse mutants*
9:45-10:15	D3-03	**Yasushi Okamura** (Onsite): *Tracing back for ancestry of vertebrate neuronal traits: clusters of sodium channels*
10:15-10:30	BREAK	
10:30-11:45	**Session 11**	(Chair: Yasushi Okamura)
10:30-10:45	D3-04	**Asuka Chokki** (Onsite): *Gravitaxis neural circuit in the ascidian larva*
10:45-11:15	D3-05	**Kohji Hotta** (Onsite): *A single motor neuron determines the rhythm of early motor behavior in Ciona*
11:15-11:30	D3-06	**Yishen Miao** (Online): *The modeling of spontaneous orienting swim in Ciona based on the interaction of two oscillators*
11:30-11:45	D3-07	**Kwantae Kim** (Onsite): *Transcriptional regulation of neuronal polarity and neurite outgrowth in the ddNs of Ciona*
11:45-16:00	LUNCH and Short Excursion	
16:00-17:45	**Session 12**	(Chair: Shuichi Wada)
16:00-16:30	D3-08	**Hidehiko Hashimoto** (Onsite): *Dynamic integration of signaling, force generation and tissue remodeling control zippering and neural tube closure*
16:30-17:00	D3-09	**Clare Hudson** (Online): *An ERF transcription factor participates in the bimodal transcriptional response of an ERK-target gene during ascidian neural induction*
17:00-17:15	D3-10	**Rossana Bettoni** (Online): *Computational model of neural induction in the ascidian embryo*
17:15-17:45	D3-11	**Sebastien Darras** (Onsite): *Highly divergent genetic programs for peripheral nervous system formation in chordates*
17:45-18:00	BREAK	

18:00-20:00	**Session 13**	(Chair: Takeshi Onuma)
18:00-18:15	D3-12	**Aki Masunaga** (Onsite): *Oikopleura dioica, the cosmopolitan appendicularian hides multiple cryptic species around the globe*
18:15-18:45	D3-13	**Charles Plessy** (Onsite): *Drastic genome remodeling across Oikopleura dioica worldwide*
18:45-19:00	D3-14	**Michael Mansfield** (Onsite): *Gene expression in the context of genomic rearrangement in Oikopleura dioica*
19:00-19:15	D3-15	**Alessandro Pennati** (Onsite): *Hmx gene conservation identifies the origin of vertebrate cranial ganglia*
19:15-19:30	D3-16	**Cristian Velandia Huerto** (Online): *A minimalist genomic toolbox? The case of bona fide microRNA evolution on tunicates*
19:30-20:00	D3-17	**Emmanuel Faure** (Online): *MorphoNet*

23:00-24:00 **Technical Session Online Q and A**

24:00-25:00 **Round Table Discussions**

Friday, July 15, 2022

9:00-10:30	**Session 14**	(Chair: Toshio Sekiguchi)
9:00-9:15	D4-01	**Cezar Borba** (Online): *Cionectome: An interactive viewer and analysis tool for the Ciona connectome*
9:15-9:30	D4-02	**Matthew Kourakis** (Online): *The Ciona AMG neuron complex and the origins of the cerebellum*
9:30-9:45	D4-03	**Hannah Gruner** (Online): *Neural Innervation of the Juvenile and Adult heart of Ciona robusta*
9:45-10:00	D4-04	**C. J. Pickett** (Online): *Dolioletta gegenbauri: a model tunicate for investigating the evolution of novel traits*
10:00-10:30	D4-05	**Honoo Satake** (Onsite): *Neuropeptides, peptide hormones, their receptors and biological roles in Ciona: new aspects of peptidergic systems in chordates*
10:30-10:45	BREAK	
10:45-12:30	**Session 15**	(Chair: Sebastien Darras)
10:45-11:15	D4-06	**Megan Wilson** (Online): *Using ATAC-sequencing to study chromatin accessibility during early whole body regeneration*
11:15-11:30	D4-07	**Satoshi Otaki** (Onsite): *Repetitive sequence DNA/TcMar-Tc1 is enriched at the boundaries of cis-regulatory regions in Ciona*
11:30-12:00	D4-08	**Jiankai Wei** (Online): *Spatiotemporal dynamics of zygotic genome activation in basal chordate revealed by interspecific hybrids*
12:00-12:30	D4-09	**Yutaka Satou** (Online): *The gene regulatory system in 32-cell embryos / the developmental program for muscle fate specification / new genome assemblies for Ciona*
12:30-14:00	LUNCH	
14:00-15:45	**Session 16**	(Chair: Hidehiko Hashimoto)
14:00-14:30	D4-10	**Wei Wang** (Online): *Primed for fate determination ? the transcriptional regulatory mechanism underlying the cardio-pharyngeal multipotent progenitor maturation*
14:30-15:00	D4-11	**Lionel Christiaen** (Onsite): *Coupling cell cycle progression, multipotent progenitor maturation and fate choices in the cardiopharyngeal lineage of a simple chordate*
15:00-15:15	D4-12	**Hongzhe Peng** (Online): *Supra extracellular bridge in Ciona notochord elongation: structure and function*
15:15-15:30	D4-13	**Wenjie Shi** (Online): *Rho GTPase signaling-depended cortex tension regulates notochord lumen growth in Ciona embryogenesis*
15:30-15:45	D4-14	**Zonglai Liang** (Online): *Ano10 mediates convergent extension and tubulogenesis during notochord formation*

15:45-16:00 BREAK

16:00-17:15 **Session 17** (Chair: Shumpei Morita)

16:00-16:15 D4-15 **Xin Zeng** (Onsite): *Computational Inference of Gene Regulatory Network in the ascidian brain by single cell RNA-seq data*

16:15-16:45 D4-16 **Remi Dumollard** (Online): *Ascidian invariant cleavage*

16:45-17:15 D4-17 **Patrick Lemaire** (Online): *Multi-modal single-cell biology of the embryogenesis of the ascidian, Phallusia mammillata*

17:15-17:30 **Closing Remarks**

18:00-22:00 **Banquet**

PRESENTATION LIST SORTED BY TOPICS

ASEXUAL REPRODUCTION AND REGENERATION

Date of Presentation	Presentation ID	Speaker and Title
July 13, 9:00-9:30	D2-01	**Ayelet Voskoboynik** (Online): *The ticking clock, an aging study of a colonial chordate linking stem cell aging to molecular decline of circadian regulation*
July 13, 10:00-10:15	D2-04	**Laurel Hiebert** (Online): *Comparing dormancy in two distantly related tunicates*
July 13, 16:30-17:00	D2-14	**Simon Blanchoud** (Online): *In-lab breeding of Botrylloides diegensis requires a suitable marine microbiome*
July 13, 17:00-17:15	D2-15	**Berivan Temiz** (Online): *Characterization of Botrylloides diegensis whole body regeneration through single-cell RNA-sequencing*
July 13, 17:15-17:45	D2-16	**Lucia Manni** (Onsite): *Stem cell niche characterization in Botryllus schlosseri*
July 13, 18:15-18:30	D2-18	**Tal Gordon** (Online): *Insights into the cellular and molecular mechanisms of Polycarpa mytiligera's central nervous system regeneration*

DEVELOPMENTAL AND CELL BIOLOGY

July 12, 9:30-10:00	D1-01	**Bradley Davidson** (Online): *Synergistic modulation of mitotic receptor trafficking through polarized degradation and recycling*
July 12, 14:00-14:30	D1-03	**Hitoshi Sawada** (Onsite): *Sperm astacin-like metalloproteases are involved in sperm penetration through the vitelline coat during ascidian fertilization*
July 12, 14:30-14:45	D1-04	**Takako Saito** (Onsite): *Insights into self-incompatibility by structural modelling*
July 12, 14:45-15:15	D1-05	**Manabu Yoshida** (Onsite): *Plasma membrane-type Ca2+/ATPase and CatSper contribute to ascidian sperm chemotaxis*
July 12, 16:15-16:45	D1-08	**Hiroki Nishida** (Onsite): *Ascidian gastrulation and blebbing activity of isolated endoderm blastomeres*
July 12, 16:45-17:00	D1-09	**Yuki Kogure** (Onsite): *Admp controls the pMLC localization to the apical side of the ventral epidermal cells and leads the ventral tail bending in ascidian tailbud embryo*
July 12, 17:00-17:15	D1-10	**Lisa Thomann** (Online): *Role of Pi3K signaling during Phallusia mammillata endoderm invagination*
July 12, 18:45-19:15	D1-15	**Takeshi Onuma** (Onsite): *Transcriptomes of the fast-evolving chordate, Oikopleura dioica, uncover drastic difference in transcription factor and post-plasmic RNA composition that is expressed in early embryos from those of ascidians*
July 13, 9:30-9:45	D2-02	**Chiara Anselmi** (Online): *Botryllus schlosseri, an emerging evo-devo model for the study of neurogenesis, neurodegeneration, and aging*
July 13, 10:45-11:15	D2-06	**Atsuko Sato** (Onsite): *How developmental buffering stabilizes development in the face of thermal stress?*
July 13, 15:30-15:45	D2-11	**Xueping Sun** (Online): *A microRNA Cluster-Lefty Pathway is Required for Cellulose Synthesis During Ascidian Larval Metamorphosis*
July 13, 19:45-20:00	D2-21	**Laura Drago** (Onsite): S*tress granules in ascidians: an overview*
July 14, 16:30-17:00	D3-09	**Clare Hudson** (Online): *An ERF transcription factor participates in the bimodal transcriptional response of an ERK-target gene during ascidian neural induction*
July 14, 17:15-17:45	D3-11	**Sebastien Darras** (Onsite): *Highly divergent genetic programs for peripheral nervous system formation in chordates*

July 14, 19:00-19:15 D3-15 **Alessandro Pennati** (Onsite): *Hmx gene conservation identifies the origin of vertebrate cranial ganglia*

July 15, 9:45-10:00 D4-04 **C. J. Pickett** (Online): *Dolioletta gegenbauri: a model tunicate for investigating the evolution of novel traits*

July 15, 11:30-12:00 D4-08 **Jiankai Wei** (Online): *Spatiotemporal dynamics of zygotic genome activation in basal chordate revealed by interspecific hybrids*

July 15, 14:00-14:30 D4-10 **Wei Wang** (Online): *Primed for fate determination ? the transcriptional regulatory mechanism underlying the cardio-pharyngeal multipotent progenitor maturation*

July 15, 14:30-15:00 D4-11 **Lionel Christiaen** (Onsite): *Coupling cell cycle progression, multipotent progenitor maturation and fate choices in the cardiopharyngeal lineage of a simple chordate*

July 15, 15:00-15:15 D4-12 **Hongzhe Peng** (Online): *Supra extracellular bridge in Ciona notochord elongation: structure and function*

July 15, 15:15-15:30 D4-13 **Wenjie Shi** (Online): *Rho GTPase signaling-depended cortex tension regulates notochord lumen growth in Ciona embryogenesis*

July 15, 15:30-15:45 D4-14 **Zonglai Liang** (Online): *Ano10 mediates convergent extension and tubulogenesis during notochord formation*

July 15, 16:15-16:45 D4-16 **Remi Dumollard** (Online): *Ascidian invariant cleavage*

ECOLOGY AND ENVIRONMENT

July 12, 15:15-15:45 D1-06 **Serina Lee** (Online): *Fertilization and embryogenesis of two tropical ascidians in varied salinities and temperatures*

July 12, 15:45-16:00 D1-07 **Daisuke Shimizu** (Onsite): *Photoreceptive tissues and photoresponse in the adult of Ciona intestinalis type A*

July 13, 10:30-10:45 D2-05 **Marcel Velasquez Sayago** (Online): *Oceanographic drivers of ascidian biogeographic patterns (Tunicata: Ascidiacea) along the Western Atlantic coast*

July 13, 11:15-11:45 D2-07 **Shuichi Wada** (Onsite): *Towards an understanding of detoxification mechanisms induced by polycyclic aromatic hydrocarbons in the ascidian Ciona intestinalis*

July 13, 11:45-12:15 D2-08 **Euichi Hirose** (Onsite): *Nipple array of tunic cuticle: a review of the multifunctional nanostructure under water*

July 13, 20:00-20:30 D2-22 **Noa Shenkar** (Onsite): *Ascidians as model organisms for assessing the extent and impact of anthropogenic pollutants in marine environments*

July 13, 20:30-20:45 D2-23 **Raz Platin** (Online): *Styela on the edge: life cycle and reproductive patterns of the solitary ascidian Styela plicata along the Mediterranean coasts of Israel*

EVOLUTION AND TAXONOMY

July 12, 19:15-19:45 D1-16 **Thomas Stach** (Online): *Beyond Oikopleura dioica - evolution of appendicularian diversity*

July 12, 19:45-20:15 D1-17 **Cristian Canestro** (Online): *Deconstruction of the cardiopharyngeal gene regulatory network in appendicularians and the evolution of their complete free-living lifestyle from an ascidian-like biphasic ancestral tunicate*

July 13, 12:15-12:30 D2-09 **Naohiro Hasegawa** (Onsite): *Graveyards of Giant Pandas at the Bottom of the Sea? A Strange-Looking Undescribed Species of Colonial Ascidians in the Genus Clavelina (Tunicata: Ascidiacea)*

July 14, 18:00-18:15 D3-12 **Aki Masunaga** (Onsite): *Oikopleura dioica, the cosmopolitan appendicularian hides multiple cryptic species around the globe*

July 15, 9:15-9:30 D4-02 **Matthew Kourakis** (Online): *The Ciona AMG neuron complex and the origins of the cerebellum*

GENE REGULATION

July 15, 10:45-11:15 D4-06 **Megan Wilson** (Online): *Using ATAC-sequencing to study chromatin accessibility during early whole body regeneration*

July 15, 12:00-12:30 D4-09 **Yutaka Satou** (Online): *The gene regulatory system in 32-cell embryos / the developmental program for muscle fate specification / new genome assemblies for Ciona*

GENOME AND GENOMICS

July 13, 19:15-19:45 D2-20 **Marta Pascual** (Online): *Lessons from the invasive species Styela plicata. From the genome to the holobiome*

July 14, 18:15-18:45 D3-13 **Charles Plessy** (Onsite): *Drastic genome remodeling across Oikopleura dioica worldwide*

July 14, 18:45-19:00 D3-14 **Michael Mansfield** (Onsite): *Gene expression in the context of genomic rearrangement in Oikopleura dioica*

July 14, 19:15-19:30 D3-16 **Cristian Velandia Huerto** (Online): *A minimalist genomic toolbox? The case of bona fide microRNA evolution on tunicates*

July 15, 11:15-11:30 D4-07 **Satoshi Otaki** (Onsite): *Repetitive sequence DNA/TcMar-Tc1 is enriched at the boundaries of cis-regulatory regions in Ciona*

July 15, 16:00-16:15 D4-15 **Xin Zeng** (Onsite): *Computational Inference of Gene Regulatory Network in the ascidian brain by single cell RNA-seq data*

IMAGING AND MODELLING

July 14, 16:00-16:30 D3-08 **Hidehiko Hashimoto** (Onsite): *Dynamic integration of signaling, force generation and tissue remodeling control zippering and neural tube closure*

July 14, 17:00-17:15 D3-10 **Rossana Bettoni** (Online): *Computational model of neural induction in the ascidian embryo*

July 14, 19:30-20:00 D3-17 **Emmanuel Faure** (Online): *MorphoNet*

July 15, 9:00-9:15 D4-01 **Cezar Borba** (Online): *Cionectome: An interactive viewer and analysis tool for the Ciona connectome*

July 15, 16:45-17:15 D4-17 **Patrick Lemaire** (Online): *Multi-modal single-cell biology of the embryogenesis of the ascidian, Phallusia mammillata*

IMMUNE SYSTEM

July 13, 9:45-10:00 D2-03 **Henry Rodriguez-Valbuena** (Online): *Evolutionary origin and genomic dynamics of Botryllus schlosseri allorecognition genes*

July 13, 18:45-19:15 D2-19 **Loriano Ballarin** (Onsite): *The complement system of ascidians: clues from the colonial ascidian Botryllus schlosseri*

NEURAL DEVELOPMENT AND PHYSIOLOGY

July 12, 17:15-17:30 D1-11 **Ayana Maruo** (Onsite): *Molecular and functional diversity of the nerve cord ependymal cells in Ciona larvae*

July 12, 17:30-17:45	D1-12	**Nanako Okawa** (Onsite): *Investigation of neuron-glia interactions using a combination of optogenetics and calcium imaging in Ciona swimming larvae*
July 12, 17:45-18:00	D1-13	**Athira Athira** (Online): *Comprehensive analysis of behavioral dynamics in the protochordate Ciona intestinalis*
July 12, 18:15-18:45	D1-14	**Joel Glover** (Online): *Dopaminergic neurons in Oikopleura dioica: Connectivity and regulation of movement states*
July 13, 15:00-15:30	D2-10	**Yasunori Sasakura** (Onsite): *G-protein signaling relay promotes Ciona metamorphosis*
July 14, 9:00-9:30	D3-01	**William Smith** (Online): *The mutation frimousse uncovers a cryptic oscillator that drives spontaneous orienting behavior*
July 14, 9:30-9:45	D3-02	**Jeneva Chung** (Online): *Previously uncharacterized inhibitory autonomous oscillator revealed by frimousse mutants*
July 14, 9:45-10:15	D3-03	**Yasushi Okamura** (Onsite): *Tracing back for ancestry of vertebrate neuronal traits: clusters of sodium channels*
July 14, 10:30-10:45	D3-04	**Asuka Chokki** (Onsite): *Gravitaxis neural circuit in the ascidian larva*
July 14, 10:45-11:15	D3-05	**Kohji Hotta** (Onsite): *A single motor neuron determines the rhythm of early motor behavior in Ciona*
July 14, 11:15-11:30	D3-06	**Yishen Miao** (Online): *The modeling of spontaneous orienting swim in Ciona based on the interaction of two oscillators*
July 14, 11:30-11:45	D3-07	**Kwantae Kim** (Onsite): *Transcriptional regulation of neuronal polarity and neurite outgrowth in the ddNs of Ciona*
July 15, 10:00-10:30	D4-05	**Honoo Satake** (Onsite): *Neuropeptides, peptide hormones, their receptors and biological roles in Ciona: new aspects of peptidergic systems in chordates.*

