



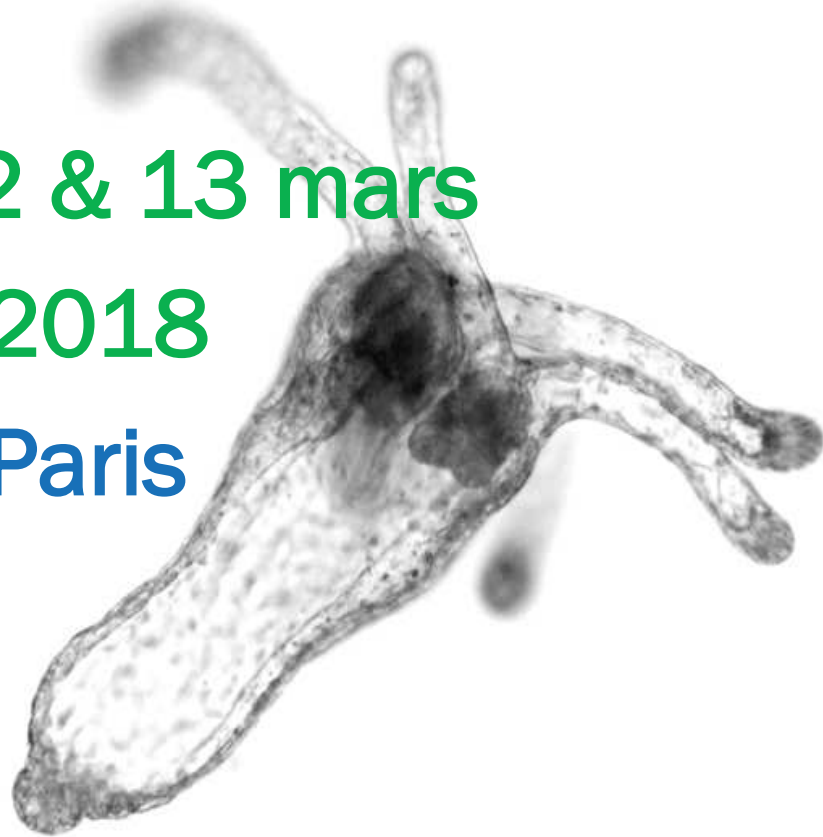
**Journées du réseau**

**André Picard**

**12 & 13 mars**

**2018**

**Paris**



*« Keynote talks:  
Grigory Genikhovich  
Evelyn Houliston »*

<http://www.reseau-andre-picard.org/>

**Sorbonne Université, Campus  
Jussieu, Amphi Charpak (Tour 22,  
niveau bas)**

**12 mars : 9h-18h30**

**13 mars : 9h-16h30**

**Le Réseau André Picard** a été créé en 2010 à l'initiative de Patrick Cormier afin de structurer la discipline « Biologie du Développement » de l'UPMC. Les « Journées André Picard-JAP », organisées alternativement sur le site de Paris et dans l'une des stations marines UPMC, ont permis dès la création de favoriser les échanges des chercheur.e.s. autour de ce thème. Les 9<sup>ème</sup> JAP s'ouvrent sur le regroupement de l'UPMC et Paris Sorbonne en un unique établissement, Sorbonne Université, dont le MNHN reste un partenaire associé.

Historiquement composé de laboratoires de l'UPMC localisés sur 4 sites géographiques éloignés (Campus de Jussieu à Paris, et les stations marines de l'université à Roscoff, Banyuls-sur-Mer et Villefranche-sur-Mer), le réseau André Picard a intégré des laboratoires du MNHN et Paris Sorbonne, afin de structurer la discipline « biologie du développement » au sein de la ComUE Sorbonne Universités.

Son action s'est progressivement élargie aux disciplines attenantes, Evolution-Développement (Evo-Devo), Ecologie-Développement (Eco-Devo) mais aussi aux mathématiques/physique et philosophie. Les recherches développées par ces laboratoires sont liées par leur thématique et sont complémentaires par leurs approches multi échelle et transdisciplinaire. De plus, la diversité des modèles biologiques utilisés par les membres du réseau, favorise les échanges interactifs et comparatifs et constitue un atout majeur et unique pour la communauté.

A l'heure actuelle, le Réseau André Picard compte 45 Groupes de Recherche de 11 UMRs différentes

**André Picard's network** was created in 2010 on the initiative of P. Cormier to structure the Developmental Biology Field within P. and M. Curie University (UPMC). The « Journées André Picard or JAP », which are alternately organised in Paris and within one of the UPMC marine station, allowed researchers to exchange around this field from their creation. The 9th JAP open at the time of the merge between UPMC and Sorbonne Université, to form one institution, that includes the MNHN as an associate member.

Historically composed of UPMC units from 4 distant geographical sites (Jussieu Campus in Paris, Marine stations from Roscoff, Banyuls-sur-Mer and Villefranche sur Mer), the André Picard network has integrated some research units from the MNHN and the ComuE Sorbonne Universités. Its interests have widened progressively to closely related fields, Evolution and Development (Evo-Devo), Ecology and Development (Eco-Devo) but also to mathematics-physics and philosophy. Projects developed by these teams are linked by their topics and are complementary thanks to their multi-scale approaches and trans-disciplinarity. Furthermore, the diversity of model organisms studied by the network favours interactive and comparative exchanges which constitute a major and unique asset for the scientific community. At present, André Picard's network consists of 45 research groups from 11 UMRs.

Organisateur/trices des JAP 2018 : Christine Vesque, Jean Michel Gibert, Anne Cardoso et Isabelle Mouas.



