Proposal of PhD project - October 2015 – September 2018
"Investissement d'avenir"
LABEX *MitoCross*

Title: Human mitochondrial aminoacyl-tRNA synthetase: structure, function, evolution and connections to mitochondrial disorders.

Project: Mitochondria play an important role in cellular energetic metabolism. They are implicated in major functions such as respiration, energy production, and metabolites synthesis. Consequently, a mitochondrial (mt) dysfunction is usually deleterious for the cell, and can be associated with human neurodegenerative and muscular disorders. Mitochondria have their own translation machinery dedicated, in human, to the synthesis of only 13 proteins, all sub-units of the respiratory chain complexes. While 22 tRNAs and 2 rRNAs are encoded by the mt-genome as well, all other macromolecules requires for protein biosynthesis are encoded by the nuclear genome, synthesized within the cytosol and imported into the mitochondria thanks to the presence of a dedicated targeting sequence. Among the imported proteins, the aminoacyl-tRNA synthetases (aaRSs) are key enzymes within the translation process, responsible for the specific attachment of the 20 amino acids to the corresponding tRNAs.

Recently, mutations within nuclear genes coding for the all set of mt-aaRSs have been correlated to pathologies, with an unexpected variability of phenotypic expressions **[1].** The host laboratory contributes cases reported for enzymes specific for aspartic acid (mt-AspRS, **[2]**) and asparagine (mt-AsnRS, **[3]**). In some of the cases, the absence of major defects within canonical properties of the mt-aaRSs calls for the existence of alternate functions. Alternate functions have already been described at several instances for aaRSs of cytosolic location. In contrast, there is solid knowledge about neither organization nor alternate function(s) of aaRSs in mitochondria. Also, work performed in the host laboratory, mainly focusing on mt-AspRS, allowed to establish the crystallographic structure and the thermodynamic properties of the enzyme **[4]**, as well as to decipher its cellular characteristics and sub-mitochondrial location (unpublished results). The impact of pathology-related mutations has been investigated on all these properties

We now wish to widen our field of investigation, and to take advantage of the acquired expertise to undertake the analysis of another system: the one specific for arginine. This system turns out to be of interest because for instance of the evolutionary complexity of ArgRSs, and because of the presence of a structural module, found exclusively in ArgRSs and very likely involved in the interaction with the elbow of $tRNA^{Arg}$. It is nowadays established that mt-$tRNA^{Arg}$ display one of the largest structural diversity, so that to reach in some species the status of world's smaller ("armless") tRNA **[5]**. In addition, numerous mutations within the nuclear gene coding for the human mt-ArgRS have been found and correlated to the severe Pontocerebellar hypoplasia type 6 (reviewed in **[1]**).

The PhD candidate will contribute to the characterization of the human mt-ArgRS, to decipher its structural and functional properties (using recombinant proteins expressed in a bacterial strain), to establish its organization within mitochondria (using sub-fractionation of mitochondria, extracted from mammalian cells in culture), and to search for cellular partner so that to identify possible alternate function(s). The PhD candidate will also investigate the impact of pathology-related mutations on all of these aspects.

Altogether, the acquired results obtained during the PhD will help to reveal peculiarities, alternate/novel functions of mt-aaRSs, their possible involvement into different cellular pathways, and ultimately should shed light into molecular mechanisms underlying some the pathologies.

[1]. Schwenzer H. *et al*. (2014) Top. Current Chemistry
[2]. Scheper G.C. *et al*. (2007) Nature Genetics
[3]. Simon M. *et al*. (2015) Plos Genetics
[4]. Neuenfeldt *et al*. (2013) NAR
[5]. Wende *et al*. (2014) Biochimie

Wished skills: This PhD project is intended for a candidate with a strong motivation and strong background in biochemistry, molecular and cellular biology (expertise in cell culture is required). The ability to integrate and deepen new concepts, as well as to be able to search for and analyze information within scientific publications will be indispensable skills, quite as the control of scientific English.

Expertises which will be acquired during the training: The PhD student will be immersed in a competitive subject at the international level. He will deepen his knowledge on cell culture, immune-precipitation, mitochondria fractionation, biochemical and biophysical characterization of macromolecular complexes, *in vivo* and *in vitro* characterization of molecules. Also, the PhD student will have opportunities to present his achievements within national and international meetings, and will be capable of drafting his work in English.

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Candidates have to send as soon as possible **complete CV** and **Letter in support of application** at M.Sissler@ibmc-cnrs.unistra.fr

Deadline for application is Thursday the 23rd of July 2015

Selected candidates will be interviewed Thursday the 30th of July 2015