POST-EMBRYONIC DEVELOPMENT

July 12, 10:00-10:30	D1-02	**Alberto Stolfi** (Online): *Evolution of a chordate-specific mechanism for myoblast fusion*
July 13, 15:45-16:00	D2-12	**Nozomu Totsuka** (Onsite): *Timing of replacement of the non-self-test cells and the self-tunic cells during Ciona metamorphosis*
July 13, 16:00-16:15	D2-13	**Gabriel Krasovec** (Online): *D-serine controls epidermal vesicle release via NMDA receptor allowing tissue migration during the metamorphosis of Ciona*
July 13, 17:45-18:15	D2-17	**Maria Sirakov** (Online): *Ciona robusta as model system for Intestinal Stem Cells biology*
July 15, 9:30-9:45	D4-03	**Hannah Gruner** (Online): *Neural Innervation of the Juvenile and Adult heart of Ciona robusta*

TECHNICAL SESSION

July 12, 10:30-10:35	Tech-01	**Alberto Stolfi** (Online): *Opening remarks*
July 12, 10:35-12:05	Tech-02	**Chen Cao** (Online): *Single-Cell Transcriptional Analysis*
July 12, 12:05-12:25	Tech-03	**Takeo Horie** (Onsite): *Regulatory cocktail for dopaminergic neurons in ascidian identified by single cell transcriptomics*
July 12, 12:25-12:30	Tech-04	**Takeo Horie** (Onsite): *Closing remarks*
July 14, 23:00-24:00		*Online Q and A*

ROUND TABLE DISCUSSION

July 14, 24:00-25:00 **The ANISEED Team**: *Towards a tunicate community data management plan*

Technical Session

July 12th, 10:30-12:30, Technical Session
July 14th, 23:00-24:00, Online Question Session

Opening by Alberto Stolfi (Georgia Institute of Technology)

Single-Cell Transcriptional Analysis

Chen Cao*
Lewis-Sigler Institute for Integrative Genomics, Carl Icahn Laboratory, Princeton University

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The development of single cell sequencing technology provides valuable insights into cells previously unachieved by bulk cell analysis, and has revolutionizing our understanding of many biological processes. Here I will provide a broad introduction to transcriptome analysis at the single-cell level by presenting the state-of-the-art in method development, technology platforms, computational analysis methods and data application, while highlighting the considerations for designing and interpreting a study using single-cell RNA sequencing.

Regulatory cocktail for dopaminergic neurons in ascidian identified by single cell transcriptomics

Takeo Horie*
Laboratory for single-cell Neurobiology, Graduate School of Frontier Biosciences, Osaka University

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Dopaminergic (DA) neurons mediate a variety of reward behavior in vertebrate. There are several classes of DA neurons in vertebrate brain including midbrain DA neurons and hypothalamus. Characterization of the molecular programs controlling differentiation of DA neurons is crucial for understanding this important neuronal cell type. For this purpose, we sought to identify regulatory genes for differentiation of DA neurons in the ascidian, *Ciona intestinalis*.

The CNS of the *Ciona* consists of 177 neurons that share a number of similarities with vertebrate brains. *Ciona* larvae contains single cluster of DA neurons which located in ventral region of the sensory vesicle. Here we perform whole embryos single cell RNA-seq assays to elucidate the regulatory network underlying the specification of DA neurons. We identified the transcription factor *Ptf1a* is the most strongly expressed cell-specific transcription factor in DA neurons. Knock down experiment of *Ptf1a* results in loss of DA neurons, while misexpression of *Ptf1a* results in the appearance of supernumerary DA neurons. Photoreceptor cells and ependymal cells are the most susceptible to transformation, and both cell types express high levels of *Meis*. Co-expression of both *Ptf1a* and *Meis* caused the wholesale transformation of the entire CNS into DA neurons. We therefore suggest that the reiterative use of functional manipulations and single cell RNA-seq assays is an effective means for the identification of regulatory cocktails underlying the specification of specific cell identities.

Closing by Takeo Horie (Osaka Univ.)

Round Table Discussion

July 14th, 24:00-25:00

Towards a tunicate community data management plan

The ANISEED Team[1,2]

1. Lemaire Team, CRBM, CNRS, 1919 Rte de Mende, 34293 Montpellier, France
2. Bioself Communication, 502B route de l'esterel, 83600 Les Adrets-de-l'Esterel, France

The biomedical literature is currently marked by an extreme fragmentation of published research data, which makes it singularly difficult to aggregate, to integrate into a broad context and to reuse, particularly in the context of artificial intelligence projects. While large-scale genomic data are generally formatted according to international standards that allow them to enter a FAIR process, the more traditional "small-scale" publications, which constitute the majority of articles, are generally inaccessible in a standardized form that can be searched, reused and aggregated. Yet these small-scale data are of very high added value and crucial to the effective interpretation of large-scale data. Aware of the problem, some publishers are requesting that the data from the articles they publish be deposited in public databases.

In this context, we are working on integrating and making available online, in accordance with FAIR principles, small- and large-scale data from publications and unpublished experiments of the global ascidian community, using ANISEED.

Several strategies for adhering to the FAIR principles have already been put in place, but we would like to propose new directions to the community:

1. Increase the proportion of the domain's literature that is manually curated to near completeness.

2. To set up a reward and tracking system for unpublished submissions to traditional journals. Much of the data produced by laboratories is never published in traditional journals, because its interest for the laboratory is insufficient to motivate the writing of a long article. This "dark matter", often of high quality, includes both new discoveries and negative results or reproductions of previously published experiments. Its aggregation makes it possible to refine and make more robust the description of the system. We are developing a partnership with microPublication (https://www.micropublication.org/), to publish very short articles describing single results, peer-reviewed and identified by a DOI. Authors, who will be required to provide formatted and annotated data with a controlled vocabulary, will be rewarded and cited for work that would otherwise remain in their experimental notebooks or hard drives.

3. Assign permanent DOIs to direct submissions for publication in a mainstream scientific journal. An increasing number of researchers are asking us to make their datasets available in ANISEED in order to meet publishers' demands that, as part of a data management plan, the datasets be accessible in a reference database and identified by a DOI. To reinforce this process, we are currently in contact with the Opidor team to finalise the method (https://opidor.fr/identifier/).

We need feedback from the community on whether these axes could be useful.

Our project is a case study for the future evolution of model organism databases. The inclusion of microPublication will lead to collaboration with other global information systems such as Wormbase or ZFIN in order to use the same guidelines and standards needed to develop the bridge between ANISEED and microPublication data.

The project also brings better visibility to ANISEED with publishers so that i) ANISEED is recommended to researchers to submit their data before publication (e.g. suggest to the publisher PLOS that ANISEED is part of the list of recommended biological repositories https://fairsharing.org/FAIRsharing.t2exm); ii) Perennial object identifiers make the link between published articles and underlying datasets. Their implementation will allow ANISEED to continue to integrate in an international environment by linking datasets and publications. These efforts will strengthen ANISEED's international role.

Oral Presentations

D1-01 to D4-17

D1-01

Synergistic modulation of mitotic receptor trafficking through polarized degradation and recycling

Hannah Gruner[1], Camryn Slosky[1], Christina Cota[2] and Bradley Davidson[1]*
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2 Colby College, Waterville ME, USA

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Asymmetric division is essential for cell fate diversification in both embryonic and stem cell lineages. Although polarized mitotic segregation of cytoplasmic factors has been the focus of intensive research, the polarized trafficking of trans-membrane signaling factors also impacts daughter cell fate and this process remains poorly characterized. We use the asymmetric division of the cardio-pharyngeal founder cell lineage (B7.5) in *Ciona robusta* to investigate mitotic trafficking of transmembrane receptors. In dividing founder cells, polarized re-distribution of Fibroblast Growth Factor Receptors (FGFRs) drives differential induction of the cardio-pharyngeal progenitors (trunk ventral cells, TVCs). Our previous studies have delineated cell-cycle specific roles for cyclin-dependent kinase 1 (CDK1) and Aurora Kinase (AurK) in modulation of FGFR trafficking (Cota et al., 2021). Our most recent results have substantially refined our model, delineating distinct, cell-cycle specific contributions for FGFR degradation and AurK-dependent recycling in polarized FGFR trafficking. We first determined that FGFR degradation is dictated by a small C-terminal cytoplasmic domain. Removal of this domain blocks FGFR degradation allowing us to perform live, in-vivo imaging of mitotic traffic patterns. During prophase and metaphase, full length FGFR is highly enriched on the ventral side of dividing founder cells (which gives rise to the TVC daughter). Strikingly, truncated FGFR was internalized and stored in uniformly distributed endosomes indicating that FGFR degradation is necessary for polarized storage of FGFR during mitotic entry. However, as cells exit mitosis, we observe a robust polarized redistribution of truncated FGFR along the ventral membrane. Thus, it appears that FGFR degradation is not required for polarized FGFR re-distribution during mitotic exit. We have also shown that application of an AurK inhibitor disrupts polarized FGFR redistribution. Based on these results we propose a two-step model. During mitotic entry, FGFR is internalized and subjected to differential degradation, leading to biased accumulation of FGFR containing endosomes on the ventral side. During exit, AurK potentiates differential recycling of stored FGFR towards the ventral membrane leading to differential induction. We are currently exploring the pathway by which AurK impacts FGFR recycling. We are also investigating whether our model is applicable to mitotic trafficking of different trans-membrane proteins and if similar regulatory patterns pertain to other cell types, including mammalian cell lines.

References

Cota CD, Dreier MS, Colgan W, Cha A, Sia T, Davidson B. Cyclin-dependent Kinase 1 and Aurora Kinase choreograph mitotic storage and redistribution of a growth factor receptor. PLoS Biol. 2021 Jan 4;19(1):e3001029. doi: 10.1371/journal.pbio.3001029. PMID: 33395410; PMCID: PMC7808676.

D1-02

Evolution of a chordate-specific mechanism for myoblast fusion

Haifeng Zhang[1], Renjie Shang[1,2], Kwantae Kim[3], Wei Zheng[4,5], Christopher J. Johnson[3], Lei Sun[6], Xiang Niu[7], Liang Liu[8,9], Jingqi Zhou[2], Lingshu Liu[2], Zheng Zhang[1], Theodore A. Uyeno[10], Jimin Pei[11], Skye D. Fissette[12], Stephen A. Green[13], Sukhada P. Samudra[2], Junfei Wen[1], Jianli Zhang[14], Jonathan T. Eggenschwiler[2], Douglas B. Menke[2], Marianne E. Bronner[13], Nick V. Grishin[11,15], Weiming Li[12], Kaixiong Ye[2,9], Yang Zhang[4,5], Alberto Stolfi[3]*, Pengpeng Bi[1,2]

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[5]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA
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[10]Department of Biology, Valdosta State University, Valdosta, GA, USA
[11]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA
[12]Department of Fisheries & Wildlife, Michigan State University, East Lansing, MI, USA
[13]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA
[14]College of Engineering, University of Georgia, Athens, GA, USA
[15]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, USA

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Vertebrate myoblast fusion allows for multinucleated muscle fibers to compound the size and strength of individual mononucleated cells, but the evolution of this important process is currently unknown. The phylum Chordata hosts closely related groups that span distinct myoblast fusion states: no fusion in cephalochordates, facultative fusion and multinucleation in tunicates, and extensive, obligatory fusion in vertebrates. To elucidate how these differences may have evolved, we studied the evolutionary origins and function of membrane-coalescing agents Myomaker and Myomixer in various groups of chordates. Here we report that *Myomaker* likely arose through gene duplication in the last common ancestor of tunicates and vertebrates, while *Myomixer* appears to have evolved *de novo* in early vertebrates. Functional tests revealed an unexpectedly complex evolutionary history of myoblast fusion in chordates. A pre-vertebrate phase of muscle multinucleation driven by Myomaker was followed by the later emergence of Myomixer that enables the highly efficient fusion system of vertebrates. In *Ciona,* facultative activation of *Myomaker* in multinucleated juvenile siphon and body wall muscles (but not mononucleated larval tail muscles) is carried out by a post-metamorphic myogenesis program based on the cooperative activity of MRF and Ebf. Our findings suggest an evolutionary model of chordate-specific fusogens and illustrate how new genes can shape the emergence of novel morphogenetic traits and mechanisms.

D1-03

Sperm astacin-like metalloproteases are involved in sperm penetration through the vitelline coat during ascidian fertilization

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We previously reported that the sperm proteasome and two sperm trypsin-like proteases, acrosin and spermosin, are involved in fertilization of the stolidobranch ascidian *Halocynthia roretzi,* particularly in the sperm binding to and/or penetration of the vitelline coat (VC) (see review 1). In contrast, sperm proteasome and chymotrypsin-like protease appear to be involved in sperm penetration though the VC in the phlebobranch ascidian *Ciona intestinalis* (2). In order to identify the proteasome subunits or chymotrypsin-like protease on the sperm surface, proteomic analysis of the sperm surface protein was investigated using the ascidian *C. intestinalis.* Unexpectedly, instead of proteasome subunits or chymotrypsin-like protease, several astacin-like metalloproteases with thrombospondin type-1 repeats (designated as tast) were identified in the sperm surface in *C. intestinalis* (3, 4). A metalloprotease inhibitor GM6001, but not GM6001NC (negative control), inhibited the fertilization of intact eggs but not VC-free eggs. Since GM6001 showed no apparent inhibition toward sperm binding to the VC, it is thought that sperm metalloprotease (tast) must play a key role in sperm penetration of the VC as a lysin in *C. intestinalis.*

In the present report, we examined the effects of GM6001 on fertilization of *H. roretzi.* GM6001, but not GM6001NC, strongly inhibited the fertilization. Similar inhibitory ability to fertilization was observed by GM6001 analog TAPI-1, but another analog TAPI-2 showed much weaker inhibition. Both GM6001 and TAPI-1, but not TAPI-2, have aromatic group at P2' subsite, the metalloprotease responsible for ascidian fertilization (tast) may prefer aromatic residues at P2' site. When the isolated VC was treated with sperm extract, a new band was observed by SDS-PAGE. The appearance of this band was inhibited by GM6001. The newly appeared band was identified by mass spectrometry as vitellogenin, which is a binding partner for sperm acrosin in *H. roretzi.* ANISEED database showed the existence of at least 4 gene models of tast, having an astacin-like metalloprotease domain with a single N-terminal-side transmembrane domain and C-terminal-side two or three thrombospondin-type1-repeats. In addition, mRNA expressions of these genes were confirmed by RT-PCR. These results suggest that several novel astacin-like metalloproteases may play a key role in sperm penetration of the VC, probably as a lysin or one of the lysin system in *H. roretzi.*

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D1-04

Insights into self-incompatibility by structural modelling

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Fertilization is a fundamental biological event in living organisms to generate new life with a mixed genetic background. To achieve successful fertilization, sperm and egg undergo a complex sequence of processes. *C. intestinalis* is a hermaphrodite and inbreeding depression is a serious problem. Therefore, ascidians possess a self-incompatibility (SI) system to avoid self-fertilization. We identified the genes responsible for SI: sperm-side polycystin 1-like receptors *s-Themis-A/B/B2* and egg-side fibrinogen-like ligands on the vitelline coat (VC) *v-Themis-A/B/B2*[1,2]. Since *s/v-Themis* genes are polymorphic[1,2], their genetic variability could control identification. In fact, fertilization is blocked when SI allelic gene pairs are matched[2]. After self/nonself-recognition on the VC, only self-recognized sperm are rejected because self-recognition triggers an increase in intracellular Ca^{2+} of sperm, which causes sperm quiescence[3].

However, it is still unknown how SI proteins individually identify gametes. To understand the molecular mechanism of self/nonself-recognition with respect to protein structure and due to the lack of available crystal structures, we attempted to predict the structures of s/v-Themis proteins. Recently, artificial intelligence tools, such as AlphaFold2[4], have become more and more powerful at predicting the structure of proteins with unknown folds at high accuracy. These AlphaFold2 predictions can be used to in the design of new constructs for expression of recombinant proteins to perform functional assays. Moreover, AlphaFold2 structural predictions have already been successful at indicating specific domains and regions of s/v-Themis that are extremely polymorphic and could be responsible for their identification.

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D1-05

Plasma membrane-type Ca^{2+}/ATPase and CatSper contribute to ascidian sperm chemotaxis

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The sperm motility including chemotactic behavior is regulated via changes in the intracellular Ca^{2+} concentration, and the sperm-specific Ca^{2+} channel, CatSper, have been shown to play an important role in the regulation of Ca^{2+} in the sperm of many animals. On the other hand, we found that the plasma membrane-type Ca^{2+}/ATPase (PMCA) act as the receptor for the sperm-activating and -attracting factor (SAAF) in the ascidian *Ciona intestinalis* (*1*). Although its existence of the *Catsper* genes has been reported, it is not clear whether CatSper is the Ca^{2+} channel that actually functions in ascidian sperm. Thus, we have tried to examine the *Catsper3* KO sperm and found that CatSper plays an important role in the ascidian as well as in mammals, and is involved in spermatogenesis and basic motility mechanisms.

In ascidians, sperm chemotaxis is generally species-specific, and actually species-specific sperm attractants have been identified in some species (*2, 3*). However, the mechanisms recognizing species-specific attractants have not been well studied. Therefore, we identified PMCAs in six Phlebobranchia species. Finally, we found the testis-specific splice variant of PMCA in all the species investigated. Moreover, the extracellular loops 1, 2, and 4 in ascidian PMCA underwent a positive selection (*4*). These findings suggest that PMCA recognizes the species-specific structure of SAAF at the extracellular loops 1, 2, and 4, and its testis-specific C-terminal region is involved in the activation and chemotaxis of ascidian sperms.