## 9 ÈME JOURNÉES ANDRÉ PICARD 2018

**Monday 12<sup>th</sup> March**  
(Amphi Charpak)



09:00-09:30

**Bienvenue/Wellcome**

### **Axis formation**

9:30-10:30

**Grigory GENIKHOVICH** University of Vienna, Austria

**Keynote lecture:** Evolution of animal bilaterality: once or more than once?

10:30-11:00

**Yann LE PETILLON** UMR 7232-CNRS-Banyuls

Anterior neural ectoderm formation in the hemichordate *Ptychodera flava*

**11:00-11h30**

**Pause-café/Coffee break**

### **Regeneration and asexual reproduction**

11:30-12:00

**Anabelle PLANQUES** UMR 7592-CNRS-Paris

Posterior regeneration in the annelid *Platynereis*

12:00-12:30

**Marta SCELZO** UMR 7009-Sorbonne Université-Villefranche

Novel form of asexual development in Styelid ascidians: vasa budding in *Polyandrocarpa zorritensis*

**12:30-14:15**

**Lunch and poster session (Caves Esclangon)**

**Posters presented by Julien CALLOCH, Isabelle DOMART-COULON,  
Rémi DUMOLLARD, Jérôme LACOSTE**

14:15-14:50

**Laure BONNAUD-PONTICELLI** UMR 7208-MNHN-Paris

**and Bénédicte CHARRIER** UMR 8227-Sorbonne Université-Roscoff

Le réseau André Picard : bilan 2016-2017

14:50-15:50

**Evelyn HOULISTON** UMR 7009-Sorbonne Université-Villefranche

**Keynote lecture:** Light-mediated oocyte maturation and spawning in the jellyfish *Clytia*

### **Nervous system development**

15:50-16:10

**Dhikra SOUIDENNE** UMR 7208-MNHN-Paris

Dynamics of the development of the nervous central system in the cuttlefish *Sepia officinalis* Linnaeus, 1758

16:10-16:30 **Morgane BONADÈ** UMR 7208-MNHN-Paris  
Influence of light on the setting-up of the dopaminergic system in *Sepia officinalis*

**16:30-17:00** *Pause-café/Coffee break*

17:00-17:30 **Sylvie MAZAN** UMR 7232-Sorbonne Université-Banyuls sur Mer  
Neurogenetic asymmetries during habenular development in the catshark *S. canicula*: evolutionary implications

17:30-18:00 **Pierre-Luc BARDET** ICM Sorbonne-Université-Paris  
Reissner's fiber in the cerebrospinal fluid controls morphogenesis of the body axis

### ***Bio-mechanics***

18:00-18:30 **Vlad COSTACHE** UMR 7622-Sorbonne Université-Paris  
Formin dynamics and cortical actomyosin contractility in the early *C. elegans* embryo

## **Tuesday 13<sup>th</sup> March**

(Amphi Charpak)

### ***Post-transcriptional modifications: methylation***

09:00-09:20 **Margarita ANGELOVA** UMR 7622-Sorbonne Université-Paris  
Crosstalk between tRFs and sncRNA pathways in an RNA methylase mutant

09:20-09:40 **Dilyana DIMITROVA** UMR 7622-Sorbonne Université-Paris  
RNA methyltransferase mutant links tRFs to small non-coding RNAs

09:40-10:00 **Lorane LE FRANC** UMR 7208-UCN-Caen  
Epigenetic regulation of development: focus on RNA methylation in a distant model of economic and ecologic significance: the oyster, *Crassostrea gigas*

10:00-10:30 **Hélène THOMASSIN-BOURREL** UMR 7622-Sorbonne Université-Paris  
Mutation of a single lysine residue in Ribosomal Protein uL11 is sufficient to confer *Minute*-like phenotypes

### ***Genomics and transcriptomics***

10:30-11:00 **Hector ESCRIVA** UMR 7232-Sorbonne Université-Banyuls  
Amphioxus functional genomics reveals the evolution of vertebrate regulatory traits

**11:00-11:30** *Pause-café/Coffee break*

11:30-12:00

**Bénédicte CHARRIER** UMR 8227-Sorbonne Université-Roscoff  
Cell-specific transcriptomics along the filament of the brown alga *Ectocarpus*

**Cell cycle control**

12:00-12:30

**Michel GHO** UMR 7622-Sorbonne Université-Paris  
*Drosophila* sensory organs precursor cells are released from G2-arrest following a centrifugal wave

12:30-13:00

**Alex McDOUGALL** UMR 7009-Sorbonne Université-Villefranche  
Making an early ascidian embryo

13:00-13:20

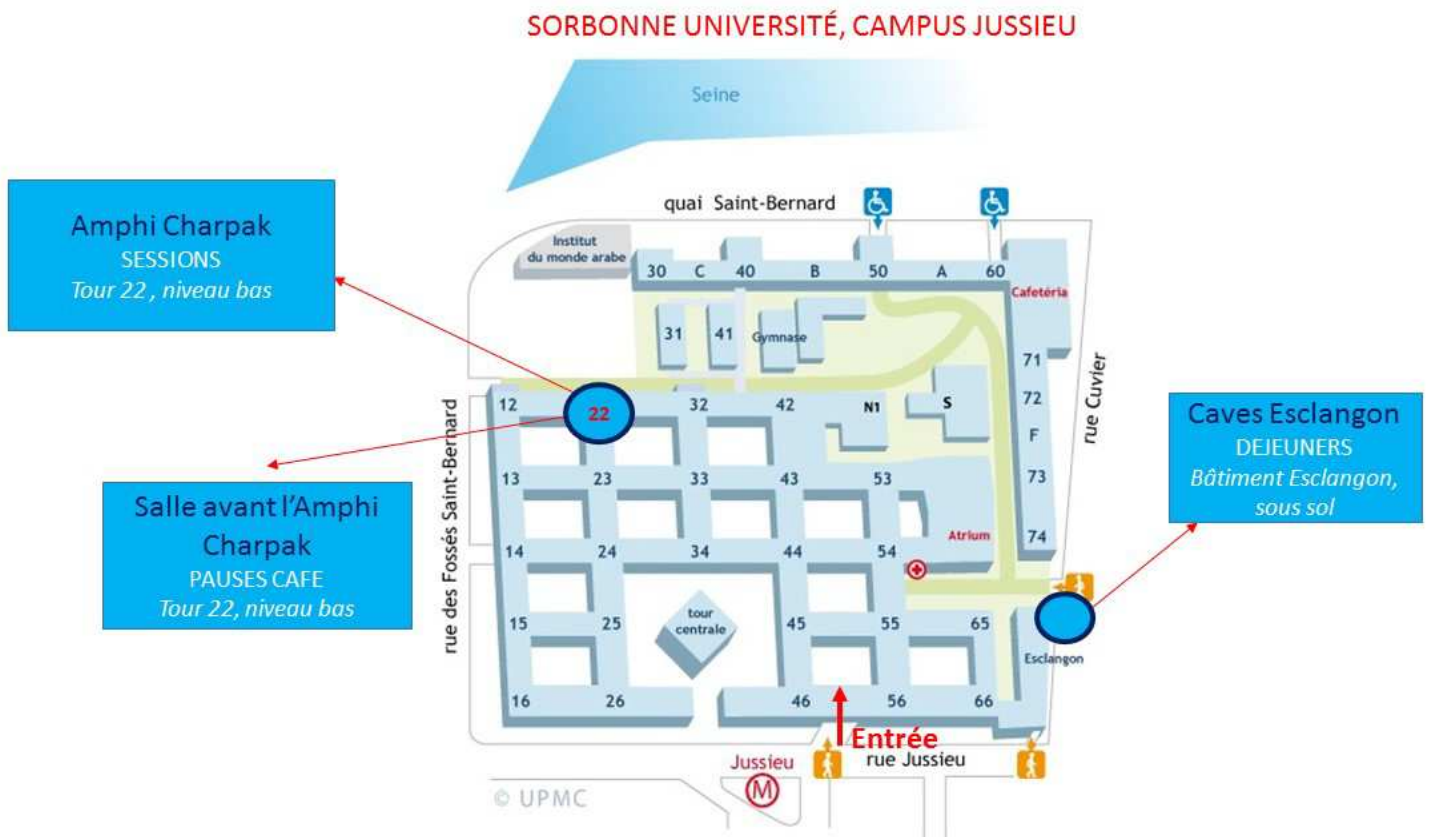
**Marianne ROCA** UMR 7009-Sorbonne Université-Villefranche  
Controls of mitosis in *Phallusia mammillata* embryos

