Ultrastructure expansion microscopy (U-ExM) to reveal the organization of the septin scaffold in cell division

Proposal for Internship call M2 SFB 2022 - Paul Sabatier University, Toulouse, France

Ewers Group – Membrane Biochemistry

The septins are a class of proteins that constitute the fourth component of the cytoskeleton, alongside with actin, microtubules and intermediate filaments. In this complex structural network, septins have a role as scaffolding proteins, interacting with actin, microtubules and the plasma membrane. This is of special interest in dynamic cellular processes such as mitosis.
Here, not only the DNA has to be duplicated and distributed between the two daughter cells. Equally as important is the correct distribution of cytoplasm and the organelles and proteins it contains. This happens during cytokinesis, in which the plasma membrane is deformed between the daughter cells to build a cleavage furrow and later on the intercellular bridge. Septins are largely present in the aforementioned structures and act as scaffold for other mitotic proteins such as anillin and myosin. Later on, septins recruit abscission factors to the intercellular bridge, which allows the daughter cells to separate. Septins are required for successful cytokinesis in most cell types and are thus targets for cancer therapy.

However, we do not fully understand how septins recruit and interact with the other mitotic components. This is due to the variable structural properties of septins. There are 13 different septins in human, which can align into palindromic hexa- or octamers. These in turn, can constitute more elaborate structures, like rings, filaments and gauzes.

The aim of this project is to understand the nanoscopic organization of the septin oligomers in relation to mitotic and cytokinetic proteins. We employ different super-resolution (STORM, U-ExM, DNA-PAINT) imaging techniques to generate a nanoscopic, temporally resolved map of the cytokinetic machinery.
Joining this project, you will learn how to synchronize cultured mammalian cells in their cell cycle. You will optimize the protocol for multi-protein staining in U-ExM (ultrastructure expansion microscopy). Finally, you will perform confocal microscopy to visualize the architecture of septins and other cytoskeletal proteins in mitosis and cytokinesis.

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References

Gambarotto, D., Hamel, V., and Guichard, P. (2021). Ultrastructure expansion microscopy (U-ExM). Methods Cell Biol 161, 57-81.

Karasmanis, E.P., Hwang, D., Nakos, K., Bowen, J.R., Angelis, D., and Spiliotis, E.T. (2019). A Septin Double Ring Controls the Spatiotemporal Organization of the ESCRT Machinery in Cytokinetic Abscission. Curr Biol *29*, 2174-2182 e2177.

Mostowy, S., and Cossart, P. (2012). Septins: the fourth component of the cytoskeleton. Nat Rev Mol Cell Biol *13*, 183-194.

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