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D1-06

Fertilization and embryogenesis of two tropical ascidians in varied salinities and temperature

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There are few reports of the effect of environmental stress on ascidian fertilization and embryonic development. Most of these are reported for temperate species (Nomaguchi et al. 1997; Thiyagarajan & Qian, 2003; Pineda et al., 2012; Renborg et al., 2014; Kim et al. 2019). This study investigates the effects of temperature and salinity on fertilization, through to settlement stage for two common tropical species of ascidians – *Phallusia nigra* and *P. philippinensis*. The effect of temperature and salinity was examined for three temperatures (25, 28, 32°C) at three salinities (25, 30, 35 psu). It was observed that larval development of both species failed at 25 psu, whereas in open sea conditions of 30 to 35 psu, fertilization and development was successful between 25 – 32°C for both species. Embryogenesis was highest at 28°C and 32°C for *P. nigra*, whereas for *P. philippinensis,* the optimum temperature was 28°C. The maximal temperature tolerance for embryo development appeared to be ≥ 32°C and < 32°C for *P. nigra* and *P. philippinensis* respectively. The higher temperature tolerance range for embryo development in *P. nigra* may favour its dominance in the tropics in face of rising seawater temperature.

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D1-07

Photoreceptive tissues and photoresponse in the adult of *Ciona intestinalis* type A

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The life history of ascidians consists of distinct larval and adult stages. The adult ascidians are sessile animals, bearing an extremely modified version of the chordate body plan with a simple nervous system. In contrast, the body plan of the free-swimming, tadpole-like larvae shares basic features with the body plan of vertebrates, including a CNS derived from a dorsal neural tube. The brain of the ascidian larva contains two sensory organs with a pigment cell: an eyespot (ocellus) and a gravity sense organ (otolith). Swimming behavior of the tadpole larva is controlled by photoreception and gravity sensing of these sensory organs. The ocellus contains ciliary photoreceptor cells, similar to those of the retina and the pineal organ of vertebrates. The photoreceptor cells uses a vertebrate-type visual pigment opsin as the photoreceptor molecule. Development and function of the ocellus of the ascidian larva have been elucidated in detail. In contrast, molecular and cellular mechanisms of photoreception in adult ascidians are still poorly understood. Three types of light-responsive behaviors have been described in the adult ascidians: siphon retraction, phototropism and gamete release (spawning). The pigmented spots around the siphon openers, the epithelial cells of the sperm duct and the cerebral ganglion have been suggested to be candidate photoreceptor organs underlying these behaviors. The siphon retraction triggered by flashlight in adults of the ascidian *Ciona intestinalis* was reported by Hecht about 100 years ago (Hecht, 1918). He reported that the cerebral ganglion was the photoreceptor organ for this behavior. Since then, however, no further studies have been conducted on this photic response. In this study, we re-examined light-evoked siphon retraction in the adults of *Ciona intestinalis* type A (*Ciona robusta*) by using laser pointers. Siphon retraction was efficiently induced when a green laser (532 nm) was irradiated to the neural complex, including the cerebral ganglion and the neural gland, whereas it was not induced when a red laser (635 nm) was irradiated to the neural complex. Green laser irradiation to tissues other than the neural complex rarely induced siphon retraction. These results suggest that green-sensitive photoreceptors in the cerebral ganglion are responsible for the light-evoked siphon retraction in adult ascidians

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D1-08

Ascidian gastrulation and blebbing activity of isolated endoderm blastomeres

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Gastrulation represents the first dynamic cell movement during embryogenesis. Endoderm and mesoderm cells are internalized into embryos during this process. Ascidian embryos provide a simple system for studying gastrulation in chordates. Gastrulation starts in spherical late 64-cell embryos with 10 endoderm blastomeres. The mechanisms of gastrulation in ascidians have been investigated and a two-step model has been proposed [1,2]. The first step involves apical constriction of endoderm cells. The second step is apicobasal shortening. In this study, isolated ascidian endoderm progenitor cells displayed dynamic blebbing activity at the gastrula stage, although such a dynamic cell shape change was not recognized in toto. Blebbing is often observed in migrating animal cells. In ascidians, endoderm cells only display blebbing activity. The timing of blebbing of isolated endoderm cells coincided with that of cell invagination. The constriction rate of apical surfaces correlated with the intensity of blebbing activity in each endoderm-lineage cell. Fibroblast growth factor (FGF) signaling is required and is sufficient to induce blebbing activity, independent of cell fate specification. In contrast, the timing of initiation of blebbing and intensity of blebbing response to FGF signaling are controlled by cell intrinsic factors. It is likely that the difference in intensity of blebbing activity between the A- and B-line cells could account for the anteroposterior difference in the steepness of the archenteron wall. Inhibition of zygotic transcription, FGF signaling, and Rho kinase, all of which suppressed blebbing activity, resulted in incomplete apical constriction and failure of the eventual formation of cup-shaped gastrulae. Blebbing activity was involved in the progression and maintenance of apical constriction, but not in apicobasal shortening in whole embryos. Apical constriction is mediated by two distinct blebbing-dependent and independent mechanisms. Surface tension and consequent membrane contraction may not be the sole mechanical force for apical constriction and formation of cup-shaped gastrulae. The present study reveals the hidden cellular potential of endodermal cells during gastrulation. Possible roles of blebbing in the invagination process are discussed.

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D1-09

Admp controls the pMLC localization to the apical side of the ventral epidermal cells and leads the ventral tail bending in ascidian tailbud embryo

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Chordate tailbud embryos have similar morphological features including tail bending. The recent study revealed that the asymmetrical actomyosin localization in the notochord changes the contractility and drives tail bending in the early *Ciona* tailbud embryo (Lu et al., 2020). However, the upstream regulator of this bending remains unknown. In this study, we demonstrated that *Admp* regulates this tail bending. *Admp* knock-down embryo (*Admp* MO) showed no tail bending without changing the asymmetrical localization of notochord actomyosin; the smad phosphorylation was specifically observed at the ventral midline of tail epidermis from early gastrula. The detailed observation in the morphant showed the unusual cell-cell intercalation only at ventral epidermis. In the earlier phase of this cell-cell intercalation, each ventral epidermal cell exhibited the boat-type morphology (boat cell). The myosin phosphorylation (pMLC) was accumulated at the apical side of these boat cells. Whereas the number of boat cells reduced in the *Admp* MO and pMLC was observed at the basal side. The apical localization was also observed in the ectopically *bmp2/4*-expressed epidermal cells. Finally, the laser cutting of the ventral midline epidermis during tail bending demonstrated the myosin-phosphorylation-dependent tension. From these results, we propose that *Admp* controls the pMLC localization to the apical side of the ventral epidermal cells and leads the tail bending by temporarily ventrally biased resistance to the notochord extension. Our study reveals a new function of the *Admp* that might be evolutionarily conserved in bilaterian animals.

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D1-10

Role of Pi3K signaling during *Phallusia mammillata* endoderm invagination

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Gastrulation is a fundamental event occurring during animal embryogenesis during which the embryo body plan is laid out and the main tissue types become organised. In the ascidian *Phallusia mammillata*, it starts with the invagination of endodermal precursors between the 64- and late 112-cell stages (Fiuza et al., 2020). This process involves precise coordination of cell fate decisions, cell shape changes, cell divisions and cell movements, but so far we only have a partial understanding of the ways these processes are coordinated and integrated at the transcriptional level.

Using a pharmacological approach, quantitative *in situ* hybridization chain reaction and live and fixed tissue imaging, we have uncovered a new key factor, Pi3K, in the process of endoderm invagination. I will present how Pi3K signalling acts in the control of endoderm invagination and how it is coupled/ integrates to previously demonstrated chain of cellular and molecular events at play.

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D1-11

Molecular and functional diversity of the nerve cord ependymal cells in *Ciona* larvae

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The central nervous system of the ascidian larva is divided into three parts, a brain vesicle, a motor ganglion, and a caudal nerve cord, from anterior to posterior. The brain vesicle contains sensory organs and diverse interneurons, which presumably form neural circuits for sensory information processing and motor regulation. The motor ganglion/nerve cord, in which *Hox* genes are expressed, has been suggested to be homologous to the vertebrate hindbrain/spinal cord. The motor ganglion contains cholinergic motor neurons that control contraction of the tail muscle cells for swimming. The hollow nerve cord mainly consists of four rows of glial ependymal cells: rows of the dorsal roof plate cells, the left and right lateral wall cells, and the ventral floor plate cells. Compared to the sensory organs and neurons of the brain vesicle and motor ganglion, the developmental and physiological roles of the ependymal cells of the nerve cord remain elusive. To understand developmental and physiological roles of the nerve cord ependymal cells, we performed single-cell transcriptomic profiling of each row of the nerve cord cells in the ascidian *Ciona intestinalis* type A (*Ciona robusta*). The three types of the ependymal cells (roof plate, lateral walls, and floor plate) are clearly distinguished by the transcriptome profiles. For example, the floor plate cells specifically express *hedgehog.b* and *fgf3/7/10/20*, the roof plate cells express *wnt7* and *msx.b*, and the lateral wall cells express *gnrh2* and *tbx2*. Identified ependymal cell-specific genes encode proteins involved in regulation of nervous system development and physiological functions, including molecules related to ciliary functions, axon guidance, cell-cell interaction, and intracellular signaling. In addition, the single-cell transcriptomic analysis revealed conspicuous diversity of ependymal cells along the anteroposterior axis. For example, two distinct isoforms of ephrin ligands, known to act as repulsive cues in axon guidance, are expressed in specific regions of the lateral walls and the floor plate along the anteroposterior axis. We discuss developmental and physiological roles of the glial ependymal cells of the *Ciona* nerve cord based on the gene expression profiles and experimental manipulation of selected genes.

D1-12

Investigation of neuron-glia interactions using a combination of optogenetics and calcium imaging in *Ciona* swimming larvae

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The human brain contains about 100 billion neurons. In addition, there are as many glial cells as neurons in the brain, and the glial cells occupy a large portion of the brain volume. There are four types of glial cells: astrocytes, oligodendrocytes, microglia, and ependymal cells. Glial cells were once thought to be "glue-like" cells that filled the gaps between neurons, but in recent years, a growing number of studies suggest that glial cells actively exchange information with neurons and play an important role in higher brain functions (Araque et al., 2014; Fields et al., 2015). However, it is still unclear how the glial cells are involved in higher brain functions.

The ascidian *Ciona* larva has a central nervous system homologous to that of vertebrates. Its nerve cord, the spinal cord homolog, is mainly composed of glial ependymal cells. In previous studies, when the activity of glial cells in the nerve cord of *Ciona* larvae was visualized by the calcium imaging method using G-CaMP8, we found that active Ca^{2+} transients were associated with swimming behavior and suggested that the glial cells are involved in the control of neurons and muscle activity (Okawa et al., 2020).

In this study, we analyzed the cause of glial cells activation using optogenetics in combination with calcium imaging. To analyze the relationship between neuronal activation and glial cell activity, ChrimsonR, a light-gated ion channel, was expressed in cholinergic neurons, excitatory neurons involved in tail movement, and calcium imaging of the glial cells in the nerve cord was performed while cholinergic neurons were activated by light irradiation. After activating cholinergic neurons, the Ca^{2+} concentration in glial cells increased. We also examined whether acetylcholine receptors were expressed in the glial cells of the nerve cord using the single-cell transcriptome analysis. As the result, we found that some glial cells in the nerve cord express nicotinic acetylcholine reporters. These results suggest that the glial ependymal cells receive information from activated cholinergic neurons, trigger Ca^{2+} transients. The *Ciona* larva has morphologically only one type of glial cells, the ependymal cells, but functionally, they may exhibit properties of both oligodendrocytes and astrocytes in addition to those of ependymal cells.

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D1-13

Comprehensive analysis of behavioral dynamics in the protochordate Ciona intestinalis

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Vertebrate nervous systems can generate a remarkable diversity of behaviors. However, our understanding of how behaviors may have evolved in the chordate lineage is limited by the lack of neuroethological studies leveraging our closest invertebrate relatives.

In this work, we combine high-throughput video acquisition with pharmacological perturbations of bioamine signaling to systematically reveal the global structure of the motor behavioral repertoire in the Ciona intestinalis larvae. Most of Ciona's postural variance can be captured by six basic shapes, which we term "Eigencionas". Motif analysis of postural time series further demonstrated that bioamines influence numerous stereotyped behavioral maneuvers including "startle-like" and "beat-and-glide". Employing computational modeling of swimming dynamics and spatio-temporal embedding of postural features revealed that subtle behavioural differences are generated at the levels of motor modules and the transitions between, both modulated by bioamines. Finally, we show that flexible motor module usage gives rise to diverse behaviors in response to different light stimuli.

D1-14

Dopaminergic neurons in *Oikopleura dioica*: Connectivity and regulation of movement states

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To assess the functional role of putative dopaminergic neurons in *Oikopleura dioica*, we have used behavioral and molecular biological approaches to: 1) identify putative dopaminergic neurons through fluorescence in situ hybridization (FISH) for tyrosine hydroxylase (TH) mRNA, 2) identify a putative dopamine receptor gene in the *Oikopleura* genome, 3) demonstrate functional binding of dopamine and noradrenaline to this receptor in a HEK cell expression system, 4) identify which neurons express this receptor using double FISH, 5) characterize the effect of dopamine and noradrenaline exposure on behavior, and 6) characterize the effects of TH knockdown and pharmacological disruption of dopaminergic synaptic release on behavior.

We find that the putative dopamine receptor responds to binding of dopamine and noradrenaline with G-protein-mediated Ca++ signals in HEK cells, and is expressed exclusively by putative GABAergic neurons in the cerebral and caudal ganglia, as well as transiently by the putative dopaminergic neurons. Adult *Oikopleura* during dispersion from their houses exhibited episodes of swimming interspersed by episodes of non-swimming movements (dwelling), and periods of quiescence. Exposure of adult animals to dopamine or noradrenaline decrease time spent dwelling without affecting time spent swimming, and exposure to dopamine (but not noradrenaline) increased the efficiency of swimming movements. Conversely, dsDNA-mediated knockdown of TH expression or treatment with the neurotoxin 6-OHDA increased the amount of time spent dwelling, thus increasing the overall time spent moving, but decreased the efficiency of swimming movements. We determined that dwelling consists primarily of bending movements, which typically constitute a "windup" to swimming movements. Thus, exposure to dopamine renders animals more efficient in initiating and executing swimming, whereas depletion of dopamine leads to less efficient initiation and execution of swimming: a form of hypokinesis.

We conclude that the regulation of movement by dopamine parallels that seen in vertebrates, and that dopaminergic output selectively targets GABAergic neurons. These features align neatly with the general functional organization of the nigrostriatal system in vertebrates, and suggests that the ancestral chordate version of the basal ganglia has been pared down in *Oikopleura* to a minimal circuit that remains sufficient to regulate movement states even though only a handful of neurons is involved.

D1-15

Transcriptomes of the fast-evolving chordate, *Oikopleura dioica*, uncover drastic difference in transcription factor and post-plasmic RNA composition that is expressed in early embryos from those of ascidians

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Larvaceans are planktonic chordates, and retain a tadpole shape throughout the lifetime. *Oikopleura dioica* is characterized by small number of cells (approximately 4,000 in juveniles), invariant cell linages during embryogenesis, fast development to complete morphogenesis in 10 hours after fertilization, and a compact genome which is estimated at ~ 56 Mb (The smallest genome in non-parasitic metazoans). In embryogenesis, the striking feature is that fates of most blastomeres are restricted to give rise to a single cell type by the 32-cell stage, in contrast to the cell fate restrictions at the 110-cell stage in ascidian embryos. In this sense, *O. dioica* can be regarded as "fast-developing chordate" and provides an intriguing model to investigate rapid development with small cell number, and evolution of the tadpole body plan.

We found non-canonical properties of the early development of larvaceans using RNA sequencing (RNA-seq) data and 19,277 genome-guided transcript assembly of *O. dioica* (1). First, clustering of gene expression levels at 13 developmental stages, indicated an initial zygotic gene expression of 892 genes at the 16-32 cells. Second, 24 and 51 transcription factors (TFs) were expressed in the 32-cell and 1.5 hour-post fertilization embryos, respectively. Third, RNA-seq of the isolated animal and vegetal hemispheres of the 8-cell embryos listed six localized maternal mRNAs, suggesting presence of the post-plasmic RNAs in larvaceans. Among these, it was confirmed that three mRNAs were already localized to the narrow vegetal pole region of unfertilized eggs. However, identities of most of these TFs and localized maternal mRNA were different from those known in ascidian embryos. This indicates that embryogenesis of larvaceans and ascidians share various properties, but molecules that underpin the initial embryogenesis would significantly diverge, representing developmental system drift.

The larvacean transcriptome database provides a valuable resource for genetics, developmental and evolutionary biology of chordates. The RNA-seq data were incorporated into ANISEED (https://www.aniseed.cnrs.fr/). This study supports that some maternal mRNAs are already localized to the narrow vegital pole region of unfertlized eggs, and that this fast-developing chordate lacks the first phase of ooplasmic movements (2), a fertilization-driven process to concentrate the post-plasmic RNAs to the vegital pole in ascidian embryos.