**13:20-14:30**

**Déjeuner/Lunch (Caves Esclangon)**

14:30-16:30

**General assembly of the André Picard Network (Amphi Charpak)**



## **Evolution of animal bilaterality: once or more than once?**

**GENIKHOVICH Grigory**

Dept. for Molecular Evolution and Development, UZA1  
University of Vienna Althanstr. 14, A-1090 Vienna, Austria

Body axes are systems of molecular coordinates giving each point in the organism its specific molecular address. The evolution of the Bilaterality has drastically increased the amount of positional information stored in the system, allowing for body plans far more elaborate than the ones available for radially symmetric organisms. Among animals, bilaterality is a name-giving feature of Bilateria – the clade encompassing most of the animals, except for several early branching lineages such as sponges, ctenophores, placozoans and cnidarians. However, it is at these ancient groups of animals one needs to look in order to understand how bilaterality emerged. Cnidaria are particularly interesting since they are the evolutionary sister group of Bilateria, and they may be radially or bilaterally symmetric. Strikingly, just like in Bilateria, the two body axes in the bilaterally symmetric cnidarians are controlled by Wnt/ $\beta$ -catenin and by BMP signaling, however it is not clear, whether and how cnidarian body axes correspond to bilaterian ones. Currently, with new possibilities for gene function analysis, we are trying to compare the molecular mechanisms of axis formation and patterning between Cnidaria, Bilateria and the earlier branching sponges and ctenophores in order to find out how many times bilaterality evolved and which body axes of the non-Bilateria correspond to which in Bilateria.

### Anterior neural ectoderm formation in the hemichordate *Ptychodera flava*

LE PETILLON Yann

UMR 7232-CNRS-Banyuls sur Mer

How did the “head” form and how did mechanisms sustaining this process evolved in bilaterians are key questions in the EvoDevo field. In deuterostomes, a common view to explain the first step of head formation is the establishment of an ANE (Anterior Neural Ectoderm) located at the most anterior part of the embryo. The ANE is formed in two phases. First, anterior genes such as *Six3/6* and *FoxQ2* are expressed in the anterior ectoderm and their expression is secondarily restricted to the most anterior pole of the embryo. This restriction is mainly controlled by a posterior to anterior Wnt/ $\beta$ -catenin gradient which inhibits the expression of anterior ectodermal genes except in the ANE. Nevertheless, studies on ANE formation have been mainly focused on the Wnt/ $\beta$ -catenin pathway function and the roles of other signaling pathways involved ANE formation are mostly unknown.

To better understand the evolution of the ANE formation in deuterostomes, I studied the mechanisms controlling its formation in the hemichordate *Ptychodera flava*. First, I analyzed Wnt/ $\beta$ -catenin function during the restriction of genes expression in the anterior ectoderm and showed that this mechanism is conserved between hemichordate and other metazoans. Next, I analyzed the putative function of BMP signaling pathway. Interestingly, I observed that BMP is involved in ANE patterning anterior neural cells formation. Moreover, I observed that after excision of the anterior part of the embryo during gastrulation, BMP signaling is required for ANE formation *de novo*, confirming the link between BMP signaling and ANE formation and patterning.

This study concludes that BMP signaling plays an important role in patterning the ANE, after its formation and during its restriction to the most anterior part of the embryo by the Wnt/ $\beta$ -catenin signaling pathway. Moreover, preliminary observations show that BMP signaling is also involved in ANE patterning in sea urchin embryo suggesting an evolutionary conserved mechanism in ambulacraria.



## Posterior regeneration in the annelid *Platynereis*

**PLANQUES Annabelle**

UMR 7592-CNRS-Paris

*Authors: A. PLANQUES, J. MALEM, M. VERVOORT, E. GAZAVE*

*Institut Jacques Monod, Equipe Cellules souches, Développement et Evolution, CNRS UMR 7592, Paris, France*

Regeneration is a widespread phenomenon in animals with many animals able to regenerate, upon injury, complex body structures. Despite long-lasting interest for this process, we still lack a general view of the evolution of animal regeneration and we still do not know whether regeneration processes rely on conserved principles and genetic programs. We study regeneration of the annelid *Platynereis dumerilii*, as it constitutes, due to its phylogenetic position, its belonging to a slow-evolving lineage, and the available tools, an outstanding model to address fundamental questions about the evolution of animal regeneration. After amputation of the posterior part of their body, *Platynereis* worms regenerate both the posterior most part of the body and a stem cell-rich growth zone responsible for the addition of segments. We defined five stages of posterior regeneration which correspond to particular timepoints after amputation, and identified several parameters that affect the timing of the process. Further characterization of posterior regeneration using various labellings and *in situ* hybridizations for tissue patterning, cell cycle, and stem cell genes, indicate that regeneration is a rapid process. Using EdU incorporations, labellings for cell cycle markers, and inhibitors such as Hydroxyurea, we showed that cell proliferation is required for regeneration. We also investigated the origin of the cells of the regenerating structures and conducted RNA-seq experiments at different stages of the process. Our data therefore provide a thorough characterization of *Platynereis* posterior regeneration, and pave the way, through comparative analyses, for a better understanding of the evolution of regeneration in animals.



