**Identification of molecular mechanisms involved in mitochondrial inheritance**

Project

Mitochondria are involved in several basic biological processes such as energy production, cellular homeostasis, reproduction, and ageing, so the maintenance of a viable mitochondrial genetic information is fundamental for health, fertility, and longevity [1]. Despite the deep impact of mitochondrial impairment at the organismal level, mechanisms about mitochondrial transmission and selection across generations are largely unknown.

The aim of this project is to investigate the molecular mechanisms behind mitochondrial inheritance, taking advantage of an unusual mechanism of mitochondrial inheritance present in bivalves. Indeed, more than 100 bivalve species show the so-called Doubly Uniparental Inheritance (DUI) of mitochondria [2]. DUI species possess two highly divergent mitochondrial lineages (up to 53% amino acid p-distance): i) the F-type which, similarly to what happens in species with common Strictly Maternal Inheritance (SMI) of mitochondria, is transmitted from mother to progeny; ii) the M-type which is transmitted from father to sons. One uniqueness of this system is that the 4-5 mitochondria carried from the spermatozoon follow two distinct segregation patterns according to the sex of the embryo: they are dispersed in female embryos, while in male embryos they aggregate and segregate in the D-blastomere, from which germline derives.

Factors stored in gametes are believed to be responsible for the differential mitochondrial segregation that is already observable in the first embryo divisions [2]. DUI species have progenies with sex biases (up to 9:1 sex ratio): while some DUI females produce mostly females (“female-biased”), some other DUI females produce mostly males (“male-biased”). Eggs from female-biased individuals (from now on “female-biased eggs”) after fertilization will develop female embryos with only maternally derived mitochondria; eggs from male-biased individuals (from now on “male-biased eggs”) after fertilization will develop male embryos with both maternally and paternally derived mitochondria.

The unique features highlighted above will allow unprecedent analyses for studying the mechanism of mitochondrial inheritance: there is clear evidence that DUI originated from modification of strictly maternal inheritance of mitochondria (SMI) [2,5], and it can revert to SMI [2]. Looking for differences between female-biased eggs (showing canonical inheritance of maternal mitochondria only) and male-biased eggs (showing inheritance of both maternal and paternal mitochondria) will allow us to detect genes involved in mitochondrial transmission, which would be much more challenging and expensive using canonical model species.

Activity Plan

In this project, the candidate will take advantage of the DUI system, and of the presence of sex-biased eggs to investigate molecular factors involved in the poorly characterized mechanism of mitochondrial transmission. The DUI species that will be investigated in this work is the blue mussel *Mytilus galloprovincialis*. The Postdoc will extract total RNA from 6 samples of female-biased eggs and from 6 samples of male-biased eggs. Total RNA will be used to produce total RNA libraries, that will be sequenced using Illumina® NovaSeq™ 6000. The Postdoc will therefore analyse High Throughput Sequencing data and will use bioinformatics tools to investigate differences between the sequenced conditions. Starting from raw reads, they will perform quality check of and trimming of poor-quality reads. Filtered reads will be then mapped against the *M. galloprovincialis* genome. A quality check will allow keeping only good quality mapping reads, in order to obtain the number of good quality reads mapping on each gene (raw counts). Raw counts will be then normalized to take into account differences in sequencing depth across samples and bias in library composition. Normalized counts will be then used as input data for the differential expression analysis. The final goal of these analyses will be the identification of those genes showing differential expression between female and males-biased eggs that are likely to be involved in the mechanism of mitochondrial inheritance. A characterization of RNAs differentially expressed will be therefore performed at multiple levels, including functional annotation, identification of orthologs in model species, and a possible co-interaction of factors that may be co-involved in the mechanism of mitochondrial inheritance. Particular attention will be given to genes involved in ubiquitin-related degradation pathways and cytoskeleton-associated carriers. Immunolocalization of the most promising targets will be performed together with mitochondrial staining to visualise and compare their localization pattern. The Postdoc will analyse and discuss the obtained data in a comparative framework and from an evolutionary perspective, by comparing the data obtained with evidence from literature in other DUI/SMI species.

References

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