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D1-16

Beyond *Oikopleura dioica* - evolution of appendicularian diversity

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Oikopleura dioica has emerged as an attractive model organism for evolutionary comparisons in molecular developmental studies. While appendicularians as a small taxonomic group comprising approximately 70 species only, are often considered uniform, their phylogeny and evolution is poorly understood. Here, we present first results of the diversity of appendicularian morphology grounded in comparative analyses of computer-assisted 3D-reconstructions. Based on phylogenetic considerations, we document variability and conservation in anatomical relations of organ systems, cell number, and histological composition. Within Appendicularia, the miniaturization that is thought to be the result of a pedomorphic developmental acceleration, has been reversed during the evolution of the ingroup. We show that anatomical changes rooted in miniaturization are still detectable in derived appendicularians that have become secondarily larger during evolution again. Despite the comparatively low number of species, the phenotypic diversity of appendicularians is considerable.

D1-17

Deconstruction of the cardiopharyngeal gene regulatory network in appendicularians and the evolution of their complete free-living lifestyle from an ascidian-like biphasic ancestral tunicate

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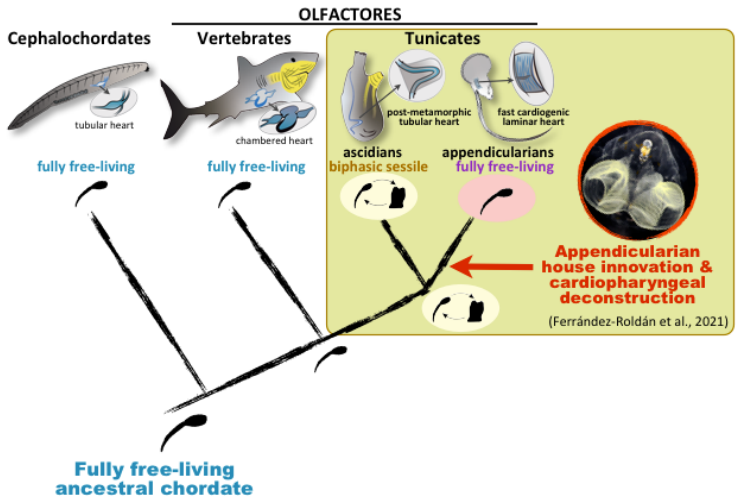
The classical Garstang's hypothesis suggested that the biphasic lifestyle of ascidians alternating free-swimming larvae and sessile adults represented the lifestyle of the last common chordate ancestor, from which free-swimming cephalochordates and vertebrates appeared by paedomorphic evolution. The bloom of phylogenomics, however, placed cephalochordates basally diverging among chordates, and tunicates as vertebrate's sister group, suggesting in contrast to Garstang's view that the chordate ancestor was free-living. A central question in tunicate evolution is the origin of sessility and whether complete free-living appendicularians could parsimoniously represent the tunicate ancestor. Considering the relevance of the "a new heart for a new head" hypothesis for the evolution of chordate lifestyles, our group has studied the development of the heart in appendicularians[1]. Our results reveal that appendicularians experienced massive ancestral losses of cardiopharyngeal genes and subfunctions, leading to the 'deconstruction' of two ancestral modules of the cardiopharyngeal gene regulatory network (GRN), which in ascidians are related to early and late multipotency involved in lineage cell-fate determination towards the first and second heart fields and siphon muscles. Our work suggests that the deconstruction of the cardiopharyngeal GRN involved the regressive loss of the siphon muscle, supporting an evolutionary scenario in which ancestral tunicates had a sessile ascidian-like adult lifestyle. In this scenario, our findings suggest that this deconstruction contributed to the acceleration of cardiogenesis and the redesign of the heart into an open-wide laminar structure in appendicularians as evolutionary adaptations during their transition to a complete pelagic free-living style upon the innovation of the food-filtering house.



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D2-01

THE TICKING CLOCK, AN AGING STUDY OF A COLONIAL CHORDATE LINKING STEM CELL AGING TO MOLECULAR DECLINE OF CIRCADIAN REGULATION

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Expression levels of circadian clock genes, which regulate 24-hour biological cycles, have been shown to change with age. However, due to limitations of tissue sampling, a comprehensive study comparing circadian gene expression across multiple time points and age groups is missing. Using the colonial chordate *Botryllus schlosseri*, a long-lived organism that exhibits weekly stem cell-mediated asexual tissue regeneration and replacement of all body organs, we studied the link between stem cell aging and circadian gene expression. Colonial tunicates, like *B. schlosseri* are unique amongst chordates in possessing a stem cell mediated asexual development pathway, termed blastogenesis. Unlike most species, where the body is long lived and maintained by cellular replacement, *B. schlosseri* regenerates new colonial units (zooids) on a weekly basis replacing the previous generation's zooids, which then die through massive apoptosis. While the colony can live for upwards of 20 years, the zooids live for only a week. The colony is thus aged according to its stem cells, which migrate from one generation to the next and are maintained throughout life through self renewal, allowing for the study of stem cell aging's effects at the organismal level. Here we characterize global gene expression changes across time and age and link them to morphological, physiological and cellular aging phenotypes. Using a computational pipeline we developed to comprehensively quantify gene expression patterns across time points and age groups, we identified unique and shared molecular characteristics at each timepoint and age. These analyses revealed that *B. schlosseri* clock and clock-controlled genes oscillate daily with age-specific amplitudes and frequencies. These age-related patterns persist at the tissue level, where dramatic variations in the cyclic gene expression link to morphological and physiological aging phenotypes. Similar cyclical expression differences were found in hundreds of pathways associated with known hallmarks of aging, as well as pathways that were not previously linked to aging. The molecular clock atlas we developed suggests alterations in circadian gene expression as a key regulator of aging, linking stem cell aging and loss of regenerative potential with molecular decline of circadian regulation.

D2-02

Botryllus schlosseri, an emerging evo-devo model for the study of neurogenesis, neurodegeneration, and aging

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Loss of the brain's functional ability is a common symptom of aging and neurodegenerative diseases[1,2]. While the genetic and molecular mechanisms underlying human neurodegeneration are studied in-depth[3–6], very little is known about the evolutionary origin of these traits and their involvement in loss of nervous system function in aged invertebrate species. Here, we characterized the nervous system of *Botryllus schlosseri*, a chordate with a simple central nervous system, as a novel model to study evolutionary neuroscience and neurodegeneration. *B. schlosseri* reproduces both sexually and asexually[7], with adult brains regenerating and degenerating multiple times throughout its adult life. Combining microscopy, transcriptomics and behavioral assays, we found that each week during colony budding, a decrease in the number of neurons in adult brains is associated with reduced response to stimuli followed by programmed cell death and removal by phagocytes (i.e 'takeover'). Moreover, we found significant changes in the expression levels of 73 mammalian homologs genes associated with neurodegenerative diseases and neural stem cells pathways during this weekly cycle. Comparing the same weekly cycle in young and old colonies, we found that old colonies contain a significantly lower number of neurons, with changes in 148 unique genes linked to neurodegenerative diseases[8]. Indeed, as a member of an evolutionary clade considered to be a sister group of vertebrates, this organism may be a fundamental resource in understanding how evolution has shaped these processes across phylogeny and obtaining insight on their mechanisms.

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D2-03

Evolutionary origin and genomic dynamics of *Botryllus schlosseri* allorecognition genes

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Marine sessile invertebrates have the ability to distinguish between self and non-self tissues within the same species. This phenomenon of allorecognition has been observed in multiple tunicates groups. In the ascidian *Botryllus schlosseri* it has been established that allorecognition is controlled by a single polymorphic locus with codominant expression called *fuhc* (fusion/histocompatibility). *Botryllus* colonies fuse if they share at least one allele at the *fuhc* locus. Within this locus at least six allorecognition genes have been isolated, which are: *fuhc-sec*, *fuhc-tm*, *fester*, *uncle fester*, *hsp40*, and *bhf*. We analyzed the evolutionary origin and genomic dynamics of these genes in different genomes and transcriptomes of tunicates. Surprisingly, we found that some of these allorecognition genes are present in solitary species that lack allorecognition abilities. In this direction, we identified that the genome of the solitary ascidian *Styela clava* has a *proto-fuhc* locus with the *bhf*, *fuhc-tm* and *hsp40l* genes. Interesting, we established that the *fester* and *uncle fester* genes are restricted to integrated colonial species of the Styelidae family. Besides, the *fester* and *uncle fester* genes are shown to be a gene family, whose members exhibit haplotype variation and alternative splicing. The genomic dynamics of the *fester* family mimic what has been observed for the KIRs and Ly49s immune receptors of human and mouse NK-cells, respectively. These findings support the idea that Fester proteins are the receptors that control the allorecognition response in the integrated colonial species of the Styelidae family, including *B. schlosseri*.

D2-04

Comparing dormancy in two distantly related tunicates

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Many asexually-propagating marine invertebrates can survive extreme environmental conditions by developing dormant structures, i.e., morphologically simplified bodies that retain the capacity to completely regenerate a functional adult when conditions return to normal. Here, we examine the environmental, morphological, and molecular characteristics of dormancy in two distantly related clonal tunicate species: *Polyandrocarpa zorritensis* and *Clavelina lepadiformis*. In both species, we report that the dormant structures are able to withstand harsher temperature and salinity conditions compared to the adult, and are the dominant forms these species employ to survive the colder winter months. By finely controlling the entry and exit of dormancy in laboratory-reared individuals, we were able to select and characterize the morphology of dormant structures associated with their transcriptome dynamics. In both species, we identified putative stem and nutritive cells in structures that resemble the earliest stages of asexual propagation. By characterizing gene expression during dormancy and regeneration into the adult body plan (i.e., germination), we observed that genes which control dormancy and environmental sensing in other metazoans, notably HIF-α and insulin signaling genes, are also expressed in tunicate dormancy. Germination-related genes in these two species, such as the retinoic acid pathway, are also found in other unrelated clonal tunicates during asexual development. These results are suggestive of repeated co-option of conserved eco-physiological and regeneration programs for the origin of novel dormancy-germination processes across distantly related animal taxa.

D2-05

Oceanographic drivers of ascidian biogeographic patterns (Tunicata: Ascidiacea) along the Western Atlantic coast

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The latitudinal diversity gradient (LDG) of many animal taxa from many geographical regions is well-known. Nevertheless, the environmental factors that affect these patterns have been scarcely documented. In ascidians, LDGs have been studied for more than two decades without a clear understanding of how environmental variables predict the biogeographic structure of species. In this study, we assess the role of oceanographic variables for predicting the latitudinal diversity gradient (LDG) of ascidian species along the Western Atlantic (WA) coast using latitudinal patterns of ascidian species diversity (228 species); and –using a random forest model and a multi-model ensemble approach– evaluate the potential effects of climate change on the distributions of six ascidians species with different life histories (i.e. solitaries and colonials) under two future climate change scenarios (RCP 2.6 and 8.5, IPCC 2014 - Intergovernmental Panel on Climate Change) for [2040-2050] and [2090-2010] time periods along the WA. The assessment of ecological variables that influence the distribution of ascidian species, is not only important for advancing biogeography, ecology and evolution research, but will also help us better understand how this group of marine invertebrates may respond to global climate change or to anthropogenic disturbances. In this era of accelerated climate change and anthropogenic pressures on the environment these research topics are becoming increasingly important for scientists worldwide to address on different animal taxa.

Keys words: Biodiversity, biogeography, species distribution modeling and climate change.

D2-06

How developmental buffering stabilizes development in the face of thermal stress?

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Canalization, or developmental buffering, is defined as developmental stability in the face of genetic and/or environmental perturbations. Understanding how developmental buffering works is important in predicting how species survives environmental change, as well as deciphering how development can be altered in the evolutionary process [1]. However, how developmental gene expression is linked to buffering remains unclear. We addressed this by co-expression network analysis [2], analyzing similarity of gene expression changes caused by heat stress during development at a whole-embryonic scale. We exploited reciprocal hybrid crosses of sibling species of the ascidian *Ciona* showing different level of buffering to thermal stress [3,4].

We identified 15 genes that are inferred to be significantly involved in developmental buffering ('MDBGs'), 14 of which belonged to a single co-expression module. We also calculated correlation coefficients of expression between MDBGs and transcription factors in the central nervous system (CNS) developmental gene network that had previously been identified experimentally [5]. We found that, compared to the correlation coefficients between MDBGs, which had an average of 0.96, all the MDBGs are loosely linked to all the CNS developmental genes (average correlation coefficient 0.45). We found only four out of 62 CNS developmental genes showed correlation coefficient > 0.9 to MDBGs, comparable to the values between MDBGs, and three of these four genes were signaling molecules: BMP2/4, Wnt7, and Delta-like. We propose that loose links to the buffering network stabilize spatiotemporally dynamic development [6].

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D2-07

Towards an understanding of detoxification mechanisms induced by polycyclic aromatic hydrocarbons in the ascidian *Ciona robusta* (*Ciona intestinalis* Type A)

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing only carbon and hydrogen and are composed of two or more aromatic rings. Some PAHs show carcinogenic, mutagenic, and teratogenic effects on animals. PAHs are released into the atmosphere as a result of fossil fuel combustion and other human activities and enter the ocean through wet and dry deposition, so there are concerns about the impact of PAHs on the marine environment. The mechanisms of PAH sensing and detoxification in vertebrates have been well studied. For example, the regulation of cytochrome P450 (CYP) gene expression by Aryl Hydrocarbon Receptor (AHR) and Pregnane X Receptor (PXR) have been elucidated. In contrast, there are few studies on the mechanisms of PAH sensing and detoxification in invertebrates, and it is unclear whether similar mechanisms exist in invertebrates as in vertebrates. In order to assess the impact of PAHs on the marine environment, it is important to understand the effects of PAHs on marine invertebrates and the mechanisms by which marine invertebrates respond to PAHs.

Ciona robusta (*Ciona intestinalis* Type A) has been studied in developmental biology, and in recent years it has also attracted attention as a model for toxicology. Therefore, we are investigating the detoxification mechanisms of PAHs in this species. We have shown that several PAHs cause abnormalities in the development of *Ciona* (Sekiguchi et al. 2020). We also examined the expression of five CYP1 family genes, four CYP3 family genes, an AHR gene, and a PXR gene and showed that these genes are expressed in regions that include the digestive tract of *Ciona*. In a reporter assay using cultured mammalian cells, the transcriptional activity of AHR was enhanced in response to benzo[*a*]pyrene, suggesting that *Ciona* AHR is a ligand-dependent transcription factor, like vertebrate AHR. We are currently conducting further functional analysis of PXR in reporter assays using cultured mammalian cells. Moreover, transcriptional regulation of CYP1 and CYP3 family genes by *Ciona* AHR and PXR is currently evaluated in a reporter assay using *Ciona* embryos. These analyses will help clarify the detoxification mechanisms of PAHs in *Ciona*.

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D2-08

Nipple array of tunic cuticle: a review of the multifunctional nanostructure under water

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Nipple array is an array of protrusions about 100 nm in height has been found on the body surface of various metazoan taxa. In ascidians, this nanostructure was originally described from botryllid ascidians as protuberances on the tunic cuticle independently from Japan and Italy in 1978. Nipple array were found in all botryllids so far studied and many of stolidobranchs, clavelinids, polyclinids, and some salps. Moreover, similar structures were reported from various marine invertebrates, including echinoderms and parasitic copepods. The occurrence in various taxa implies functional importance of this nanostructure.

In terrestrial insects, nipple array is known to be involved in anti-glare and anti-contamination, but these functions were not verified in aquatic regime. Using synthetic material mimicking the nipple array, we demonstrated several potential functions of nipple array under water: anti-glare, bubble repellency, reduction of adsorption and adhesion force, inhibition of cell extension and phagocytic activity, and anti-fouling. Nipple array can be regard as multi-functional structures that convergently occurred in various phyla, while the major function required for survival may vary among taxa depending on their habitat, body size, and mode of life (pelagic, sessile, parasitic, etc.). Although the mechanism of the nanostructure formation is unknow, occurrence of nipple array during tunic softening and cuticle regeneration may suggest a self-organization process. As nipples are comparable in size to microvilli of some species, this nanostructure might be a mimetic structure of epidermal microvilli to compensate for some physical properties in animals in which ECM cover the body surface.

D2-09

Graveyards of Giant Pandas at the Bottom of the Sea? A Strange-Looking Undescribed Species of Colonial Ascidians in the Genus *Clavelina* (Tunicata: Ascidiacea)

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An unidentified colonial ascidian called gaikotsu-panda-hoya in Japanese, literally meaning 'skeleton panda ascidian', has been attracting SCUBA divers' attention for the past few years since its strange appearance was introduced on the Internet by a diving shop in Kumejima Island, Japan. To confirm the taxonomic status of this species, fresh samples were collected from a diving point off the coast of Kumejima Island. Our morphological examination revealed that they represent an undescribed species, which can be distinguished from 44 congeners in the genus *Clavelina* Savigny, 1816 by the following five characters: i) a colony with zooids connected by basal stolons, ii) zooids up to 20 mm in length, iii) zooids white in general color, with characteristic black markings that are reminiscent of giant pandas, iv) longitudinal muscular bands running from the thorax to abdomen, and v) stigmatal rows 14 in number. This is the first record of the class Ascidiacea from Kumejima Island.

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D2-10

G-protein signaling relay promotes *Ciona* metamorphosis

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Metamorphosis is a most dramatic event of ascidian development, but its underlying mechanisms are enigmatic. Recently, we showed that the neurotransmitters GABA is essential to start metamorphosis. GABA is received by its metabotropic, G-protein coupled receptors (GPCRs) for metamorphosis. To elucidate molecular mechanisms of metamorphosis, we addressed which G-proteins are responsible for this event. We found that Gq and Gs are necessary for metamorphosis. *Ciona* larvae exhibit calcium ion (Ca) transients at the adhesive palps when they adhere to a substrate. We showed that the Ca transients are dependent on Gq which activates signaling pathways producing inositol triphosphate (IP3). Gs pathway acts in the downstream of Gq. The major role of Gs is promoting cAMP production. According to this, accumulation of cAMP is crucial for starting metamorphosis. Both Gq and Gs are suspected to function in the adhesive palps. Moreover, GABA is upstream of or acts in parallel with Gq, suggesting that GABA also acts in the palps. Taken together, we have drawn the scheme in that GABA in the palp work with Gq and Gs signaling pathways to accumulate Ca and cAMP that lead to the initiation and progression of metamorphosis.

