Fonction, structure et rôle biologique de flippases (transporteur de lipides) du parasite du paludisme.
Function, Structure and Biological role of lipid flippases from malaria parasites.

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Résumé:

Our laboratory is interested on unraveling the structure, transport mechanism and regulation of membrane transport proteins from the P4 subfamily of P-type ATPases (or lipid flippases). In eukaryotes, these transporters are responsible for maintaining the unique phospholipid composition and distribution of membranes, by transporting (or flipping) phospholipids from the extracellular (or luminal in the case of intracellular compartments) leaflet of the membrane to the cytosolic leaflet. This activity creates the asymmetric distribution of phospholipids in membranes essential for processes such as cell signaling, cell polarity and migration, vesicle formation or apoptosis. Moreover, in some eukaryotic pathogens these flippases are implicated in drug-resistance and virulence. *Plasmodium*, the malaria parasite, contains three genes coding for putative P4-ATPases (ATP2, ATP7 and ATP8) whose individual deletions (recently done on P. berghei), are incompatible with life, emphasizing the essential role of P4-ATPases for the parasite. In addition, *Plasmodium* genomes contain three genes encoding putative Cdc50 proteins, known to form heteromeric complexes with P4-ATPases in other organisms. This oligomerization is required for proper subcellular localization of the flippase and also, presumably, for their activity. To assess in detail their functional properties and to determine their 3D atomic structure, we are producing in yeast P4 ATPases-Cdc50 complexes from *Plasmodium*. We have identified two putative Plasmodium Cdc50 proteins that interact with ATP2 by combining heterologous co-expression in yeast followed by immune precipitation assays. The M2 student, in close interaction with our current PhD student, will be in charge of purifying these *Plasmodium* ATP2-Cdc50 complexes and characterizing their stability in solution. For this end, we have engineered different versions of the ATP2-Cdc50 complexes containing the green fluorescent protein (GFP) at the C-terminal ends of ATP2 and the Cdc50 subunits to (1) purify these complexes by affinity chromatography using immobilized nanobodies (camelid antibodies) against the GFP and (2) to test the stability (judged by the monodispersity in solution) of the purified ATP2-Cdc50 complexes using fluorescence size-exclusion chromatography. Our laboratory has a long-term experience in P-type ATPase transporters and P4 ATPases in particular. We have developed strategies proved to be successful for both functional characterization and 3D crystallization of P-type ATPases.We recently have generated a robust co-expression system in *S. cereviseae* to obtain functional P4 ATPase-Cdc50 protein complexes (among other proteins from Plasmodium species). Therefore, the student will be trained in the expression, purification and in the functional characterization of P-type ATPases. In addition, purified proteins will be subjected to crystallogenesis using the I2BC Crystallography Platform, where the M2 student will be fully involved.

Important remark: Due to the strict regulation to access the CEA campus, if you are interested in our proposal please contact us before the end of September/beginning October.

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