Remontée des sujets CSC 2018-2019

Ecole Doctorale / Doctoral School	BIO SPC, DGNRV department
Titre du sujet / Subject title	Limb regeneration in animals: insights from an emerging model organism
Encadrant(s) / advisor(s)	Pr. Michel Vervoort
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Etablissement /Institute	P7
Pôle / Research axis	SVS : sciences de la vie et de la santé

Description of the subject

Regeneration, the ability to restore body parts after an injury or an amputation, is a widespread and complex phenomenon in animals [1]. While having fascinated scientists for centuries, fundamental questions about the cellular and molecular basis of animal regeneration remain largely unanswered. Solving these questions requires to study new regeneration model organisms amenable to molecular, cellular and functional analyses. We study this process in the annelid worm *Platynereis dumerilii*, an outstanding model to address key questions about regeneration in animals, due to its important regeneration capabilities, its key phylogenetic position, and the availability of various advanced tools (transgenesis, functional analysis, live-imaging), as well as extensive genomic and transcriptomic resources [2]. After amputation of the posterior part of their body, *Platynereis* worms form a regeneration blastema (a structure made of undifferentiated proliferative cells) and regenerate both the pygidium (the posteriormost part of the body, which bears the anus) and a stem cell-rich subterminal growth zone that allows the addition of new segments [3]. We called this process posterior regeneration. Our preliminary data indicate that blastemal cells during posterior regeneration mainly derive from dedifferentiation of cells of the tissues abutting the amputation plane [4]. At one and two days post amputation, cells at and close to the amputation start to express various proliferation and pluripotency stem cell markers, indicating that amputation induces extensive cell reprogramming [4]. *Platynereis* is also able to regenerate various outgrowths of its body such as cirri (on both the head and the pygidium) and its swimming/crawling appendages (parapodia).

MicroRNAs (miRNAs) are small regulatory RNAs that bind to a number of target messenger RNA transcripts and block the expression of proteins [5]. Like transcription factors, they play a critical role in dynamic cell fate decisions since they simultaneously regulate a co-expressed panel of gene. miRNAs act through conserved mechanisms of action, but being tissue specific in their own expression, cell-fate switching is often driven by the expression of a few or even single miRNAs that then reset the gene expression profile of the cell. There are growing evidence for the role of miRNAs during cellular reprogramming, transdifferentiation and regeneration [6].

The main aims of this project are to characterize appendage regeneration in the annelid *Platynereis dumerilii* (Aim 1) and to study the potential involvement of miRNAs during

regeneration in this species (Aim 2). This project is part of a larger collaborative project between our team and that of Jerome Hui (Chinese University of Hong Kong), aiming at understanding the evolution of limb regeneration in animals and the involvement of miRNAs in regeneration.

AIM 1: Morphological, molecular and cellular characterization of Platynereis parapodial regeneration

The student will first characterize parapodia regeneration at the morphological level, aiming in particular to define stages in the process and to identify parameters, such as worms' size and position of the amputation site along the antero-posterior axis, which may affect the process. This descriptive work will allow to define conditions that allow efficient and reproducible regenerations, and which will be used for all subsequent studies. The student will study the mitotic behaviour of the cells during parapodial regeneration using EdU incorporations and labellings for cell cycle markers. Cell division inhibitors will be used to further assess the importance of cell proliferation during parapodial regeneration. The student will also perform various labellings and *in situ* hybridizations for markers of parapodia development and cell differentiation to establish the timing of reformation of parapodial tissues and structures during regeneration. Expression of stem cell genes will also be analyzed to define whether these genes are expressed during parapodial regeneration (as they are during posterior regeneration) or not. EdU pulse and chase and cell-lineage tracing experiments will be performed to study the cellular origin of the regenerated parapodia, in particular to assess the respective contributions of resident stem cells and dedifferentiation events to parapodia regeneration.