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D2-11

A microRNA Cluster-Lefty Pathway is Required for Cellulose Synthesis During Ascidian Larval Metamorphosis

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Synthesis of cellulose and formation of tunic structure are unique traits in the tunicate animal group. However, the regulatory mechanism of tunic formation remains obscure. Here, we identified a novel microRNA cluster of three microRNAs, including miR4018a, miR4000f, and miR4018b in Ciona savignyi. In situ hybridization and promoter assays showed that miR4018a/4000f/4018b cluster was expressed in the mesenchymal cells in the larval trunk, and the expression levels were downregulated during the later tailbud stage and larval metamorphosis. Importantly, overexpression of miR4018a/4000f/4018b cluster in mesenchymal cells abolished the cellulose synthesis in Ciona larvae and caused the loss of tunic cells in metamorphic larvae, indicating the regulatory roles of miR4018a/4000f/4018b cluster in cellulose synthesis and mesenchymal cell differentiation into tunic cells. To elucidate the molecular mechanism, we further identified the target genes of miR4018a/4000f/4018b cluster using the combination approaches of TargetScan prediction and RNA-seq data. Left–right determination factor (Lefty) was confirmed as one of the target genes after narrow-down screening and an experimental luciferase assay. Furthermore, we showed that Lefty was expressed in the mesenchymal and tunic cells, indicating its potentially regulatory roles in mesenchymal cell differentiation and tunic formation. Notably, the defects in tunic formation and loss of tunic cells caused by overexpression of miR4018a/4000f/4018b cluster could be restored when Lefty was overexpressed in Ciona larvae, suggesting that miR4018a/4000f/4018b regulated the differentiation of mesenchymal cells into tunic cells through the Lefty signaling pathway during ascidian metamorphosis. Our findings, thus, reveal a novel microRNA-Lefty molecular pathway that regulates mesenchymal cells differentiating into tunic cells required for the tunic formation in tunicate species.

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D2-12

Timing of replacement of the non-self-test cells and the self-tunic cells during *Ciona* metamorphosis

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Ascidians significantly change their body structure by metamorphosis, and the spatio-temporal cell dynamics related to metamorphosis has not been clarified. *Ciona* embryo has been surrounded by non-self-test cells, and the juvenile after metamorphosis has been surrounded by self-tunic cells derived from mesenchyme cells (Hotta et al., 2020). Mesenchymal cells migrate in below the epidermis before the metamorphosis. It has been unknown that the precise timing of the replacement of these two types of cells. We have previously designed the experimental induction system of *Ciona* metamorphosis by mechanical stimuli and shown that the two-round Ca^{2+} transients in papillae are required to initiate metamorphosis (Wakai et al., 2021). By using this system, we further investigated the dynamics of cells during metamorphosis in the precise time-course. After the metamorphosis-inducible mechanical stimulation of the palp, two-round Ca^{2+} transients occurred. During the second Ca^{2+} transients, the wave-like Ca^{2+} propagations which may be metamorphic signal to lead tail regression are observed across the entire epidermis (epithelial conduction). Interestingly, migrating mesenchymal cells released through multiple points of epidermis within 10 minutes after the second Ca^{2+} transient. We named this event as "extravasation". Using the transgenic line expressing fluorescent protein, Kaede, in mesenchymal lineages, it was revealed that all cells outside the epidermis (tunic layer) after metamorphosis were replaced into Kaede-labeled mesenchymal cells that will differentiate into tunic cells. We concluded that the replacement of test cells with tunic cells occur by extravasation at the timing after second Ca^{2+} transient in early metamorphosis.

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D2-13

D-serine controls epidermal vesicle release via NMDA receptor allowing tissue migration during the metamorphosis of *Ciona*

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D-serine is a free amino acid known to be a co-agonist of N-methyl-D-aspartate type glutamate receptor (NMDAR) modulating glutamatergic transmission in mammalian nervous system and peripheral tissues[1,2]. D-serine is also found from invertebrates including ascidians; however, its role in non-mammals is unclear. We previously found that the gene encoding serine racemase (the enzyme for D-serine synthesis) is downregulated in the mutants of *Ciona* which exhibit failure in the tail regression during metamorphosis. In this study, we discovered that D-serine is responsible for tail regression[3]. D-serine promotes the release of vesicle contents from the epidermis, which induces pocket swelling at the junction between trunk and tail. The pocket provides the space to facilitate tissue migration during tail regression. We also showed that D-serine is received by the epidermal cells through NMDAR.

The mechanisms leading to pocket formation in tunicates is similar to that of lamellar granule exocytosis in mammal skin. In mammals, vesicles exocytosis, maintaining skin homeostasis, is controlled by D-serine and NMDAR. We speculate that the role of D-serine/NMDARs in the regulation of epidermal exocytosis was already acquired by the ancestor of vertebrates/tunicates. Consequently, tunicates are ideal models to provide a better understanding of the maintenance of epidermal homeostasis in animals.

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D2-14

In-lab breeding of *Botrylloides diegensis* requires a suitable marine microbiome

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Colonial ascidians, and members of the subfamily *Botryllinae* in particular, are established models for important biological processes including allorecognition, immunobiology, aging, angiogenesis and whole-body regeneration. In our lab, we are interested in the regenerative capacity of the species *Botrylloides diegensis*. However, the closest location for sampling these animals is located in the Northern Adriatic sea, over 600 km from our location. Moreover, Switzerland being a non-coastal country, we have no direct access to natural seawater to be used for breeding colonies in the lab.

To support our study of *Botrylloides diegensis*, and to further increase the popularity of colonial ascidians in non-coastal regions, we have designed, build and developed a flexible recirculating husbandry setup for their long-term in-lab culture where specimens develop on hanging microscopy glass slides. Through more than 4 years of breeding in recirculating artificial seawater, we show that these animals can be proficiently bred in-land and suggest that our results can be extended to other species of colonial ascidians to promote research on these fascinating animals.

We also show that a suitable marine microbiome is necessary for *Botrylloides*' development with colonies regressing within a few days in sterile artificial seawater. We have found that this requirement is specific to some species of bacteria, as well as to a suitable concentration of the bacteria in the seawater. We further investigate this dramatic interplay between environmental bacteria and colony's health by studying transcriptomics and microbiomics time-courses.

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D2-15

Characterization of *Botrylloides diegensis* whole body regeneration through single-cell RNA-sequencing

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Ascidians are marine colonial chordates living in the intertidal zone as filter feeders. *Botrylloides diegensis* have interesting characteristics, including the ability to undergo whole-body regeneration (WBR). A vascular fragment (~100 cells) is enough to create a new fully-functional adult within two weeks. We carried out single-cell RNA-sequencing with the mature colony and from the regenerating vascular fragments at different points of WBR (Stage 1 - 15 hours post-amputation (hpa), Stage 2 - 24 hpa & Stage 4 - 144 hpa) to get insights on cell fate. There are at least 25 major cell clusters identified for the mature colony. *In situ* hybridisation was used to examine the expression of cluster marker genes. *Chymotrypsinogen B1* (*CTRB1)* is one of the highest upregulated genes enriched within the stomach epithelium. *CUB and sushi domains 3* (*CSMD3*) was another marker gene labelling the neural complex. We found several zooid, blood/immune tissue markers, including *NOTCH1* for stemness and *SOX11* for a stem-like cell population located inside a niche-type structure attached to vascular epithelia in the adult colony. A transient cell population increased dramatically during the early stages of WBR, suggesting a dedifferentiation process required to complete organogenesis. We predict the cells lining the vascular epithelia can dedifferentiate and build adult stem-cell niches similar to bone marrow in humans.

D2-16

Stem cell niche characterization in *Botryllus schlosseri*

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Adult stem cells (SC) are rare, undifferentiated cells found many adult tissues, essential for development and homeostasis, able of self-renewing and differentiating into a series of progenitor cells. They reside in "niches", *i.e.* microenvironments where they receive stimuli regulating their survival and proliferation (Martinez et al., 2022). SC niches have been characterized in some classical animal models (vertebrates, *D. melanogaste*r and *C. elegans*) but information on other organisms, even exhibiting well established SC mediated processes, is scant and fragmented. This is the case of the colonial ascidian *Botryllus schlosseri*, showing SC-based cyclical asexual reproduction and extensive regeneration. In *B. schlosser*i, three putative SC niches have been supposed (Voskoboynik et al. 2008; Rinkevich et al., 2013; Rosner et al., 2013). The endostyle niche is located in the anterior subendostylar sinus, which have been suggested to host putative somatic SCs. The cell islands are groups of cells located in the ventral body wall, along the endostyle, suggested to harbor putative germ SC. The gonad niche is in the lateral body wall and import/export germ cells and somatic cells for ovary and testis formation. In absence of their unequivocal morphological characterization, niches presence has been questioned. We followed the development of the putative niches and characterized qualitatively and quantitatively them by means of histology, Transmission Electron Microscopy, 3D reconstructions, *in situ* hybridization and cell transplantation. We found that the niches are close to sinuses and peripheral nerves. The endostyle niche is more extended than previously supposed and develops during early phases of bud differentiation. In the same colony, cell island distribution varies in zooids in both number and location, and in developmental stages. Within the niches, SC are round, small, with high nucleus/cytoplasm ratio, rich in free ribosomes. They are in the connective tissue, not in the hemolymph sinuses, mostly immobile. In each niche, both mitotic SC and differentiating cells are recognizable. Some SC also express the orthologues of the Yamanaka Factors SoxB1, Myc and Pou3 (Vanni et al., 2022). Cells from dissociated colonies, sorted by FACS and transplanted in compatible colonies are able to differentiate in a number of tissues and to home into the niches. Altogether, our data definitely evidence the presence of SC niches in *B. schlosseri*.

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D2-17

Ciona robusta as model system for Intestinal Stem Cells biology (CrISCs)

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The mammalian adult intestinal stem cells (ISC) maintain gut homeostasis and generate all the different intestinal cells types, either during physiological renewal as well as in response to insults.

We propose a comparative and evolutionary approach to better define the origin and the fundamental characteristics of the ISCs focusing on a non-vertebrate deuterostome, the protochordate *Ciona robusta*. This tunicate possesses a fully sequenced, high quality chromosome-scale genome and has striking analogies to the human and murine gut, which make this species as a suitable model to a comparative study.

RNA-seq analysis on Stomach, Proximal and Distal Intestine has been performed to look at the genes expressed in the gastrointestinal tract. The availability of additional gene expression public datasets will allow to further refining the set of Stomach, Proximal and Distal Intestine markers, organizing them in networks of co-regulated genes, whose putative function will be investigated in detail by functional enrichment analyses. Their placement in signaling networks described in other organisms will be investigated through comparative transcriptomic analyses, which will take into account previously published datasets for other target species.

Comparative histology and immunohistochemistry will corroborate the gene expression data, allowing the characterization of the Ciona intestinal cell type and the expression district of proliferative markers.

A better understanding via cross-comparison of the mechanisms inducing the emergence of ISCs is a pre-requisite for a comprehensive view of how these mechanisms subtend self-renewal and cell proliferation /differentiation and tissue homeostasis.

D2-18

Insights into the cellular and molecular mechanisms of *Polycarpa mytiligera*'s central nervous system regeneration

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Central nervous system (CNS) regeneration is highly diverse across the metazoans, with adult mammals showing relatively limited abilities[1]. Understanding why neurons fail to regenerate following injury remains a central challenge in regenerative medicine. Our previous study revealed the ability of the emerging model system *Polycarpa mytiligera* to regenerate all body organs and tissue types[2]. The current study aimed at investigating the cellular and molecular mechanisms involved in *Polycarpa*'s CNS regeneration. Combining transcriptome sequencing of major CNS regeneration stages with microscopy, and cell proliferation in-vivo essays coupled with FISH studies, we characterized the stepwise events that lead to whole CNS regeneration.

EdU–FISH assay demonstrated the contribution of unspecialized dividing cells to the regenerating brain. The regenerated CNS expressed key neurodevelopmental markers that are not otherwise present in the adult CNS and that the re-establishment of the nervous system, by means of the newly-formed neural progeny, was associated with the emergence of 239 conserved pathways, reflecting enhanced stem-cell related gene activity. Analyzing the expression pattern of these pathways revealed high expression of P53 and piRNA pathways preceding the activation of Notch, Wnt, and Nanos pathways at the early stages of CNS regeneration.

Our regeneration atlas revealed the basic principles and evolutionary conserved elements of CNS regeneration. The molecular and cellular mechanisms controlling regenerative capacity identified in this atlas can be used to study the evolution of stem-cell mediated regeneration and develop approaches to enhance neurogenesis in closely related species.

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D2-19

The complement system of ascidians: clues from the colonial ascidian *Botryllus schlosser*i

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The complement system of vertebrates is a complex array of soluble and membrane proteins able to sense nonself and activate an immune response. Three (alternative, lectin and classic) activation pathways are known: they lead to the cleavage of C3 to C3a and C3b. C3a is a chemokine able to recruit immunocytes at the infection site, whereas C3b acts as an opsonin favoring phagocytosis of foreign material. The lytic pathway can also be activated leading to the ultimate polymerization of C9 in the membrane of nonself cells causing their lysis.

In invertebrates, much less is known on the presence and roles of complement. As far as ascidians are concerned, a decade ago, data were limited and mainly referred to the solitary species *Halocynthia roretzi* and *Ciona robusta*, where transcripts for C3, expressed by a fraction of haemocytes, are known. Ascidian C3 is involved in the modulation of phagocytosis as well as, through its derivative C3a, in the recruitment of circulating cells.

Our interest in the immune responses of colonial ascidians led us to investigate the complement system of *Botryllus schlosseri*. Our research started ten years ago and led to the demonstration of: i) the pivotal role of circulating cytotoxic morula cells in the synthesis of the major complement components of both the alternative and lectin pathways; ii) the importance of C3 activation in modulating phagocytosis; iii) the presence of a G-protein-coupled receptor for C3a/C5a expressed by morula cells and modulating the transcription of C3; iv) the presence of a soluble C1qDC protein involved in inflammation and able to modulate the degranulation of morula cells. New ongoing studies aim to acquire information on the regulators of complement activation and their role in Botryllus biology.

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D2-20

Lessons from the invasive species *Styela plicata*. From the genome to the holobiome

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Invasive species are a major threat to biodiversity and ecosystems and at the same time constitute unique systems to study evolution in action. *Styela plicata* is responsible for one of the most successful spread invasions in ports around the globe. However, few studies analyse this species with a global perspective, and almost none rely on adaptive molecular data due to a scarcity of genomic resources, a trend reversed by our group. To extend knowledge within this field we present the chromosome-level reference genome of *Styela plicata* and the species pangenome using low-coverage WGS of 24 individuals from 6 populations. We recovered three major mitogenomic clades, differing genetically and structurally, and identified polymorphic inversions in four chromosomes, present in all populations. We further genotyped 87 individuals of 18 populations distributed worldwide using 2b-RAD methodology and recovered several genetically differentiated genomic groups, some of them related but other independent of the individual mitochondrial group. We identified several regions across the genome which differentiate the population groups, probably responsible for regional adaptation. Finally, the microbiome compositions at three different compartments (tunic, gills, digestive contents) in 30 individuals, juvenile and adults, from three Mediterranean populations were analysed. Via a multi-level approach, we have identified that the microbiome is tissue specific and differs between ontogenic stages. The time is ripe for invasive ascidians genomics that offer great opportunities to evaluate, from a holistic point of view, the role of standing genomic variation and symbiont acquisition in adaptation processes, and their role determining invasive success worldwide.

D2-21

Stress granules in ascidians: an overview

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Stress granules (SGs) are stalled translational initiation complexes preserving mRNAs for anti-stress proteins and so regulating stress responses. This is possible thanks to the presence of mRNA-binding proteins such as TIA-1 related nucleolysin (TIAR), considered an important core component of SGs [1]. They disassemble in the presence of an acute stress so to unlock the translation of mRNAs into anti-stress proteins [2].

Until now, very few works have been devoted to study SGs in invertebrates, especially in marine species. By using TIAR as SG marker we explored the possible roles of these foci in the solitary ascidian *Ciona robusta* and in the colonial ascidian *Botryllus schlosseri*, both from the Lagoon of Venice.

We started with an evaluation of their involvement in the responses to oxidative stress induced by metals, such as Cu, Zn, Fe and Cd, the impact of which on marine ecosystems is well documented. We carried out gene expression studies by qRT-PCR and *in-situ* hybridization. To validate the hypothesis of SG post-transcriptional control, we used specific anti-TIAR antibody in immunocytochemistry and immunohistochemistry and visualized their subcellular localization in immunocytes through transmission electron microscopy. In addition, the importance of SGs in the regulation of stress responses during embryonic development was investigated in *C. robusta*, through electroporation experiments with construct for reporter gene (LacZ) expression, containing the promoter region for TIAR.

Botryllus, due to its peculiar capability to reproduce sexually and asexually [3], was considered to investigate the SG role during non-embryonic development, with microinjection experiments of the anti-TIAR antibody. The latter experiments suggest that SGs is involved not only in the regulation of stress, for example the one related to the diffuse apoptosis in adult zooid tissues characterizing the weekly renewal of colony [4], but also in cell proliferation required for the full development of new adult individuals.