**Novel form of asexual development in Styelid ascidians: vasa budding in  
*Polyandrocarpa zorritensis***

**SCELZO Marta**

UMR 7009-Sorbonne Université-Villefranche sur Mer

Authors: Marta SCELZO<sup>1</sup>, Alexandre ALIÉ<sup>1</sup>, Francesco MASTROTOTARO<sup>2</sup> and Stefano TIOZZO<sup>1</sup>

<sup>1</sup> CNRS, Sorbonne Universités, UPMC Univ Paris 06, Laboratoire de Biologie du Développement de Villefranche-sur-mer, Observatoire Océanographique, 06230, Villefranche-sur-mer, France ; <sup>2</sup> Dipartimento di Biologia, Università degli studi di Bari "Aldo Moro", Italy

Colonial ascidians are the closest relatives of Vertebrates able to reproduce asexually by budding and to regenerate their entire body. These two processes vary among the different species and involve different epithelia and/or population of putative stem cells. In the Styelidae family we find species that cover heterogeneous budding ontologies. While in some species, such as in Botryllidae, the morphological events involved in the formation of the new individual are well described, in more phylogenetically distant species such as *Polyandrocarpa zorritensis*, the budding/regeneration are less or not described at all.

In order to study the budding in *Polyandrocarpa zorritensis*, we first established a laboratory culture obtaining colonies able to reproduce asexually under controlled conditions. Then, we described the life cycle of *P. zorritensis* and, by *in vivo*, histological and ultrastructural observations, we morphologically characterized and staged the onset and the bud and its early development. We identified a new kind of budding never described before in other colonial species, characterized by the participation of the vascular epithelium and circulating cells (haemocytes).

Many questions arise from these observations, as the role of cell proliferation and the haemocytes participation during the formation of the new adult. Further investigations will help to lay comparisons between related ascidian species in order to better understand the molecular mechanisms involved in budding and regeneration and to track their evolution among the group.

## Questioning Kidney regeneration in the adult: a key step to explore stem cell homeostasis

**CALLOCH Julien**

UMR 8227-Sorbonne Université-Roscoff

*Authors: Julien CALLOCH, David BUARD, Agnès BOUTET  
Traduction, Cycle Cellulaire et Développement (TCCD) – UMR 8227 - CNRS*

In adult mammals, kidney does not exhibit regenerative ability, involving that renal injuries leads to limited therapies. In case of kidney dysfunction, there are currently two available uncomfortable treatments: dialysis or organ transplant. Nephrons are the functional units of the kidney. Once there are lost in mammals, the remaining nephrons will suffer compensating hypertrophic response associated to an overload of their filtration work, resulting in kidney attrition and future additional diseases.

Some marine species (such as cartilaginous fishes) display kidney regeneration properties, an ability lost in mammalian species. In skate, it has been demonstrated that partial nephrectomy led to the growth of new nephrons at the adult stage. This capacity may be due to the persistence of self-renewing nephron progenitor cells within the adult kidney. Understanding this regenerative ability requires to apprehend the different steps of nephrons development in embryos from cartilaginous fishes.

Our goal is to define morphological and molecular steps leading to the formation of nephrons and to highlight and characterize kidney progenitor cells in cartilaginous fishes.

*Scyliorhinus canicula* is the most abundant oviparous elasmobranch belonging to chondrychtes (cartilaginous fishes) found along the coasts of northern Europe. A large quantity of eggs can then be obtained. This makes *S. canicula* a suitable species to investigate renal progenitor population using both morphological and molecular aspects at different times before hatching.

## Early skeletal colonization of the coral holobiont by the microboring Ulvophyceae *Ostreobium* sp.

**DOMART-COULON Isabelle**

UMR 7245-MNHN-Paris

Authors: A. MASSE<sup>1,2</sup>, I. DOMART-COULON<sup>1</sup>, S. GOLUBIC<sup>3</sup>, D. DUCHÉ<sup>4</sup>, A. TRIBOLLET<sup>2</sup>

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*Ostreobium* sp. (Bryopsidale, Ulvophyceae) is a major microboring alga involved in tropical reef dissolution, with a proposed symbiotic lifestyle in living corals. However, its diversity and colonization dynamics in host's early life stages remained unknown. Here, we mapped microborer distribution and abundance in skeletons of the branching coral *Pocillopora damicornis* from the onset of calcification in primary polyps (7 days) to budding juvenile colonies (1 and 3 months) that were growing on carbonate and non-carbonate substrates pre-colonized by natural biofilms, and compared them to adult colonies (in aquarium and reef settings). Surprisingly, primary polyps were already colonized by microboring filaments and their abundance depended on the nature of settlement substrate and its degree of pre-colonization by microborers. Growth of early coral recruits was unaffected even when microborers were in close vicinity to the polyp tissue. In addition to morphotype observations, chloroplast-encoded *rbcL* gene sequence analyses revealed nine new *Ostreobium* clades (OTU99%) in *Pocillopora* coral. Recruits and adults shared one dominant *rbcL* clade, undetected in larvae, but also present in aquarium seawater, carbonate and non-carbonate settlement substrates, and in corals from reef settings. Our results show a substratum-dependent colonization by *Ostreobium* clades, and indicate horizontal transmission of *Ostreobium*-coral associations.

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

DUMOLLARD Rémi

UMR 7009-Sorbonne Université-Villefranche sur Mer

Authors: Ievgeniia GAZO<sup>1,2</sup>, Isa D.L. GOMES<sup>1</sup>, Dalileh NABI<sup>1</sup>, Lydia BESNARDEAU<sup>1</sup>, Alex MCDOUGALL<sup>1</sup> and Rémi DUMOLLARD<sup>1</sup>

<sup>1</sup> Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV), UMR7009, Sorbonne Université, CNRS, Villefranche-sur-mer, France ; <sup>2</sup> University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátisí 728/II, 389 25, Vodňany, Czech Republic

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

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

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

## ***Drosophila* sensory organs precursor cells are released from G2-arrest following a centrifugal wave**

**LACOSTE Jérôme**

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*<sup>1</sup> Laboratoire de biologie du développement, UMR 7622, IBPS Sorbonne Université Paris, France ; <sup>2</sup> Centre de Recherche des Cordeliers, Sorbonne Université, 15 rue de l'Ecole de Médecine 75006 Paris, France*

G1-arrest is mainly observed in somatic quiescent cells while G2-arrest is in germ cells. Moreover, somatic G2-arrested cells are frequently associated with DNA damage or pathological situations. The sensory organ precursor cells (SOP cells) producing the mechanosensory organs in *Drosophila* are among the few cases of normal G2-arrested somatic cells. What is the biological significance of this arrest? How is G2-arrest released in these cells?