AIM 2: Involvement of miRNAs during Platynereis regeneration

Two approaches will be followed. In a first set of experiments, the student will study the expression, during posterior and parapodial regeneration, of a set of miRNAs that have been previously identified during embryonic and larval development [7]. To obtain an unbiased view of the miRNA repertoire involved in regeneration in *Platynereis* and identify putative regeneration-specific miRNAs, the student will perform deep sequencing of small RNAs at different time points of posterior and parapodial regeneration, which correspond to the main steps of these regeneration processes such as wound healing, progenitor proliferation and cell differentiation. Total RNA will be isolated from regenerating tissues and small RNA libraries will be constructed. Small RNA libraries will also be prepared from unamputated animals and immediately after amputation, to serve as a baseline control for miRNA expression levels. Illumina deep sequencing of the small RNA libraries will then be performed. Widely-used bioinformatic tools will be used to map reads to transcriptome assemblies and to the already established embryonic miRNome, as well as to identify putative novel miRNAs. Differential expression analysis will then be performed to identify miRNAs whose expression is different in regenerating tissues *versus* controls and/or whose expression is dynamic during the course of regeneration. Bioinformatic tools will be used to predict possible targets of these miRNAs and the expression profile of these targets will be evaluated using existing transcriptomic data. Expression of the most interesting miRNAs will be experimentally validated. Cross-species comparisons will be done, in particular using data about miRNAs during regeneration in arthropods, generated by the Hui's team.

To lead this ambitious project, we are expecting a highly motivated student with a good background in molecular biology and a strong interest for developmental and evolutionary biology. Working language in our team and at the Institut Jacques Monod (IJM) is English. There is no need to speak French. The IJM, located in the heart of Paris, is a leading French biological research institute, comprising about 30 interactive research groups and high-quality technological facilities. The project will be done in collaboration with the team of Jerome Hui (Chinese University of Hong Kong) and will involve research stays in the Hui's lab.

Bibliography

1. Tanaka EM, Reddien PW. Dev Cell. 2011;21:172–85.
2. Zantke J, Bannister S, Rajan VBV, Raible F, Tessmar-Raible K. Genetics. 2014;197:19–31.
3. Gazave E, Béhague J, Laplane L, Guillou A, Préau L, Demilly A, et al. Dev Biol. 2013;382:246–67.
4. Planques A, Malem J, Parapar J, Vervoort M, Gazave E. bioRxiv 352211 (submitted to Dev. Biol).
5. Hobert O. Science 2008;319: 1785–1786.
6. Li M, Izpisua Belmonte JC. 2015. Nat Struct Mol Biol 2015;22: 2–4.
7. Christodoulou F, Raible F, Tomer R, Simakov O, Trachana K, Klaus S, Snyman H, Hannon GJ, Bork P, Arendt D. Nature 2010;463: 1084–1088.

Five recent publications of the team :

-Özpolat B.D., Handberg-Thorsager M., Vervoort M., and Balavoine G. (2017). Cell lineage and cell cycling analyses of the 4d micromere using live imaging in the marine annelid *Platynereis dumerilii*. *Elife* **6**: e30463.

-Vervoort M, Meulemeester D, Béhague J, Kerner P : Evolution of *Prdm* Genes in Animals: Insights from Comparative Genomics. *Mol Biol Evol* 2016, **33**:679-96.

-Gazave E*, Behague J*, Laplane L, Guillou A, Preau L, Demilly A, Balavoine G, Vervoort M: Posterior elongation in the annelid *Platynereis dumerilii* involves stem cells molecularly related to primordial germ cells. *Dev Biol* 2013, **382**:246-267. * = equal contribution.

-Demilly A, Steinmetz P, Gazave E, Marchand L, Vervoort M: Involvement of the Wnt/beta-catenin pathway in neurectoderm architecture in *Platynereis dumerilii*. *Nat Commun* 2013, **4**:1915.

-Kerner P, Degnan SM, Marchand L, Degnan BM, Vervoort M: Evolution of RNA-binding proteins in animals: insights from genome-wide analysis in the sponge *Amphimedon queenslandica*. *Mol Biol Evol* 2011, **28**: 2289-303.

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