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D2-22

Ascidians as model organisms for assessing the extent and impact of anthropogenic pollutants in marine environments

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Coastal environments have been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current environmental stressors on the marine fauna, and of the possible potential of marine organisms as biological indicators of environmental health. Our on-going study focus on promoting the use of solitary ascidians, in particular invasive species, as biological indicators of marine environments. As highly efficient filter-feeders inhabiting both pristine and polluted environments ascidians present fundamental opportunities for ecotoxicological studies. By combining a wide variety of methods, we are currently developing tools for ascertaining the extent and impact of anthropogenic pollutants such as micro-plastics, plastic additives, pharmaceuticals, and heavy metals along the Mediterranean and Red Sea coasts of Israel. Our ability to produce lab-grown cultures and to identify a wide suite of contaminants in seawater, sediment and ascidian tissues enable us to accurately quantify a variety of contaminants of emerging concern, and conduct controlled exposure experiments. Results of our project demonstrate the applicability of invasive ascidians as biological indicators, and emphasize the immediate need for the improvement of current monitoring protocols and management plans of coastal environments.

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D2-23

Styela on the edge: life cycle and reproductive patterns of the solitary ascidian *Styela plicata* along the Mediterranean coasts of Israel

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Some solitary ascidians such as *Styela plicata* have become global nuisance species. *S. plicata* is globally distributed with an uncertain origin, considered introduced into the Mediterranean Sea. *S. plicata populations were* first observed in the eastern Mediterranean in marinas along the Israeli coast in 2015, under environmental conditions which are extreme compared to other localities: seawater temperatures as high as 31°C vs. 10-30°C, and salinity of 40 ppt, vs. 22-34 in other localities. We investigated the reproductive cycle of three populations of *S. plicata* for two years: Akko, Kishon, and Jaffa and followed populations dynamics of two populations in the field. Gamete release of 12 individuals from the Jaffa population was monitored in the laboratory. Results show significant differences between seasons in individual size distribution of the monitored two sites, even after one population (Kishon) collapsed during the winter. The Jaffa population, on the other hand, remained stable. Highest reproductive output and developing larvae were observed during winter and spring when sweater temperature range between 17-21°C. The current study shows that this species has an impressive ability to adapt to the extreme temperatures and salinities. These abilities should be further investigated in order to understand the impact of climate change on invasive marine species. Considering the accumulating evidence of the potential harm of *S. plicata* to natural fauna and artificial substrates, further monitoring of this species is required.

D3-01

The mutation *frimousse* uncovers a cryptic oscillator that drives spontaneous orienting behavior

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Ciona larvae show short spontaneous orienting swims that precede phototaxis (*1*). We observed that the time interval between spontaneous swims is highly variable, but with a peak at ~15 seconds. By imaging cholinergic neural activity using a VACHT promoter driving GCaMP7f we observed a single slow oscillating neuron in the midbrain (also known as the *posterior sensory vesicle*) with a period of ~15 seconds, and that was sporadically disrupted by trains of Ca^{2+} spikes. By contrast, the mutant line *frimousse* (*frm*) (*2*, *3*), which is lacking forebrain, shows a much more stereotyped spontaneous swim period, with spontaneous swims occurring at highly regular intervals of 9.6 seconds (*4*). In *frm* larvae we observed a single midbrain neuron with oscillating Ca^{2+} spikes matching the period of the observed spontaneous *frm* swims (~9.6 seconds). We hypothesize that the slow cholinergic oscillator is a driver of spontaneous activity. Its periodicity in wild type (15 seconds) is responsible for the observed spontaneous swim periods peaking at 15 seconds. However, the spread of observed inter-swim periods to intervals shorter and longer than 15 seconds suggest a more complex mechanism. We have previously observed that inhibition of $GABA_A$ receptors with picrotoxin greatly increases swim frequency, suggesting the spontaneous swimming is under inhibitory control (*4*). We also observed increased swim frequency, and stereotypy, in *frm* mutants. *In situ* hybridization for VGAT shows a number of inhibitory forebrain neurons that are lost in frm. These inhibitory neurons, many of which project to the midbrain, are the subject of current research.

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D3-02

Previously uncharacterized inhibitory autonomous oscillator revealed by *frimousse* mutants

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As a prelude to phototaxis, *Ciona* larvae exhibit tumbling spontaneous swims that are independent of visible light stimuli (1). The mechanism driving spontaneous swim initiation is currently not well understood. We observed that the spontaneous swim behavior of wild type *Ciona* larvae differs from *frimousse* (*frm*) larvae, a mutant which lacks the forebrain. While the spontaneous swim intervals of wild type larvae are apparently stochastic, the *frm* larvae exhibited more stereotyped swim intervals. To understand the difference in behavior between these two groups, we looked into the neural circuitry of *Ciona* to identify what the *frm* might be missing that generates their more regular spontaneous swims. Live fluorescence imaging of GABAergic activity using GCaMP driven by a VGAT promoter revealed the presence of a previously uncharacterized inhibitory oscillating neuron in the forebrain of wild type animals, but not in *frm*. This class of neuron, which we named the anterior brain vesicle oscillator (aBVO), is the only inhibitory oscillator found in the entire larva and is observed to have a period of ~2 seconds. Our lab has been able to isolate the aBVO and confirm it is an autonomous oscillator. We have tentatively identified aBVO as neuron BVIN13 of the connectome (2). Like the aBVO, BVIN13 projects to midbrain excitatory relay neurons. With this information, we are currently trying to modulate the activity of the aBVO to better understand its potential role in spontaneous swimming.

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D3-03
Tracing back for ancestry of vertebrate neuronal traits: clusters of sodium channels

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Typical invertebrate neurons achieve rapid conduction velocity by reducing axial resistance with large axon diameter, typically represented by squid giant axon for jet propulsion. On the other hand, in vertebrate brain, size and rapid conduction velocity of neurons are compromised by reduction of membrane capacitance with myelination. Such saltatory conduction in vertebrates depends on activities of voltage-gated ion channels clustered at specialized membrane regions called nodes of Ranvier and axon initial segments (AISs). Hill, Nishino et al [1] previously showed lamprey lacks myelin but contains sodium channel clusters at the proximal region of cell body, which resemble axon initial segments (AISs). This suggested that neuronal traits for rapid conduction velocity of vertebrates were acquired by sequential steps during chordate evolution; first, ion channel cluster, followed by myelination. Recent studies on molecular architecture of mammalian neurons have shown that most of molecular components are common between nodes of Ranvier and AISs. Ankyrin-G is an adaptor molecule that orchestrates clustering sodium channels, cell adhesion and regulation of cytoskeleton in nodes and AISs. It has previously been shown that *Ciona robusta* voltage-gated sodium channel, CiNav1a, and *Halocythia rorezi* Nav1 (previously called TuNa1) contain ankyrin binding motif at the cytoplasmic loop region, suggesting that they might form AIS-like clusters in ascidian neurons.

To explore for the evolutional origin of sodium channel clusters related to the architecture of vertebrate nodes and AISs, we have studied function, gene expression and subcellular localization of CiNav1a. CiNav1a exhibited rapidly activating and inactivating sodium current upon heterologous expression in Xenopus oocyte but it was TTX-insensitive. Immunostaining with polyclonal antibody against CiNav1a showed panneuronal expression both in tadpole larva and juvenile. Signal was also found in the cerebral ganglion of adult. We are also currently studying whether ankyrin is colocalized with CiNav1a in any membrane domain of ascidian neurons.

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D3-04

Gravitaxis neural circuit in the ascidian larva

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The central nervous system of the *Ciona intestinalis* larva contains 177 neurons. The larva utilizes simple neural circuits to perceive environmental stimuli and change their behavior. The sensory organs, otolith, and ocellus are involved in gravity sensing and photoreception. Sensory neurons named antenna cells are associated with otolith and are thought to be gravity-sensing neurons.

The ascidian larvae change swimming patterns during the larval stage. First, the larvae swim upward with a negative gravity response in the initial period of the larval stage. Then, four hours after hatching, the larvae swim downwards with a photophobic behavior. Finally, 8 hours after hatching, the larvae settle to the substrate and start metamorphosis. This change in swimming direction may involve the development of the photophobic response and changes in the neural circuit that senses gravity. However, the structure and function of the gravitaxis neural circuit remain unclear.

To understand the structure and function of the gravitaxis neural circuit in the ascidian larva, we constructed an experimental system to express foreign genes, specifically in antenna cells. Previous studies have shown that vesicular glutamate transporter (*VGLUT*) is expressed in antenna cells. The Kaede reporter driven by 2.8 kb of *VGLUT* promoter was expressed in glutamatergic neurons, including antenna cells in the larval stage. We performed deletion analysis of the *VGLUT* promoter and identified antenna cells' specific enhancer.

Furthermore, we labeled antenna cells with this enhancer and performed Ca^{2+} imaging of glutamatergic neurons. The results revealed that the activity of the antenna cells starts immediately after hatching when the larvae swim upward with gravity response. On the other hand, photoreceptor cell activity was not observed immediately after hatching but intensified about 2 hours later. These results are consistent with the swimming behavior of the ascidian larvae, suggesting that antenna cell activity is involved in the gravity response and downward swimming of them.

We are currently conducting single-cell transcriptome analysis in the ascidian embryos labeled with antenna cells. Based on the results, we will discuss the functions and characteristics of antenna cells.

D3-05

A single motor neuron determines the rhythm of early motor behavior in *Ciona*

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Recent work in tunicate supports the similarity between the motor circuits of vertebrates and basal deuterostome lineages. To understand how the rhythmic activity in motor circuits is acquired during development of protochordate *Ciona*, we investigated the coordination of the motor response by identifying a single pair of oscillatory motor neurons (MN2/A10.64). The MN2 neurons had Ca^{2+} oscillation with an ~80-s interval that was cell autonomous even in a dissociated single cell. The Ca^{2+} oscillation of MN2 coincided with the early tail flick (ETF) observed at stage (St.) 23 to 24. The spikes of the membrane potential in MN2 gradually correlated with the rhythm of ipsilateral muscle contractions in ETFs. The optogenetic experiments indicated that MN2 is a necessary and sufficient component of ETFs. These results indicate that MN2 is indispensable for the early spontaneous rhythmic motor behavior of *Ciona*. Furthermore, we have succeeded in long-term imaging of MN2 neural activity after St. 24, strongly suggesting that MN2 determines not only in the rhythms of ETFs but also in rhythms during swimming behavior. Our findings shed light on an understanding of the development and evolution of rhythmic locomotion of chordates.

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D3-06

The modeling of spontaneous orienting swim in *Ciona* based on the interaction of two oscillators

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By using VGAT- and VAChT-promoter driven GCaMP7f, we measured the calcium ion flux in the neurons of the protochordate *Ciona robusta*. We observed two oscillating neurons in the forebrain and the midbrain of the wild type larva respectively. Fourier transform of the GCaMP recording shows the forebrain oscillator (FO) has an oscillation frequency of ~0.4 hertz and the midbrain oscillator (MO) a frequency of ~0.07 hertz. The MO frequency closely matches the most frequent swim-to-swim interval in the spontaneous orienting swim preceding the phototactic swim, ~15 seconds (1). The forebrain is absent in the mutant *frimousse* (*frm*) while the midbrain, hindbrain, and the caudal nerve are still intact (2, 3). Yet *frm* still performs a highly stereotyped spontaneous swim with the two most frequent swim-to-swim intervals in *frm* mutant being ~2 second and ~9 seconds (4). The GCaMP recording of the *frm* MO shows a different frequency compared to the wild type. Fourier transform of the *frm* GCaMP recording shows two frequencies of interest at ~0.11 hertz and ~0.57 hertz. Both frequencies also closely match the swim-to-swim interval. Since the FO is GABAergic and the MO cholinergic, we proposed a model to explain the changes in oscillation frequency and swim-to-swim interval due to the presence of FO in wild type larva.

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D3-07

Transcriptional regulation of neuronal polarity and neurite outgrowth in the ddNs of *Ciona*

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Ciona is a good model organism for neurodevelopmental studies. It has well-conserved chordate-specific genome and gene regulatory networks, and miniaturized but typically chordate central nervous system consisting of 177 neurons (Ryan et al., 2016). In *Ciona*, Descending Decussating Neurons (ddNs) are a single left-right pair of motor ganglion neurons proposed to be homologous to vertebrate Mauthner cells (Ryan et al., 2017). Here, we investigate the spatiotemporal deployment of a gene regulatory network for specification, differentiation, and unique axon trajectory of the ddNs. The ddNs show noteworthy features of cell behaviors during their development, such as inversion of apicobasal polarity and polarized axon outgrowth towards and across the midline (Gibboney et al. 2020). As we previously revealed, Pax3/7 is necessary and sufficient for ddN specification and polarized axon growth (Stolfi et al. 2011). Pax3/7 also activates the expression of downstream genes encoding other transcription factors in the ddNs, including Pou4, Lhx1/5, and Dmbx (Imai et al. 2009). Here we show that Pax3/7 and Pou4 activate different candidate effector genes in the ddNs. While Pax3/7 is necessary and sufficient for the expression of *ddN-expressed peptide (Ddpep)*, Pou4 activates *Stabilizer of axonemal microtubules (Saxo)* and *Sel1 domain-containing membrane protein (Seldom)*. We also show that Pax3/7 regulates *Ddpep* in the ACINs (variably called Anterior Caudal Inhibitory Neurons or Ascending Contralateral Inhibitory Neurons), while Pou4 also activates *Saxo* in various neurons of the peripheral nervous system. This distributed regulatory logic may be important for activating different combinations of effector genes in distinct neuronal subtypes. We present preliminary evidence that Ddpep might be an autocrine cue for axon guidance, while Saxo may have diverse roles related to its microtubule-stabilizing properties, from ciliogenesis to axon growth.

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D3-08

Dynamic integration of signaling, force generation and tissue remodeling control zippering and neural tube closure

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We use zippering during neural tube closure in the simple chordate *Ciona robusta* to study how dynamic coupling of signaling and force generation patterns collective cell movements. During zippering, the neural folds meet and fuse from posterior to anterior, replacing heterotypic junctions between neuroectodermal and epidermal cells (Ne/Epi junctions) with homotypic Ne/Ne and Epi/Epi junctions. We previously showed that zipper progression is powered by sequential RhoA/Myosin II activation and rapid contraction of Ne/Epi junctions, in a posterior to anterior wave just ahead of the moving zipper. Here, we show that wave of RhoA/Myosin II activation is tightly coordinated with local separation of Ne/Epi junctions ahead of the advancing zipper. Local detachment of apical (tight and adherens) junctions correlate with activation of RhoA and myosin II. Acute induction of Ne/Epi junction separation by inhibiting binding of cell-cell adhesions or applying mechanical force leads to rapid accumulation of RhoA/Myosin II activity wherever separation occurs, suggesting that separation of Ne/Epi junctions induces RhoA/Myosin II activity. We show that the structural components of tight junction and adherens junction are depleted specifically along Ne/Epi junctions ahead of the advancing zipper, and that junction separation is locally biased to multicellular vertices and to the zipper itself – regions where junctional forces pulling perpendicular to the Ne/Epi boundary are concentrated. These observations suggest that local patterns of adhesion and junction tension are dynamically and reciprocally coupled ahead of the advancing zipper. Therefore, we propose that zipper progression is governed by a tissue-level feedback loop in which: junction tensions separate local Ne/Epi junctions at zipper, inducing rapid and local activation of RhoA/myosin II and junction contraction, moving the zipper forward and bringing more anterior Ne/Epi junctions into signaling range for junction separation.

D3-09

An ERF transcription factor participates in the bimodal transcriptional response of an ERK-target gene during ascidian neural induction

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During the initiation of neural induction in ectoderm cells of the 32-cell stage ascidian embryo, graded FGF signalling is converted into the bimodal transcriptional output of *Otx*, an 'immediate-early' target gene. Focusing on the 8 ectoderm cells of the 'a-line', we show that quantitative measurements of nuclear ERK activation levels scale with the area of cell surface contact measured between the ectoderm cells and the FGF-expressing mesendoderm cells. In contrast, quantitative in situ hybridisation of *Otx* reveals a bimodal profile, with ectoderm cells having either high or low numbers of transcripts. Neural precursors have the largest area of cell surface contact with FGF-expressing mesendoderm cells, exhibit the highest levels of ERK activation and have high *Otx* transcript counts. Activation of *Otx* depends upon the ETS1/2 transcription factor and ETS- binding sites in the upstream regulatory sequences of *Otx*. Another member of the ETS family of transcription factors, the ERF2 repressor, acts to repress *Otx* in non-neural ectoderm cells. Mathematical models support the hypothesis that competition between ETS1/2 and ERF2 for the ETS binding sites increases the sensitivity of the *Otx* response to ERK. Our data suggests that non-linear nuclear export of ERF2 could contribute to generating ultrasensitivity in the *Otx* transcriptional response to ERK.

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D3-10

Computational model of neural induction in the ascidian embryo

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Embryonic development is a highly reproducible process that relies on the ability of cells to interpret signals from their environment and activate specific genetic programs. The role played by the geometrical arrangement of the cells in the embryo in determining cell fate is poorly understood. Because the embryogenesis of ascidians proceeds with an invariant cell division pattern, such that cellular configurations and cell cycle progression are quasi-invariant, this organism provides a unique tool to investigate the impact of the geometrical arrangement of cells on the level of signaling, and consequently on cell fate induction.

We focus on neural induction in early ascidian embryogenesis. During this process, precisely two ectoderm cells adopt the neural fate, characterized by the expression of the neural marker *Otx*. The inputs of the signaling cascade, FGF and ephrin, are converted via the surfaces of contact of the cells with the signaling molecules into different levels of ERK activity that are then transduced into a bimodal *Otx* expression.

We developed a computational model to provide a full mathematical description of FGF and ephrin-signaling control of neural fate induction in the 32-cell stage ascidian embryo. Together with *in vivo* observations, our results lead us to conclude that the process of neural induction is controlled by the cell surface contacts with FGF and ephrin. The model also provided quantitative information about signal processing and highlights how a moderately nonlinear ERK signalling is transduced into an ON or OFF expression of the neural marker *Otx*.