In the dorsal thorax, mechanosensory organs are located at regular intervals and aligned in well-defined rows parallel to the midline. All of them arise from the asymmetric cell division of SOP cells. SOP cells are selected from G2-arrested proneural cells and maintained in this arrest for at least 10 hours before re-enter the cell cycle. In order to study the exit from this G2-arrest, we have precisely analyzed the dynamic of SOP divisions in the notum by live imaging microscopy.

We show that the SOP cells do not re-enter into division simultaneously or randomly. Instead, they divide according to a temporal specific order. Particularly, they follow a wave in which SOP cells centrally-located divide earlier than those posteriorly- and anteriorly-located.

Our working hypothesis is that the progressive division of SOP cells along the A/P axis is due to signals propagating along each row that release SOP cells from their G2-arrest. We are currently investigating the molecular mechanism controlling the transition from G2 to M phase in this tissue. We are also modeling the dynamics of the resumption of the SOP in control and mutant genetic contexts. Our findings would shed light on basic developmental mechanisms that coordinate cell differentiation and division. This work aims to reveal the biological significance of a non-canonical cell cycle arrest for somatic cells.

## Light-mediated oocyte maturation and spawning in the jellyfish *Clytia*

**HOULISTON Evelyn**

UMR 7009-Sorbonne Université-Villefranche sur Mer

Authors: Gonzalo QUIROGA ARTIGAS, Pascal LAPEBIE, Lucas LECLERE, Carine BARREAU, Tsuyoshi MOMOSE and Evelyn HOULISTON  
CNRS, Sorbonne Université

In the hydrozoan jellyfish *Clytia hemisphaerica* oocytes grow, mature and are released every day in response to dark/light cues, even when the gonad is isolated from the medusa. Using a combination of bioinformatics and functional approaches, we have identified some of the key molecular and cellular regulators of oocyte maturation and spawning in this experimental model species. Specialised neurosecretory cells in the gonad ectoderm cells respond to a dark-light transition by secreting a small neuropeptidic Maturation Inducing Hormone (MIH; identified in collaboration with N. Takeda, Asamushi Research Center for Marine Biology, and R. Deguchi, Miyagi University of Education, Sendai). We found that one of 10 opsin photopigments identified from the *Clytia* genome, Opsin9, is expressed selectively in these cells and is essential for light-mediated MIH secretion and thus for oocyte maturation and spawning, as demonstrated by CRISPR/Cas9 knockout of the Opsin9 gene. We have also identified a putative GPCR receptor for the MIH activates a specific GPCR (collaboration with G. Jékely, MPI Tübingen) to initiate oocyte maturation, and are analysing its function.

### References

- Takeda, N., Kon, Y., Quiroga Artigas, G., Lapébie, P., Barreau, C., Koizumi, O, Kishimoto, T., Tachibana, K., Houliston, E.\* & Deguchi R.\* (2018). Identification of jellyfish neuropeptides that act directly as oocyte maturation-inducing hormones. *Development* 145(2). pii: dev156786. doi: 10.1242/dev.156786.
- Quiroga Artigas, G., Lapébie, P., Leclère, L., Takeda, N., Deguchi R., Jékely, G., Momose, T.\* & Houliston, E.\*(2018). A gonad-expressed opsin mediates light-induced spawning in the jellyfish *Clytia*. *Elife* 7 pii: e29555. doi: 10.7554/eLife.29555.

# Dynamics of the development of the nervous central system in the cuttlefish *Sepia officinalis* Linnaeus, 1758

**SOUIDENNE Dhikra**

UMR 7208-MNHN-Paris

*Authors: Dhikra SOUIDENNE, Boudjema IMARAZENE, Sandra E. DOS SANTOS, Laure BONNAUD-PONTICELLI  
UMR MNHN, CNRS 7208, Sorbonne Université, IRD 207, UCN, UA*

The common cuttlefish, like other cephalopods, has a continuous growth throughout adult life and performs some of the most complex behaviors and cognitive abilities among mollusks. Its central nervous system consists of a brain, two optic lobes and peripheral nervous system composed of a ganglionic network spread in the arms and a pair of specific stellate ganglia.

Hence, thanks to this highly centralized nervous system and its direct development process, *Sepia officinalis* present a relevant model to study the dynamic of neurogenesis during the development.

The aim of our study is to test the correlation between the dynamic of neurogenesis and the acquisition of cognitive abilities during embryonic and posthatching development of *Sepia officinalis*.

To this end, we focused on the dopaminergic system, which is most responsible of learning and memorizing processes among vertebrates, but remains to be explored in non-vertebrates animals.

Therefore, we evaluated the variation of cell number as structure mass increases during development using the “isotropic fractionator method”.

By using the immunostaining method, we evaluate between the development stage E24 to the adulthood:

- 1- the number of neuronal cells corresponding to the fraction of cells stained with a neuronal nuclear protein marker (NeuN) versus the total cell number.
- 2- the number of dopaminergic cells stained with a nuclear receptor expressed in dopaminergic neurons (Nurr).

First results show that cell proliferation inducing mass increase is continuous in nervous structures. The number of Nurr-positive cells in brain is enhanced with age. However, no such significant enhancement has been observed in the optic lobes and stellate ganglia suggesting a precocious set up of the sensory system control in cuttlefish. The brain seems thus to be strongly implicated in the development of the dopaminergic system.



**Influence of light on the setting-up of the dopaminergic system  
in *Sepia officinalis***

**BONADÈ Morgane**

UMR 7208-MNHN-Paris

*Authors: Morgane BONADÈ, Boudjema IMARAZENE, Laure BONNAUD-PONTICELLI  
UMR MNHN, CNRS 7208, Sorbonne Université, IRD 207, UCN, UA*

Cephalopods produce large amounts of neurotransmitters including dopamine in their highly centralized central nervous system, composed of a brain and two optic lobes. Dopamine is involved in visual learning and cognition in many Metazoans and might have the same role in Cephalopods. Nevertheless there are only few data available on the characterization and development of the dopaminergic system in relation to the acquisition of learning in Cephalopods.

