OFFRE DE STAGE

Laboratoire, équipe: LMGM/ Regulation and transport of proteins across cell membranes
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Titre du projet : **Decoding Mitochondrial Protein Import Signals in Health and Diseases**

Description du projet (2000 caractères max) :

Mitochondria are essential organelles that fulfill central functions in cell metabolism and signaling. Mitochondrial dysfunction has been implicated in human neurological disorders, cancer and cardiovascular disease.

About 1500 proteins are distributed among the innermost mitochondrial matrix, the inner membrane (IM), the intermembrane space (IMS), and the outer membrane (OM). Nearly 70% of the mitochondrial proteome is synthesized on cytosolic ribosomes as preproteins with an amino-terminal cleavable presequence. After transport across the OM, the presequence signal is transported by the presequence translocase (TIM23 complex) into the mitochondrial matrix. Internal targeting signals determine the preprotein final destination. Hydrophilic preproteins are transported across the IM into the matrix with the help of the matrix-localized import motor (PAM machinery). Other preproteins possess hydrophobic stop-transfer signals that halt translocation into the matrix and promote the opening of a lateral gate into the surrounding IM. The mechanisms by which internal targeting signals are recognized by the translocase and how their targeting information influences the TIM23-PAM association or the opening of the TIM23 lateral gate remain largely elusive.

This M2 project investigates how the TIM23 complex deciphers internal preprotein signals. We have described the role of three TIM23 regulatory subunits that are critical for the faithful transport of preproteins. These include the presequence receptor Tim50, the TIM23-PAM coupling factor Pam17 and the lateral gatekeeper Mgr2 [1-3]. The M2 student will use yeast genetics and biochemical methodologies to dissect how Tim50, Pam17 and Mgr2 contribute 1) to recognize internal preprotein targeting signals and 2) to remodel the TIM23 complex to accurately transport these signals into the matrix or IM. The project will help to understand how the information contained in preprotein targeting signals is converted into functional tasks mediated by the TIM23 complex. The M2 student will integrate a young and dynamic group at the LMGM/CBI.

Techniques : *S. cerevisiae* mutagenesis; epifluorescence microscopy; phylogenetic analysis of protein motifs; protein mutagenesis; site-directed photocrosslinking; *in vivo* protein proximity assays; native protein immunoprecipitation; protein purification by affinity chromatography; reconstitution of native complexes into proteoliposomes.

Références (5 max) :

1. S. Lee *et al.*, The Mgr2 subunit of the TIM23 complex regulates membrane insertion of marginal stop-transfer signals in the mitochondrial inner membrane. *FEBS letters*, (2019).
2. C. Moulin, A. Caumont-Sarcos, R. Ieva, Mitochondrial presequence import: Multiple regulatory knobs fine-tune mitochondrial biogenesis and homeostasis. *Biochimica et biophysica acta. Molecular cell research* **1866**, 930-944 (2019).
3. A. Caumont-Sarcos *et al.*, Transmembrane Coordination of Preprotein Recognition and Motor Coupling by the Mitochondrial Presequence Receptor Tim50. *Cell reports* **30**, 3092-3104.e3094 (2020).