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D3-11

Highly divergent genetic programs for peripheral nervous system formation in chordates

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Vertebrates develop their peripheral nervous system (PNS) from transient unique embryonic structures, the neural crest and the ectodermal placodes that are located at the border of the forming central nervous system. By contrast, in the invertebrate chordates, amphioxus and ascidians, a large part of the PNS originates at the opposite of the embryo, in the ventral ectoderm. In both groups, a biphasic mechanism regulates ventral PNS formation: high BMP levels specify a neurogenic territory within which glutamatergic epidermal sensory neurons formation is controlled by the Notch pathway. Given these similarities and the phylogenetic relationships within chordates, it is likely that ventral PNS is an ancestral feature in chordates and that it has been lost in vertebrates. In order to get insights into the molecular control of ventral PNS formation and to test the hypothesis of their homology, we undertook a close comparison of ventral PNS formation in the ascidian *Phallusia mammillata* and the amphioxus *Branchiostoma lanceolatum*. Using timed RNA-seq series we identified novel markers of the ventral PNS during different phases of its development in both species. Then, by extensively determining the expression of paralogous and orthologous genes, we observed that only a small fraction of the genes have a conserved expression in the ventral PNS. This suggests two opposing interpretations. Either the amphioxus ventral PNS and the ascidian ventral PNS are not homologous, or they are but extensive drift has occurred in their regulatory mechanisms given the long period (600My) of separate evolution and accelerated evolution in the ascidian lineage. Interestingly, a large fraction of the ventral PNS orthologous genes are actually expressed in the dorsally forming PNS of vertebrates, suggesting that ancestral sensory neurons gene networks have been redeployed in vertebrates.

D3-12

Oikopleura dioica, the cosmopolitan appendicularian hides multiple cryptic species around the globe

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Cryptic species are often hidden under one species name because of their great morphological similarities. Identifying cryptic species is fundamental to fully understand Earth's biodiversity. Appendicularian tunicates are one of the most abundant organisms of the mesozooplankton with key role in marine trophic webs and global carbon flux. Like most appendicularians with cosmopolitan distributions, *Oikopleura dioica* (Fol 1872) is considered a single species worldwide based on morphological features that distinguish them from other species. The results of our comparative genomic analyses across distant populations of *O. dioica,* including phylogenetic analysis of nuclear (18S and ITS) and mitochondrial (COI) genes and syntenic analysis (see the accompanying abstract) revealed at least three genetically distant lineages (Ryukyu Archipelago, the North Pacific, and the North Atlantic/Mediterranean) that may conform different species despite their great morphological similarities. *In vitro* crosses of two neighboring populations from Japan that belonged to different lineages showed total prezygotic reproductive isolation. Our results, therefore, reveal that *O. dioic*a likely hides multiple cryptic species, and provides an attractive model to understand how strong morphological conservation can be maintained despite extensive genetic distance and genomic structural variation.

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D3-13

Drastic genome remodeling across *Oikopleura dioica* worldwide

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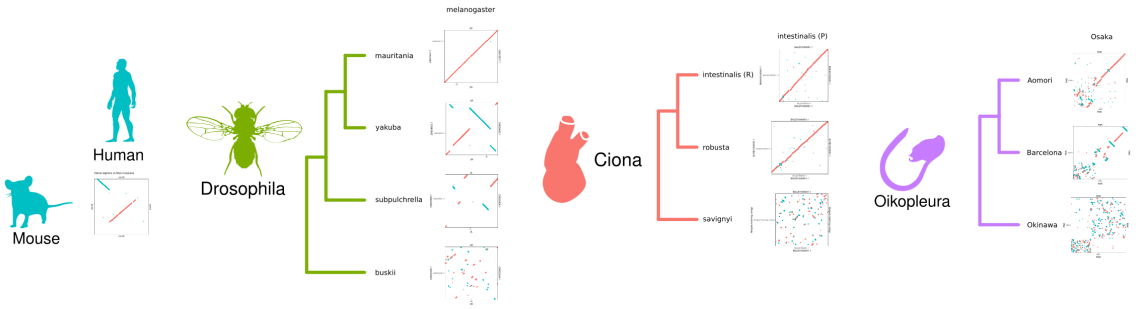
The conserved arrangement of genes on chromosomes (synteny) reflects the existence of genomic mechanisms relying on proximity and is also essential to the formation of crossovers during meiosis. Some synteny remains between animals having their last common ancestor 500 million year ago. The zooplankton *Oikopleura dioica* has one of the fastest-evolving animal genomes, due to its fast life cycle of only a few days, and the lack of the non-homologous end joining pathway (NHEJ) for repairing DNA damage such as UV-induced DNA breaks. Its genome is one of the smallest in the animal kingdom (65 Mbp), allowing us to sequence it at a reasonable cost.

We sequenced genomes of single *Oikopleura dioica* individuals from the North Atlantic, Mediterranean sea, North Pacific, and Subtropical Pacific (one chromosome assembly plus one validation draft per region) and developed a bioinformatics pipeline for automating pairwise genome comparisons using the Nextflow system. Applying this pipeline on our data, we observed an unexpected extent of chromosome rearrangements given the extraordinarily conserved morphology of these animals (see the accompanying abstract). We report here a quantitative analysis of the structural differences between 6 genomes, sequence features in the regions flanking the synteny breakpoints, and the discovery a palindromic motif that might be implicated in the formation of these structural changes. We computed molecular clocks and estimated possible speciation events at the scale of a few 10s of millions of years.

Our study shows that gene order in genomes of distinct *Oikopleura dioica* in different places around the globe is subject to considerable changes, suggesting that regulatory circuits in *Oikopleura dioica* are less dependent on long-range interactions than other chordate animals. Even in comparison with other pairs of species that diverged at a similar time scale, the number of breaks of syntenies in *Oikopleura dioica* genomes remains among the highest ever reported so far across animals. We propose that the *Oikopleura dioica* genome can be used as a contrast model to decipher the principles constraining gene order, with the prospect of applying these rules to the synthesis of artificial genomes reduced in size and designed *in silico*.

Alignment of 10-Mbp windows in pairs of increasingly distant species.



D3-14

Gene expression in the context of genomic rearrangement in *Oikopleura dioica*

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Animal genomes exhibit patterns of conservation and variation that are typically related to the functions encoded by different genomic regions. Conversely, functional regions of the genome can be considered to constrain the allowable space of genetic variability. Although the relationship between sequence conservation and function can be complex, some patterns are clear: genic regions are typically under strong purifying selection and therefore conserved, particularly within exonic regions, and further patterns emerge at the level of individual codons. Genes and genomes must also encode functional elements necessary to successfully transcribe and translate coding sequences, and thus regulatory regions impose their own constraints on genetic variation. In all cases, mutations that persist to fixation within a population or species can be considered to have survived some level of selective filtering, and may in fact represent adaptations.

Recent efforts by our lab and others have produced high-quality, chromosome-scale genome assemblies of *Oikopleura dioica*, including representatives from the North Pacific and Subtropical Pacific. Comparative genomic analyses between these populations revealed a substantial degree of genomic rearrangement, which is particularly surprising given both populations are largely morphologically indistinguishable. Conservation of morphology with variation in gene order implies the existence of compensatory gene expression mechanisms.

We have evaluated the conservation and variation of gene expression through side-by-side comparison of *O. dioica* populations from the North Pacific and Subtropical Pacific. By combining transcriptomic data (RNAseq) with chromatin accessibility data (ATACseq), we have evaluated gene expression throughout embryonic development, spanning fertilization to tailshift. We have also employed single-library CAGE to annotate operon sequences. Ultimately, we anticipate that these data will contribute to understanding how transcriptional mechanisms are able to accommodate the extensive degree of genetic variation observed between populations of *O. dioica*.

D3-15

Hmx gene conservation identifies the origin of vertebrate cranial ganglia

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The evolutionary origin of vertebrates included innovations in sensory processing associated with the acquisition of a predatory lifestyle. Vertebrates perceive external stimuli through sensory systems serviced by cranial sensory ganglia, whose neurons predominantly arise from cranial placodes; however, understanding the evolutionary origin of placodes and cranial sensory ganglia is hampered by the gulf between living lineages and difficulty in assigning homology between cell types and structures. To address this question, we used the *Hmx* gene family that links vertebrate cranial ganglia to tunicate bipolar tail neurons.

We showed that *Hmx* is a constitutive component of vertebrate cranial sensory ganglia development and that the single *Hmx* gene found in *Ciona intestinalis* marks specifically the bipolar tail neurons. Through gain and loss of function we also demonstrated that *Hmx* in *Ciona* is necessary and sufficient to drive the differentiation program of Bipolar Tail Neurons, cells previously thought to be homologues of neural crest[1,2]. Using *Ciona* and lamprey transgenesis we found that a unique, tandemly duplicated enhancer pair regulate *Hmx* in the stem-vertebrate lineage. Strikingly, we also showed robust vertebrate *Hmx* enhancer function in *Ciona*, demonstrating that deep conservation of the upstream regulatory network spans the evolutionary origin of vertebrates.

These experiments demonstrate regulatory and functional conservation between *Ciona* and vertebrate *Hmx*, and point to bipolar tail neurons as homologues of cranial sensory ganglia.

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D3-16

A minimalist genomic toolbox? The case of *bona fide* microRNA evolution on tunicates

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The advent of high-throughput sequencing technologies provided a unique opportunity to reconstruct evolutionary histories from coding and non-coding RNAs along the evolution of chordates. This study focused on the evolution of the microRNA (miRNA) complement of tunicates and related deuterostomes. As widely conserved post-transcriptional regulators, miRNAs have been reported as essential key players with tunicate-specific developmental roles, including: cellulose synthesis[1], mechanisms of larval metamorphosis[2], neural fate determination[3], and so forth[4]. Evolutionary features like faster miRNA family innovation and an extensive loss of conserved families are correlated with the overall morphological simplification of tunicates[5,6]. In this regard, the inclusion of all available genomic information is critical to correctly decipher the miRNA evolutionary history in chordates.

Curation and reconstruction of 1807 miRNA probabilistic models enabled us to perform a comprehensive annotation of canonical miRNAs covering 16 tunicate and 13 additional deuterostomes species using the miRNAture pipeline[7]. Furthermore, thorough comparison with established annotations and short RNA-seq data allowed us to identify a set of *de novo* families. Finally, gain/loss events were calculated using Dollo's parsimony and extended miRNA structural alignments were enhanced by syntenic anchors.

Compared to other deuterostomes, tunicates exhibit a dramatic loss of canonical miRNAs. Despite this reduction at species level, larger numbers of miRNA families were shared within Olfactores in comparison to cephalochordates and vertebrates, or the ancestral miRNA repertoire of chordates. In this study, the miRNA complement in tunicates and clade-specific families was further extended and improved.

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D3-17

MorphoNet

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We have developed an innovative online morphodynamics browser, MorphoNet, to share, curate and interact with large-scale segmented meshed data, in a FAIR philosophy. It permits interactive visualization, annotation and sharing of complex segmented 3D and 3D plus time datasets with an intuitive graphical user interface, running on a standard web browser. In a similar way as genomic browsers display genetic features and epigenetic or gene expression data as traces onto the primary genome sequence, quantitative and qualitative information can be imported and projected onto individual or grouped segmented objects in MorphoNet. These "morphological augmentations" can be saved and shared with other users.

Moreover, MorphoNet offers more flexibility than genome browsers. While the DNA base pair is the universal unit of information in genome browsers, the relevant units of information can vary within imaging datasets, from complex organs down to molecular complexes. The choice of the unit of information is therefore left to the user. Objects can also be hidden, made translucent or hierarchically grouped: spatially (e.g., by tissue), temporally (e.g., by cell lineages), by imaging channels (e.g., nuclei and plasma membranes), and identified with specific color labels. We have recently improved robustness, speed, added many new features and the application can now be easily integrated with OMERO and FIJI.

Moreover, MorphoNet offers now a complete panel of exploration of the gene expression database ANISEED. You have now to possibility to colorize each individual cells by their gene expression profile. But you can also combine multiples profiles in order to either visualize the difference or the union of some specific selected cells.

An example of a fully annotated Phallusia Mammillata embryo http://morphonet.org/TO1r1t8T.

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D4-01

Cionectome: An interactive viewer and analysis tool for the *Ciona* connectome

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The *Ciona* larva is the only chordate for which a complete synaptic connectome has been derived (*1*). The connectome details 6618 synapses, 1206 being electrical synapses, between the 231 neurons making up the central and peripheral nervous systems in a single *Ciona* larva. Because of its small size, the *Ciona* nervous system is ideal for the analysis of sensory-motor circuits driving behaviors. Moreover, as the sole representative of a deuterostome connectome, it is valuable for comparative analysis of circuit logic and architecture to the other available connectomes (*i.e.*, *C. elegans* and *Drosophila*). In order to make the *Ciona* connectome data more accessible and useful, we are developing an interactive connectome viewer housed in *MorphoNet* (*2*) that will be synchronized to the databases on ANISEED. The user interface of Cionectome consists of an interactive three-dimensional depiction of the larval nervous system in which the individual neurons can be viewed as spheres corresponding to soma centroids, full cell reconstruction given by the serial section EM, or a mix of the two. Synaptic connections are shown as connecting lines with color-coded directionality. Each neuron is identified with the name given in the connectome publication (*1*), and by hovering over the neurons with a mouse a pop-up shows the known attributes of that neuron [*e.g.,* neurotransmitter use, receptor expression, transcriptome data]. The neuron representations can also be highlighted or hidden by these attributes for ease of viewing and analysis. A preview of Cionectome can be found at tinyurl.com/cionectome .

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D4-02

The *Ciona* AMG neuron complex and the origins of the cerebellum

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The vertebrate cerebellum lies over the anterior hindbrain where it serves as a center of sensory processing, coordinating peripheral input to fine-tune motor function *(1)*. While the cerebellum's origins have been traced to as early as agnathan fish *(2)*, we propose that tunicates may reveal still deeper roots. The *Ciona* AMG (Ascending Motor Ganglion) neuron assemblage is found dorsally in the larval motor ganglion, at the positional equivalent of the vertebrate anterior dorsal hindbrain. Seven cells make up this complex, including a single excitatory cell - through which most sensory input is routed - and six cells with inhibitory output, mirroring the circuitry of the vertebrate cerebellum *(3)*. The synaptic connectivity which characterizes the AMGs suggests these neurons integrate peripheral with visual inputs to influence motor function; to better quantify their contribution to swim behaviors, we are using targeted laser ablation to disable AMG function. Candidate inducers of the AMG complex will be assayed using drugs applied in discrete temporal windows during tailbud stages, potentially revealing common developmental requirements with the vertebrate cerebellum. While many studies establishing homology have relied on single or few diagnostic genes, the extensive differences accumulated since the tunicate/vertebrate split, and an apparent lack of exclusively cerebellar markers in vertebrates, suggest to us a different strategy, comparative transcriptomics. Homology is not a binary state, determining to what extent the AMGs and cerebellum share ancestry will help to reconstruct the history of innovations which characterize the chordate CNS.

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D4-03

Neural Innervation of the Juvenile and Adult heart of *Ciona robusta*

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The dynamic process of neural development must be precisely orchestrated in order for neurons to innervate their target cells, such as the heart. The process of cardiac innervation is not well documented, especially in the developing *Ciona robusta* juvenile. Moreover, the role of these neurons in the adult heart is mysterious. Here we use the neuronal construct PC2>Kaede to closely characterize heart innervation across developing juvenile stages. Using live and fixed imaging approaches we identified a small population of PC2+ cells early in juvenile development superficially positioned on the developing heart. The PC2+ cells possess neurite-like projections by day 9 of development. To investigate the role of neuronal signaling on heart function in *Ciona robusta*, we used pharmacological approaches to block neuronal signaling in the heart. We injected a Neurokinin receptor antagonist, Aprepitant, to block neuronal Tachykinin signaling specifically in the adult heart. We find a significant reduction in cell proliferation observed in the heart upon inhibiting Tachykinin. These data suggest proper neuronal innervation promotes proliferation of cardiomyocytes in *Ciona robusta*. We are currently using super-resolution imaging techniques to generate a time-course of cardiac innervation in juveniles. Additionally, we are in the process of uncovering the mechanisms underlying neural development through applying CRISPR-Cas9 genome editing in juveniles.

D4-04

Dolioletta gegenbauri: a model tunicate for investigating the evolution of novel traits

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The evolution of novel traits involves regulatory network rewiring and the reallocation of cell fates often during embryogenesis. Doliolids (thaliacea) evolved a life history requiring the development of unique morphological features, such as circumferential muscle bands supporting propulsion within their pelagic lifestyle. Utilizing culturing techniques that promote successful procurement of fertilized eggs, we have begun to analyze Doliolid embryonic development on several levels. Fertilized egg clusters were obtained for live-imaging and confocal microscopy analysis. Several intriguing behavioral and morphological features were observed, including exceedingly long, slow-moving tails. Notochord cell quantity varied, up to 74, while tail muscle quantity totaled 36, the same as phlebobranch Cionids. We sequenced the *Dolioletta gegenbauri* genome and transcriptome and completed an initial transcriptome analysis of various cardiopharyngeal genes over embryonic timepoints and major life stages. We also generated reporter constructs of putative regulatory elements using Doliolid genomic DNA. Minimalized elements were determined using reporter assays in *Ciona robusta* and showed expression in cardiopharyngeal lineages. *FoxF* regulation in *C. robusta* is ETS-dependent, however a *D. gegenbauri FoxF* minimal element driving expression in the *Ciona* cardiopharyngeal lineages did not contain any ETS binding sites. Curiously, transcriptomic analysis detected expression of the *D. gegenbauri* ETS homolog only in larval and adult stages, suggesting that Doliolids have abandoned that particular route of MapK-dependent cell specification during embryonic development. We are currently performing single-cell sequencing analysis of embryonic development with the goal of understanding alterations in transcriptional programs leading to reallocated cell fates during tissue and organ morphogenesis.

