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Host laboratory:
LPCV www.lpcv.fr

Host group/team:
Lipid

Title of the M2 research internship:
Structural characterization of AtVPS13M1, a lipid transfer protein involved in plant response to phosphate starvation

Project summary:
Membrane biogenesis involves a massive exchange of lipids between organelles. Lipids can be transported between cell compartments by vesicular or non-vesicular routes, the latter is thought to occur between virtually all organelles. This pathway involves the direct exchange of lipids between two membranes that are closely apposed at a distance below 30nm and is mediated by Lipid Transfer Proteins (LTPs) that are soluble proteins bearing a hydrophobic cavity accommodating one or several lipid molecules. VPS13 are LTPs forming hydrophobic channels between membranes and they appear to be key players in membrane biogenesis in yeast and mammals.
The model plant Arabidopsis thaliana has four VPS13 proteins but their structure and function are poorly characterized. The lab recently started the study of AtVPS13M1, a 4200 amino acids long VPS13, and demonstrated 1) that the protein is able to bind lipids and to transport them between membranes and 2) that AtVPS13M1 is involved in membrane lipid remodeling occurring in response to phosphate starvation. Structural predictions showed that AtVPS13M1 forms a 22 nm-long tunnel with a hydrophobic concave surface decorated with several domains. The goal of the project is to optimize an AtVPS13M1 purification protocol in order to study its 3D structure by cryo-electron microscopy (cryo-EM) and single particle analysis. More specifically, the purification of the AtVPS13M1(1-800) fragment from insect cells has already been set up in the lab; this internship aims to optimize the current protocol (buffers, detergents, lipids, ...) and also to clone and test different fragment sizes. Homogeneity and oligomerization states of the purified fragments will be characterized by mass photometry and negative staining before going to cryo-EM with the most promising samples. Overall, the project will increase our knowledge about the structural organization of plant VPS13 and how they interact with lipid molecules.

Keywords:
lipid transport, VPS13 structure, cryo-EM

Relevant publications of the team:

Leterme, S., O. Bastien, R.A. Cigliano, A. Amato, and M. Michaud. 2023. Phylogenetic and Structural Analyses of VPS13 Proteins in Archaeplastida Reveal Their Complex Evolutionary History in Viridiplantae. Contact (Thousand Oaks). 6:1–23. doi:10.1177/25152564231211976.

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Michaud, M., V. Gros, M. Tardif, S. Brugière, M. Ferro, W.A. Prinz, A. Toulmay, J. Mathur, M. Wozny, D. Falconet, E. Maréchal, M.A. Block, and J. Jouhet. 2016. AtMic60 Is Involved in Plant Mitochondria Lipid Trafficking and Is Part of a Large Complex. Current Biology. 26:627–639. doi:10.1016/j.cub.2016.01.011.

Zhang M, Jungblut A, Kunert F, Hauptmann L, Hoffmann T, Kolesnikova O, Metzner F, Moldt M, Weis F, DiMaio F, Hopfner KP, Eustermann S. (2023) Hexasome-INO80 complex reveals structural basis of noncanonical nucleosome remodeling. Science 381(6655).

Weis F, Beckers M, von der Hocht I, Sachse C. (2019) Elucidation of the viral disassembly switch of tobacco mosaic virus. EMBO Reports 20(11).