It has been shown that light induces an increase of dopaminergic neurons in Vertebrates. Therefore we hypothesised, in *S. officinalis* embryo, a role of the light in brain maturation, before hatching through the egg capsule and after hatching. Indeed, visual learning has been reported in cuttlefish in late embryonic stages when light reaches the embryo.

In order to gain a better understanding of the setting-up of the dopaminergic system, we are characterizing the dopaminergic receptors and localizing them in different stages of *Sepia* embryos. We also designed a protocol where eggs are submitted to different photoperiods. Then the expression of dopamine receptors and other photosensitive molecules such as cryptochromes and rhodopsin will be quantified through qRT-PCR in central nervous system in the different conditions. This work will give us first informations about the role of light in the setting-up of the dopaminergic system and the acquisition of visual learning.

**Neurogenetic asymmetries during habenular development in the catshark  
*S. canicula*: evolutionary implications**

**MAZAN Sylvie**

UMR 7232- Sorbonne Université-Banyuls

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Analysis of the establishment of epithalamic asymmetry in two non-conventional model organisms, a cartilaginous fish and a lamprey, has highlighted an essential role for Nodal signalling, likely to be ancestral in vertebrates but largely lost in zebrafish. In order to decipher the cellular mechanisms underlying this mechanistic divergence, we have characterised neurogenetic asymmetries during habenular development in the catshark *Scyliorhinus canicula* and addressed the role of Nodal in this process. This analysis highlights Nodal dependent asymmetries in the timing of neuronal differentiation, proliferation rates of neural progenitors and in the maintenance of their pool between the left and right habenulae. It also suggests an asymmetric, temporal regulation of neuronal cell fate choices between the left and the right habenulae. Some of these asymmetries are reminiscent of those reported in the zebrafish while others are reported for the first time in the developing habenulae. Taken together, these data suggest that the cellular mechanisms controlling habenular asymmetry formation substantially differ between the catshark and the zebrafish, possibly as a result of constraints related to slow development and large organ size in the former. Such differences between species may account for the variability of epithalamic asymmetries observed across vertebrates.

## **Reissner's fiber in the cerebrospinal fluid controls morphogenesis of the body axis**

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The cerebrospinal fluid (CSF) is a complex fluid that circulates in the CNS ventricular system. There is a recent regain of attention in neuroscience about the role of the CSF circulation and composition, especially during development. Here, we unravel the role of a long-known and rather mysterious structure present in the CSF of most vertebrates, referred to as Reissner's fiber (Reissner, 1860). The Reissner's fiber is an extracellular fibrillary structure forming in the CSF via the aggregation of the SCO-spondin glycoprotein, secreted by cells of the third ventricle wall and spinal floor plate. Although multiple roles have been suggested for this structure, the lack of a genetic model prevented to ascertain any hypothesis.

Here we have generated zebrafish *scospondin* mutants, leading to the absence of Reissner's fiber from early stages of development. This mutation was associated with an abnormal curvature of the embryonic posterior axis, reminiscent the "curled-down" phenotype observed in zebrafish mutants of ciliary genes. So far, no model explained how the regulation of CSF physical and chemical properties by ciliary function impacts the development of the posterior axis. Our *scospondin* mutants allowed us to reexamine this problem.

We could show that the *scospondin* mutation did not affect ciliogenesis, nor cilia motility and cilia-generated CSF flow. Reciprocally, we analyzed whether zebrafish ciliary mutants exhibiting the curled-down phenotype properly formed a Reissner's fiber along the ventricular space during development. Our results show that four ciliary mutants develop abnormal Reissner's structures early in development. Altogether, our results indicate that motile cilia promote the formation of Reissner's fiber, which is critical for body axis morphogenesis. We postulate the existence of a relay signal requiring the Reissner's fiber, and we are currently looking for this signal.

# Formin dynamics and cortical actomyosin contractility in the early *C. elegans* embryo

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Proper development and morphogenesis relies on fine-tuned spatial and temporal deployment of forces inside cells. The actomyosin cytoskeleton is determinant for the deployment of these forces and so, for the mechanical properties of embryonic cells and tissues.

In *C. elegans* early embryos, the cell cortex includes at least two types of cytoskeletal structures that simultaneously coexist, competing for the actin monomer pool as well as for their various binding partners. Strikingly, these structures co-exist in space and time, competing for the actin monomer pool as well as for their various binding partners. Indeed, actin network homeostasis is not only a balance between cytoplasmic G- and cortical F-Actin, but also a tug-of-war between these different actin structures for the control of the actin monomer pool (Burke *et al.*, 2014). The distribution between these different populations is critically important for the mechanical properties of the cortex (Pujol *et al.*, 2012 ; Chaigne *et al.*, 2013).

Formins are responsible for the polymerization of new actin filaments. Using HILO imaging and the SmPreSS method (Robin *et al.*, 2014), we can track single molecules of actin or actin-binding partners (such as the processive formin CYK-1) in order to understand the biochemical interactions *in vivo* within cortical actomyosin and the organisation of the active meshwork during contractility pulses (orientation of actin filaments). I will also present results of the analysis of CYK-1 at the cortex, as proxy for measuring actin filament length *in vivo* and how it scales with cell size in the embryo.

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## Crosstalk between tRFs and sncRNA pathways in an RNA methylase mutant

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In a genomic screen aiming to unravel new factors involved in small RNA pathways in *Drosophila melanogaster* we identified a novel gene that encodes a protein orthologous to yeast and human tRNA-methyltransferases. Dysfunctions of this human ortholog cause Intellectual Disability (ID), a pathology that is genetically linked to cancer. We found that *Drosophila* mutants exhibit severe phenotypes, such as reduced lifespan, ovarian size reduction, and decreased viral resistance. Furthermore its mutation results in defects in the three small non-coding RNA pathways (mi-, si- and piRNA). Consistently, we detected increased transposon levels in *Drosophila* heads. Interestingly, we also observed an abnormal accumulation of tRNA fragments (tRFs) originating from substrates conserved for the human ortholog protein. Therefore our results support an unexpected link between RNA modifications, ncRNA pathways, tRFs and disease etiology, such as neurodegeneration and cancer. We are currently investigating whether this functional conservation is conserved in human, using patients cells carrying mutations in the corresponding tRNA methylase.