D4-05

Neuropeptides, peptide hormones, their receptors and biological roles in *Ciona*: new aspects of peptidergic systems in chordates

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Over the past fifteen years, we have identified more than thirty *Ciona* neuropeptides (80% of all identified ascidian neuropeptides) mainly using peptidomic approaches combined with reference to the genome sequences [1, 2]. These neuropeptides are classified into two groups [1, 2]. The first group includes homologs or prototypes of vertebrate neuropeptides, and the second group includes *Ciona*-specific novel neuropeptides. We have also identified the cognate receptors for these peptides [2]. In particular, we elucidated multiple receptors *Ciona*-specific neuropeptides by a combination of our original machine learning system and experimental evidence for specific signaling by the predicted neuropeptide-receptor pairs, and verified unprecedented evolutionary relatedness of receptors for neuropeptides [3]. Moreover, several neuropeptides were found to responsible for the regulation of ovarian follicle growth and maturation. *Ciona* tachykinin specifically participates in the growth of vitellogenic follicles via up-regulation of enzymatic activities of proteases [4,5], and *Ciona* vasopressin triggers oocyte maturation and ovulation via activation of maturation promoting factor- and matrix metalloproteinase-directed collagen degradation, respectively [4,6,7]. *Ciona* cholecystokinin was also shown to activate ovulation via up-regulation of receptor tyrosine kinases and the following activation of matrix metalloproteinase [4,8]. These results revealed that the peptidergic nervous system plays major roles in ovarian follicle growth, maturation, and ovulation in *Ciona*. Combined with the critical phylogenetic position of *Ciona*, these novel findings pave the way for investigating novel molecular mechanisms and evolutionary processes of various neuropeptidergic systems in chordates.

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D4-06

Using ATAC-sequencing to study chromatin accessibility during early whole body regeneration

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Botryllid ascidians like *Botrylloides diegensis* have various interesting life history characteristics, including the ability to undergo whole-body regeneration (WBR), hibernation/aestivation, blastogenesis, metamorphosis, and natural chimerism. A small vascular tissue fragment from a *B diegensis* colony (~100-200 cells) is enough to create a new fully-functional adult within 8-14 days. WBR ability is restricted to a small number of species, such as hydra and planaria, with botryllids as the only chordates that can undergo this type of regeneration. *B. diegensis* WBR differs from other WBR examples, notably by the emergence of regeneration niches that involve the clustering of progenitor cells to form a regeneration bud that forms the organ systems of the adult.

Previously, we demonstrated that histone deacetylation (HDAC) activity is essential for completing WBR (Zondag et al., 2019). Histone protein modifications, such as acetylation, regulate transcription factor access to the genomic DNA by changing chromatin structure. To understand this in-depth, we performed ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) on control and HDAC inhibited colonial groups during the early stages of regeneration. Over 300 differentially accessible regions were found, and the functional annotations linked these regions to genes involved in cell proliferation regulation and differentiation, such as *RIG1*, *NOS1, FCN2* and *DLK1*. These genomic regions were also enriched for FOX-binding motifs, a class of transcription factors know to alter chromatin accessibility.

In addition to genome-wide studies, the roles of the chromatin remodellers during WBR, including histone variants and chaperone targets, are currently being investigated. We hypothesize that rapid changes to chromatin dynamics are essential for early cell differentiation from progenitor aggregates.

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D4-07

Repetitive sequence DNA/TcMar-Tc1 is enriched at the boundaries of cis-regulatory regions in Ciona

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A number of previous research conducted using reporter-gene assay has revealed that cis-regulatory regions located just close to their flanking genes work as their enhancers in Ciona robusta.[1,2,3] Such experimentally validated cis-regulatory regions are stored in public databases, including DBTGR, CITRES, and ANISEED, but they are not complete.[4,5,6] Furthermore, although construct-truncation studies have revealed their boundaries and have defined the undividable units of enhancers, such information has not always been added to such databases.[7,8,9]

We have manually surveyed cis-regulatory regions from literature published from 1997 to 2020.[10,11,12,13] The number of curated articles is 282. These experimentally validated cis-regulatory regions were mapped to the reference genome in JGI 1.0, JGI 2.1, Ensembl, RefSeq, KH2012, and HT2019, respectively.[14,15,16,17,18] The total number of mapped sequences is over 2,200 in FASTA format. We also store the results of truncation studies whose total number is over 780.

By the VISTA plot, we found that some of them share around 100bp consensus sequences which are likely to be parts of repetitive elements.[19,20] It is known that major repetitive elements in the entire genome are SINE/CORE, DNA/TcMar-Tc1, and Low complexity regions, in descending order.[21,22,23,24] In our preliminary study, repetitive elements are found rarely in exons. However, DNA/TcMar-Tc1 and Low complexity regions abundantly overlap with cis-regulatory regions, and we observed a peak of DNA/TcMar-Tc1 around a region which is a few kb away from enhancers.

When a gene contains several types of enhancers, repetitive sequences are frequently found in their boundaries. In higher eukaryotes, insulators are known to delimit the range of enhancer actions.[25,26,27] Our result may indicate that repetitive elements demarcate the boundary of enhancers in Ciona.

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D4-08

Spatiotemporal dynamics of zygotic genome activation in basal chordate revealed by interspecific hybrids

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Zygotic genome activation (ZGA) is a universal process for early embryogenesis, in which the embryo handover the maternal control to zygotic machineries. Accumulated knowledge has been acquired from different model organisms in last semi-century. However, a settled explanation of mechanism underlies this critical process, especially for the housekeeping gene reactivation, is still missing. Here, using hybrids derived from two distinct ascidian species (*Ciona robusta* and *C. savignyi*), which is divergent more than 120 Mya with significant divergence between most orthologous genes, we symmetrically documented unique dynamics of ZGA in urochordate, and found two coordinated waves of ZGA, representing early developmental and housekeeping gene reactivation during 8-cell to 110-cell stages. Comparative analysis revealed the regulatory connection between maternal genes and zygotic genes, which also presented allelic specific expression in a species- rather than parental effect, attributing to the divergence of cis-regulatory elements. The single-cell RNA sequencing revealed that spatial differential-reactivation of paternal housekeeping genes significantly correlated with the mechanical property of each cell type. These findings suggest that a potentially novel strategy account for the ZGA from basal chordate evolutionary perspective.

D4-09

The gene regulatory system in 32-cell embryos / the developmental program for muscle fate specification / new genome assemblies for *Ciona*

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In the 32-cell ascidian embryos, genes encoding transcription factors and signaling molecules are expressed in specific patterns. Recently, we succeeded in determining mathematical functions that represent the regulatory logics of all regulatory genes that begin to be expressed at the 32-cell stage (1). We showed that the expression of the regulatory genes became reproducible by calculation and controllable by experimental manipulations. The only exception was *Nodal*, whose expression was controllable at the 32-cell stage but not at the 16-cell stage. This observation indicated that a regulatory gene that began to be expressed at the 16-cell stage was involved in *Nodal* regulation. We found that *Friend of Gata* (*Fog* or *Zfpm*) was indeed involved in activation of *Nodal*. With the updated mathematical function for *Nodal* as well as the other 12 mathematical functions, we can now fully calculate and control the expression of all of the regulatory genes that begin to be expressed at the 32-cell stage.

Our group has also been working on deciphering the gene regulatory mechanism in the primary lineage muscle cells. On the basis of many studies, we have revealed a genetic pathway that begins with maternal factors Macho-1 and β-catenin, is mediated by the *Zic-r.b*/*Tbx6-r.b*/*Mrf* loop, and ends with expression of muscle structural genes (2). Recently, we also showed that the role of *Tbx6-r.b* is taken over by *Tbx15/18/20* (3). Although a majority of genes specifically expressed in muscle cells are controlled by this pathway, a minor fraction of muscle-specific genes are not. Here we will report our recent progress on the genetic pathway that regulates these genes.

I will also introduce new *Ciona* genome assemblies and a new gene model set. These assemblies are much more continuous and contains fewer gaps than the KH assembly (4-6). These assemblies were made in collaboration with Nori Satoh (OIST, Japan), Atsuko Sato (Ochanomizu Univ., Japan), Hitoyoshi Yasuo (Sorbonne Universite, France), and their colleagues.

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D4-10

Primed for fate determination – the transcriptional regulatory mechanism underlying the cardio-pharyngeal multipotent progenitor maturation

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Cardiogenesis initiates from mesodermal cardio-pharyngeal multipotent progenitors. The mechanism underlying how those progenitors acquired the multipotency and prepared themselves for fate determination remains unclear. We have observed that in tunicate *Ciona,* – a marine invertebrate model animal, the cardio-pharyngeal multipotent progenitor-truck ventral cells (TVCs) will enter a mature cell state that is primed with the multilineage transcripts before committing the cardiac or pharyngeal fate. To achieve a system-level understanding of the regulatory mechanisms that direct cardio-pharyngeal multipotent progenitor to maturation, we developed a single-cell multiplexing strategy, which enables us to capture the single-cell transcriptional dynamics with minimal batch effects and high temporal resolution. We obtained the trajectory of TVC maturation and identified the stepwise cell states from the birth of founder cells to the initiation of cardio-pharyngeal fate determination. We also established the correlation between the gene expression patterns and cell state transitions. This study has provided a global view of the transcriptional regulation driving the TVC towards maturation, thus identifying the key players in the TVC maturation event.

D4-11

Coupling cell cycle progression, multipotent progenitor maturation and fate choices in the cardiopharyngeal lineage of a simple chordate

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As development proceeds, successive fate decisions must follow cell divisions in an orderly manner for the single celled zygote to generate the observed diversity of cell identities. In vertebrate embryos, relatively loose coupling between cell divisions and fate choices allow pluri- and multipotent progenitor populations to expand without differentiating, presumably fostering plasticity and by extension evolvability. By contrast, in early embryos of the tunicate model Ciona, as well as in other ascidian species, cell divisions are tightly coupled with fate decisions, in a highly canalized ontogenetic sequence that is remarkably conserved among distantly related species. Here, I will present our work on cardiopharyngeal development, which lead us to focus on specific cell fate decisions, namely heart vs. pharyngeal muscle lineages, and their dependence upon mitosis; I will describe the dynamic "maturation" of the multipotent progenitor transcriptomes through one interphase and during collective migration; and I will present evidence that said maturation also follows cell cycle progression, and leads to the acquisition of competence, in part by conferring progenitors the ability to orient their divisions with respect to a cardiopharyngeal niche that conditions heart vs. pharyngeal muscle fate choices. In summary, I will discuss the potential of research using tunicates to uncover molecular mechanisms governing the coupling between cell cycle progression, multipotency and asymmetric fate decisions in chordates, and their potential relevance for the evolution of cardiovascular systems.

D4-12

Supra extracellular bridge in Ciona notochord elongation: structure and function

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Extracellular matrix (ECM) is a kind of macromolecules secreted by cells assembling and functioning extracellular, which is necessary for cell shape and behavior regulating such as polarization, migration and shape change. ECM can assemble into an organized network to provide anchoring sites and inducing signaling for cells in both physical and chemical pathway. Collagen, as a widely distributed family of extracellular fibrous proteins, is the main component of ECM to provide elasticity in tissue morphogenesis. Collagens together with other ECM molecules can form complicated higher structure, but how these structures interact with cell membrane and coordinate with the around cells to construct tissue structure is still unclear. Here, we observed a supra extracellular ECM bridge structure that linked the notochord sheath to epidermis at Ciona larval tail bud stage. We then described the dynamic process for its formation, and proved the specific structure formation based on the collagens flow along the tissue gap and the stable cell junction sealing the muscle cells' intercellular space. Furthermore, we found this structure contributed to cell elasticity to transmit mechanical force from F-actin contractile ring to entire notochord tissue to drive notochord elongation, whose disruption lead to the shape change of notochord cells' basal domain and the elongation failure of entire tail bud. Besides, this ECM bridge linked the notochord with epidermis, serving to coordinate multi-tissues elongation together with long distance. This work provides a reference of how cells build ECM environment to indirectly regulate it shape and movement.

D4-13

Rho GTPase signaling-depended cortex tension regulates notochord lumen growth in *Ciona* embryogenesis

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Tubular organs make a tubular network, performing transport and exchange functions within an organism. The cellular processes of tubulogenesis is distinct and complicated, including cell shape change, cell proliferation, mesenchyme-to-epithelium transition, cell polarization, cell migration and differentiation[1]. Formation of *Ciona* notochord tube is demonstrated to be a novel type of tubulogenesis, different from the existed ones in other creatures. It begins with *de novo* formation of the centrally extracellular lumen pockets between each two adjacent cylindrically-like notochord cells. Then, it does not undergo lumen connection involving either membrane fission or fusion, but experiences cell migration and an overall cell rearrangement process, accompanying with the growth of lumen[2]. Based on our previous work that demonstrates that *Ciona* lumenogenesis is an actomyosin-regulated process, we further found that *Ciona* notochord cells formed three contractile rings regulated by two independent pathway and authenticated the upstream Rho GTPase. We also showed that the stability of tight junctions was required for lumen opening and expansion. we described the function of cortex tension in lumen formation and built up an *in vivo* lumen expansion model in *Ciona* notochord tubulogenesis. The quantitate statistics of cortex tension showed a high correlation with the lumen geometry. Numerical simulation indicates a high correlation between lumen opening velocity and net actomyosin-mediated stress applied on lumen. Moreover, combining computational and experimental methods, our model connects the lumen geometry with vesicle trafficking velocity and apical surface tension. Overall, our results demonstrate that the balance between cortex thickness distribution, vesicle trafficking, ion transport and tight junction stability is essential to establish a stabilized lumen dynamic.

D4-14

Ano10 mediates convergent extension and tubulogenesis during notochord formation

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During embryonic development cells are organized into complex tissues and organs. A widespread component of organs across metazoans are epithelial tubes. However, it is largely unknown what molecular mechanisms underly the diversity of cell behaviors exhibited during tube morphogenesis. Here we show that *Ciona intestinalis* TMEM16K/Ano10 is required for convergent extension and lumen formation during notochord morphogenesis. Loss of Ano10 hampered the ability of notochord cells to regulate their intracellular Ca^{2+} dynamics. Finally, we use heterologous expression to demonstrate that Ano10 acts as an ion channel capable of Ca^{2+} signaling.

D4-15

Computational Inference of Gene Regulatory Network in the ascidian brain by single cell RNA-seq data

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The ascidians have been excellent model systems in neuroscience due to their simple nervous system. Although single cell expression profiles of neuronal cells that span the entire development of the *Ciona intestinalis* Type A larva have been determined[1], the underlying regulatory mechanism among cell types remains unclear. To reconstruct the gene regulatory network in the brain of the *Ciona* larva, we first identify co-expression modules between 200 transcription factors and their potential target genes by using single-cell RNA-seq data[2]. The co-expression modules are then pruned by checking whether the promoter region of genes contain the binding motifs of their upstream regulators. The remaining co-expression modules are called regulons. Finally, the enrichment of regulons in each cell are estimated. As a result, we identify 16 regulons with 1298 genes for neuronal cells in larva stage. Diverse activity of these 16 regulons in cell types indicate that their different intrinsic regulatory networks. Interestingly, the homeobox factor Lhx3 and its target genes are highly enriched in Rx+ anterior sensory vesicle (aSV), which might be the potential regulator of Rx[3]. Together, our analysis provides new insights into regulatory mechanism in the central nervous system of *Ciona*.

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D4-16

Ascidian invariant cleavage

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Following fertilization embryos often follow a predictable pattern of early cleavage divisions that are conserved within certain classes of animals (Wilson, 1900). An unresolved question in the field is what rules govern the pattern of cleavage divisions up to the blastula stage? The mechanistic processes that generate cleavage patterns operate at several scales: at the micro level of proteins and protein complexes at the cell cortex or intracellular organelles (e.g. centrosome and mitotic spindle) and at the cellular level via adhesion and cortical tension determining cell shape.

One of the most remarkable features of ascidian embryos is that their compact embryos (no prominent blastocoel) display an invariant cleavage pattern that is also perfectly conserved between distantly-related ascidian species allowing Conklin's nomenclature (1905) to be used for all studied ascidian species. Invariant cell positioning is accompanied by invariant cell shape which is important for cell-cell signaling starting at the 32-cell stage when 4 animal blastomeres are induced by vegetal blastomere derived FGF to become neural precursors. In order to uncover the cell biological and biomechanical mechanisms and features that underlie this invariant cleavage pattern we have so far found a number of mechanisms that work together to create the invariant cleavage pattern: cell cycle asynchrony, unequal cell division, long apical length dependent spindle positioning and mitotic apical relaxation. I will present our published and unpublished data on these cell biological mechanisms and biomechanical features that together contribute to the invariant cleavage pattern up to the blastula stage.

D4-17

Multi-modal single-cell biology of the embryogenesis of the ascidian, *Phallusia mammillata*

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Why do some taxa undergo rapid morphological changes while others, such as coelacanths or horseshoe crabs, keep very similar morphologies over hundreds of millions of years? Is this correlated with the rates of divergence of their genomes, or are there specific mechanisms that buffer or enhance the impact of genetic divergence on morphology? Ascidians, a group of marine tunicates, have kept a quasi-identical embryonic development over nearly 400 million years of evolution. Yet, they are fast evolvers at the molecular level.

To shed light on this paradox, it is necessary to understand how genomic changes are propagated and interpreted at the transcriptional, cell mechanical and anatomical levels. Our recent work[1] on the computational reconstruction of embryogenesis with single cell resolution revealed a relationship between the range of cell communication and the scale at which development is reproducible, a study which has been summarised in a small animation movie[2]. In my talk, I will present an update on our efforts to automatically reconstruct and quantify cell lineage trees and the dynamics of single-cell shapes during ascidian embryogenesis, and how such reconstruction can inform cell communication and the mechanical control of embryogenesis.

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