Labex PhD project in the frame of task 2: Membrane dynamics and proliferation:

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The general objective of task 2 is to understand common metabolic pathways involved in membrane biogenesis and proliferation across evolution and to study them in two biological models: *Escherichia coli* and *Chlamydomonas reinhardtii*. The proposed PhD project will focus on the bacterial model and is divided in three parts.

1. Analysis of membrane proliferation in *Escherichia coli* by fluorescent microscopy and flow cytometry. The *E. coli* mutant host C43λ(DE3) (Miroux and Walker, 1996) undergoes massive membrane proliferation upon the homologous overexpression of the *atpF* gene encoding the b subunit of the F1Fo ATP synthase. In this bacterial host, b expression is not toxic and therefore cells react to the overproduction of a membrane protein by synthetizing more lipids (here cardiolipids) that are organized in a network of membranes (Arechaga et al., 2000) closed to those found in mitochondria and chloroplasts. Where membrane proliferation initiates within the cell, what is the time course of expression of lipid synthesis and is it correlated with b expression? These are the questions we wish to answer using time lapse fluorescent microscopy and flow cytometry. Federica Angius, an erasmus master student is currently constructing b-GFP fusion protein and b/GFP co-expression vectors that will use in combination with fluorescent marker of lipids ([10-N-nonyl-3,6-bis(dimethylamino)acridine]) (Mileykovskaya and Dowhan, 2000, 2009)

2. Identification of early regulation steps leading to membrane proliferation by omics and bioinformatics approaches

Once the time course of membrane proliferation is well defined, we will attempt to identify regulatory functions that are involved in the early steps of membrane proliferation using RNA sequencing approaches and the HT sequencing platform at ENS. In parallel, label free proteome analysis will be performed thanks to the Q-exact mass spectrometer being installed at IBPC. Putative genes coming up from these analyses will be either inactivated or overexpressed in C43λ(DE3) in order to test their putative role in membrane proliferation. Search for orthologs in bacterial phyla, including cyanobacteria and *Chlamydomonas reinhardtii* will be undertaken and ectopic expression in bacteria attempted.

3. Setting up a genetic screen for the isolation of bacterial mutants overproducing membranes.

This task is independent of tasks 1 and 2. C41/C43λ(DE3) mutants have been selected based on the survival of the cells upon overexpression of the b subunit. This primitive approach does not allow the rapid identification of the mutations in those mutants (complete genomic sequencing is being undertaken by competitors (Wagner et al., 2008)). Our objective is to set up a genetic screen enabling the rapid identification of bacterial mutants exhibiting constitutive membrane proliferation with or, ideally, without AtpF overexpression. Preliminary results suggest that NAO could be used as marker of cardiolipin content in bacteria to select bacterial mutants by flow cytometry in cell sorting.
experiments. As a proof of principle we will mix C43\textlambda (DE3) and BL21(DE3) at different ratio and try to recover C43\textlambda (DE3) cells by flow cytometry cell sorting (Broussais Necker/Platform). If successful, we will use this approach using transposon insertion or plasmid overexpression libraries of BL21\textlambda (DE3) strain to isolate mutant exhibiting high lipid content (high NAO green fluorescence in cytometry).

Feasibility of the project.

This research project is a new topic for the unit but we will take advantage of the recent arrival of two microbiologists Philippe Delepelaire and Marc Uzan in our group and from the expertise in mass spectrometry and flow cytometry. In term of equipment we will make use of the new instrumentation being installed at IBPC ie fluorescent microscope, mass spectrometer but we will probably ask the LABEX for financial support to cover the cost of RNA sequencing and cell sorting, experiment that will be done in specialised platform.

Outcome and perspectives

We hope to identify key elements that govern membrane proliferation in bacteria. The data obtained from our simple bacterial model may help understanding more elaborated sytems in cyanobacteria or Chlamydomonas. This project is also complementary of the structural approach developed by Dror Warschawski and Xavier Warnet (PhD student) in our unit. They use solid state NMR to establish the structure of the b subunit in those native membranes as proof of principle of in cell membrane protein structure determination.

Long term perspectives are to design or isolate bacterial mutant in which the amount of membrane and the machinery of folding and insertion of membrane protein is adapted to the large scale production of membrane proteins.

References