## RNA methyltransferase mutant links tRFs to small non-coding RNAs

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tRNA fragments (tRFs) are 18-26 nucleotide small RNAs derived from specifically cleaved mature tRNA transcripts. In *Drosophila melanogaster*, our team has discovered a putative conserved tRNA methyltransferase and linked it to accumulation of specific tRFs and dysregulation of the three small non-coding RNA pathways (sncRNA) *i.e.* si-, mi- and piRNA. Dysfunctions in the human ortholog cause a specific case of Intellectual Disability (ID). The mutant flies exhibit intriguing phenotypes, such as reduced lifespan, ovarian size reduction, decreased viral resistance, and most importantly, increased transposon levels in heads. Therefore, our results support an unexpected link between RNA modifications, sncRNA pathways, tRFs, and disease etiology, such as ID. We are currently investigating the functional and mechanistic conservation in ID patient cells carrying mutations in the corresponding tRNA methylase.

*Keywords:*

*RNA methylation, sncRNA pathways, Intellectual Disability, tRFs*

### **Epigenetic regulation of development: focus on RNA methylation in a distant model of economic and ecologic significance: the oyster, *Crassostrea gigas***

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Parallel to epigenetic processes in the nucleosome, the methylation of RNA has recently emerged as a crucial regulator of metazoan development. Indeed, N6-methyladenosine (N6-mA or m6A) governs maternal to zygote transition, stabilizes X chromosome silencing and controls splicing in vertebrates, and also sex determination in insects. N6-mA is the most abundant modification in eukaryotic RNAs and depends on a machinery comprising a methyltransferase complex ('writers'), erasing enzymes ('erasers') and mediation proteins ('readers'). However, the evolutionary conservation of this critical regulator of early development and of its molecular machinery is unknown to date in lophotrochozoan animals such as the oyster *Crassostrea* (eg. *Magallana*) *gigas*. *C. gigas* is a bivalve mollusk whose indirect development is under the strong epigenetic influence of DNA methylation. Oyster maternal RNAs accumulate in the oocyte during the gametogenesis, where environmental conditions induce life traits that can be inheritable and impact the development of the offspring. Therefore, we hypothesized that N6A-RNA could constitute an epitranscriptomic regulator of oyster development.

To test this hypothesis, we investigated the presence and variations of m6A during oyster early life using a m6A antibody. In parallel, we searched and characterized *in silico* the putative conserved m6A regulators as well as their expression levels in oyster embryos. Our results indicate that m6A is present and exhibits a biphasic decay with high levels in oocytes and after gastrulation. In addition, oysters present a complete putatively conserved associated machinery which expression is regulated during the early life. This suggests that m6A may regulate oyster development and could be implicated in MZT and organogenesis. This work brings pioneer fundamental data in a distant model towards a better understanding of the evolution and regulation of epigenetic mechanisms of development. Future directions will aim at deciphering whether m6A constitutes a vector of 'epitranscriptomic' inheritance in the context of global environmental changes.



# Mutation of a single lysine residue in Ribosomal Protein uL11 is sufficient to confer *Minute*-like phenotypes

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Ribosome biogenesis is an essential yet highly energy-demanding process that requires the proper coordination of hundreds of factors. Mutations in genes encoding Ribosomal Proteins (RPs) have been shown to cause an array of cellular and developmental defects in a variety of organisms. In *Drosophila melanogaster*, disruption of RP genes lead to a recognizable common pattern of dominant, haploinsufficient phenotypes referred to as the “*Minute*” syndrome. *Minute* phenotypes are characterized by developmental delay, small body size, short and thin bristles, poor fertility and viability, and have been attributed to a reduced overall protein synthesis. However, a growing body of evidence shows that RPs also possess additional regulatory functions outside of the ribosome.

We discovered that the *Drosophila* Ribosomal Protein uL11 may possess such an extra-ribosomal function. When tri-methylated on lysine 3, uL11 (uL11K3me3) interacts with the chromodomain of Corto, a member of the Enhancer of Trithorax and Polycomb (ETP) family of epigenetic cofactors. uL11 and Corto bind chromatin at the same *loci* on polytene chromosomes and regulate a subset of genes implicated in ribosome biogenesis. Hence, in addition to its *bona fide* role in ribosome biogenesis and translation, uL11 also participates in transcriptional regulation of ribosomal protein genes and could be involved in the dynamic coordination of ribosome biogenesis.

To specifically address the role of the tri-methylation of uL11 lysine 3 *in vivo*, we used the CRISPR/Cas9 technology to generate uL11 variant proteins whose lysine 3 has been deleted or replaced by an alanine. Whilst the  $\Delta$ K3 mutants exhibit less severe phenotypes than the K3A mutants, both alleles display the characteristics of *Minute* mutants. Whether these uL11 mutations affect the transcriptional regulation of ribosomal protein genes or the translational functions of uL11 is under investigation.

## **Amphioxus functional genomics reveals the evolution of vertebrate regulatory traits**

**ESCRIVA Hector**

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All chordates share a fundamental bodyplan that was greatly elaborated in vertebrates. Vertebrates also evolved highly distinctive genomes, sculpted by two whole genome duplications (WGD). To investigate the evolution of genome regulation in chordates, we characterized promoters, methylation, chromatin accessibility, histone modifications and transcriptomes in multiple tissues and throughout development of the cephalochordate amphioxus. These data revealed an intermediate stage in the evolution of differentially methylated enhancers, and high conservation of gene expression and its underlying *cis* regulatory logic between amphioxus and vertebrates, maximally at a developmental phylotypic period. We also unraveled the principal route of regulatory evolution following WGD: over 80% of gene families with multiple paralogs in vertebrates have members that restricted their ancestral expression, undergoing specialization rather than subfunctionalization. Counter-intuitively, vertebrate genes that underwent expression restriction increased the complexity of their regulatory landscapes. Altogether, these data pave the way for a better understanding of the regulatory principles underlying key vertebrate innovations.

### Cell-specific transcriptomics along the filament of the brown alga *Ectocarpus*

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*Ectocarpus* is a tiny brown alga made of uniseriate filaments. In the early stages, at least 5 cell types, which are defined by both their position along the filament and their age, compose this string of cells (Le Bail *et al.*, 2008). This ranges from the elongated apical cells, ensuring growth by elongation and cell division, to the spherical cells at the centre of the filament, from which branches emerge.

