Selection and quality control of mitochondria

Mitochondria are a fundamental component of the eukaryotic cell. They are involved in many biological processes; of all, a central role is the production of ATP through oxidative phosphorylation (OXPHOS). Mitochondria possess their own genome, the mitochondrial DNA (mtDNA), which is commonly uniparentally transmitted by the mother, and it is essential to encode part of the genes involved in OXPHOS.

It is well known that within each cell of an individual there are up to hundreds of thousands of mitochondria, each of them possessing multiple copies of mtDNAs. It was first believed that these copies were identical to each other, but modern sequencing techniques revealed that each individual possesses a heterogeneous population of mtDNAs, a condition known as heteroplasmy [1]. As any population, also the mitochondrial one undergoes events of selection [2, 3], but how mitochondria are selected and transmitted to next generations are largely unknown. However, this is a crucial topic, since the spread of some mitochondrial mutations can compromise the functioning of respiratory complexes, and, in turn, potentially undermining fitness, fertility and lifespan [4]. Therefore, studying the mechanisms of mitochondrial selection and quality control is essential both to fill our gap in understanding mitochondrial biology and in preventing the transmission of mitochondrial diseases.

A unique model for studying the dynamics of selection and inheritance of mitochondria is provided by some species of bivalve molluscs. In such species, we find the only known exception in animals to the maternal inheritance of mitochondria, the so-called doubly uniparental inheritance (DUI) [5]. Contrary to any other species, mitochondria from the spermatozoon are not eliminated or excluded from the embryo, but they are actively maintained in males and eventually carried to the blastomeres that, during development, will give rise to the male gonad. In females, instead, mitochondria from the spermatozoon are likely eliminated. The result of such mechanism is the presence, in male individuals, of two distinct and very divergent mitochondrial populations (one coming from the mother, called F-type, and one coming from the father, called M-type).

The aim of this project is investigating DUI species for studying the dynamics of mitochondrial selection. For this purpose, we can take advantage of the unique feature of DUI species: M-type mitochondria are actively maintained in males. This peculiarity makes DUI species an excellent model, since genes involved in selection and quality control of mitochondria are likely characterized by different evolution compared to their orthologs in species with strictly maternal inheritance of mitochondria (SMI).

Despite the role of mitochondria in several diseases, most of the mechanisms beneath selection and quality control of these organelles are largely unknown. This project may provide new insights into mitochondrial biology by using species that, in spite of their peculiarity in mitochondrial inheritance, have never been investigated for such topic.

The project will be structured in two different parts: the first part will be performed using High Throughput Sequencing data and bioinformatics tools and aim to detect candidate genes involved in selection and quality control of mitochondria; the second part will require the use of specific

antibodies against the candidate target proteins, and will consist of immunofluorescence protocols and confocal visualization, as well as immunoelectron microscopy approaches, to document the localization and possible interactions of the candidate proteins in the above mentioned processes.

In the first part of the project, the PostDoc will query online databases to retrieve short reads from RNA-Seq experiments from both DUI and SMI bivalves. After quality control and trimming of the raw reads, filtered data will be used to obtain a *de novo* transcriptome for each species. Starting from transcriptomes, open reading frames (ORFs) will be first predicted and annotated, and then used as input to infer orthology among the investigated species. The analysis of sequence evolution will allow detecting genes and sites with different evolution patterns in DUI and SMI species. A functional enrichment of these genes will allow selecting those involved in mitochondrial functions. Once obtained a list of candidate genes involved in the selection of M-type mitochondria, the presence of differential expression between male and female gonads from DUI species will be investigated.

In the second part of the project, the localization of protein products of candidate genes involved in mitochondrial selection will be investigated using immunolocalization *in situ* with confocal and electron microscopy. More in detail, the localization of proteins involved in selection of M-type mitochondria is expected in the gonads during the proliferation of male germline. Males and females of DUI species (e.g. the Manila clam *Ruditapes philippinarum*) will be collected at different stages of gonad maturation. For each individual, both somatic tissues and gonads will be sampled. Samples will be stained with antibodies and localization of target genes will be performed using confocal microscope. Details about localization at call ultrastructural level will be performed by ising immunoelectron microscopy.

References:

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