The aim of the study was to assess to which extent cells experience differentiation along this filament, in response either to a genetically coded programme of cell fate, or simply to ageing. The approach was to use their genome-wide transcriptomic profile as a proxy. We performed laser-capture microdissection on fixed WT *Ectocarpus* filaments and isolated individual or groups of cells, from which RNAs were extracted, amplified and further sequenced as described in Saint-Marcoux *et al.* (2015).

Similar experiments were performed on 2 cell differentiation mutants, *etoile* (Le Bail *et al.*, 2011), altered in apical growth, and *knacki*, impaired in cell rounding.

The talk will present and discuss comparisons of the absolute and relative expression profiles both between the different cells making the WT filament, i.e. over a ~ 100 µm in length, and between the WT and the 2 mutants for a single cell type.

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***Drosophila* sensory organs precursor cells are released from G2-arrest following a centrifugal wave**

**GHO Michel**

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G1-arrest is mainly observed in somatic quiescent cells while G2-arrest is in germ cells. Moreover, somatic G2-arrested cells are frequently associated with DNA damage or pathological situations. The sensory organ precursor cells (SOP cells) producing the mechanosensory organs in *Drosophila* are among the few cases of normal G2-arrested somatic cells. What is the biological significance of this arrest? How is G2-arrest released in these cells?

In the dorsal thorax, mechanosensory organs are located at regular intervals and aligned in well-defined rows parallel to the midline. All of them arise from the asymmetric cell division of SOP cells. SOP cells are selected from G2-arrested proneural cells and maintained in this arrest for at least 10 hours before re-enter the cell cycle. In order to study the exit from this G2-arrest, we have precisely analyzed the dynamic of SOP divisions in the notum by live imaging microscopy.

We show that the SOP cells do not re-enter into division simultaneously or randomly. Instead, they divide according to a temporal specific order. Particularly, they follow a wave in which SOP cells centrally-located divide earlier than those posteriorly- and anteriorly-located.

Our working hypothesis is that the progressive division of SOP cells along the A/P axis is due to signals propagating along each row that release SOP cells from their G2-arrest. We are currently investigating the molecular mechanism controlling the transition from G2 to M phase in this tissue. We are also modeling the dynamics of the resumption of the SOP in control and mutant genetic contexts. Our findings would shed light on basic developmental mechanisms that coordinate cell differentiation and division. This work aims to reveal the biological significance of a non-canonical cell cycle arrest for somatic cells.

## **Making an early ascidian embryo**

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Even though we can easily recognize early embryos from each other (e.g. a sea urchin versus a spiralian versus an ascidian, etc.) it is not entirely clear what cell biological mechanisms lead to these emergent shapes of each embryo. To determine how the pattern of cell division is controlled up to the blastula stage has been difficult to assess because it often relies on biophysical constraints coupled with maternal mechanisms, and thus cannot be addressed by traditional in situ or transcriptomic based approaches. For many organisms the early stages of cleavage are important for the setting-up of cell-cell contacts or the segregation of maternal determinants to specific blastomeres, both of which can influence cell fate choices. To address this problem we have been using the ascidian embryo because ascidians display a highly conserved invariant cleavage pattern up to the blastula stage.

We have uncovered three cell biological mechanisms, one that control cell cycle duration and a second that controls cell size, which together with a third apical cell shape sensing mechanism control the position of all cells up to the blastula stage. Cell cycle duration is controlled by a  $\beta$ -catenin-dependent mechanism, while cell size during unequal cell division of two posterior vegetal blastomeres is controlled by microtubule dynamics. The third mechanism is a default one that integrates mitotic spindle positioning in the apical plane across the whole embryo. Perturbing either unequal cell division or cell cycle duration perturbs the invariant cleavage pattern. Finally, we have tested and verified a computational model that predicts mitotic spindle positioning in the apical cellular plane in every cell up to the blastula stage.

### Controls of mitosis in *Phallusia mammillata* embryos

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The Spindle Assembly Checkpoint (SAC) controls mitosis by delaying the metaphase-anaphase transition until all chromosomes are properly attached to microtubules to avoid erroneous chromosome segregation<sup>1</sup>. Chromosome segregation defects can lead to aneuploidy, a condition deleterious for development. However, by comparing SAC response during cleavage in several metazoan species, we observed that while some embryos, like those of sea urchin, have an active SAC control, other embryos, like those of ascidian, do not. We aim at understanding the regulation of SAC activity in embryos of the ascidian *P. mammillata*.

To determine when a SAC-dependent anaphase delay appears during development in *P. mammillata*, we compared the effect of microtubule depolymerizing drugs (eg. nocodazole) on mitotic progression at different developmental stages. We could show that prior to gastrulation mitotic timing is unaffected by lack of spindle microtubules. A delay in anaphase is observed only from a stage between gastrula and neurula. The dependence of this delay on SAC activation is being tested using SAC inhibitors.

To understand why the SAC is inefficient in cleaving *P. mammillata* embryos, we are now analysing the spindle assembly checkpoint response at the molecular level. Transcripts encoding all main SAC components are present throughout embryogenesis even when the SAC is not efficient (Aniseed2 and Octopus database). At the protein level, we focused on Mad2, the central regulator of SAC signalling. We find that Mad2 protein is present in embryos already from the egg. In addition, exogenous Mad2 protein localizes to unattached kinetochores, but its overexpression does not induce a mitotic block. We are now testing the localization of other SAC components as well as the protein complexes associated with those factors. Using mass-spectrometry we have already analysed Mad2 associated proteins in eggs and have identified 5 new potential regulators of SAC signalling in *P. mammillata* embryo.

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*Photo de couverture* : Potograph shows a control polyp of the sea anemone *Nematostella vectensis* in the upper left and three polyps with the primary, oral-aboral body axes forming as a result of the experimental upregulation of Wnt/beta-catenin signaling. Upper right polyp has two oral ends, because it developed from an embryo placed into a GSK3-beta inhibitor at late gastrula stage. Lower left polyp develops extra heads and tentacles because it is a mosaic APC mutant. Lower right is a double-headed polyp which developed from dissociated and reaggregated aboral hemispheres of embryos. Normally, such aggregates form ciliated balls without any axes, however, this aggregate was rescued and formed a polyp because some of the aboral hemispheres used for the aggregate formation were expressing Wnt1 and Wnt3 from plasmids injected into single blastomeres at the 8-cell stage. **Grigory Genikhovitch**