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Project description :

Ultrastructure of the cytokinetic intercellular bridge by cryo-electron tomography

An intercellular bridge (ICB) connects dividing mammalian cells, in late cytokinesis, before they eventually split in two. At the ICB different sets of proteins are recruited. Mis regulations at late cytokinesis can lead to multinucleated cells and thus diseases. Among the regulating factors, septins are recruited by anillin and self-assemble into two ring-like structure on both sides of the central midbody (Figure 1, left). Septins are filamentous proteins interacting with the inner plasma membrane, are ubiquitous in eukaryotes and involved in membrane remodeling. ESCRT-III protein complexes are then recruited in between those septin rings before being relocalized to the future abscission site.

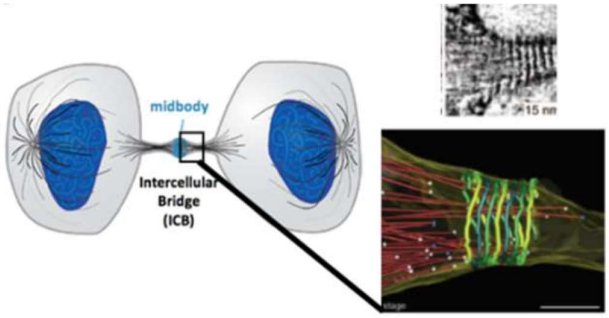

Figure 1: Intercellular Bridge between the mother and daughter cell. A 17 nm proteins filament is observed by electron microscopy (Top right image). Bottom right: 3D reconstruction of the abscission structure

Electron microscopy analysis at the ICB revealed that ESCRT-dependent 17 nm-thick filaments form a conical structure from the midbody to the abscission site (Guizetti et al; Science (2011). However, the nature of these filaments, remains poorly understood, since individual ESCRT-III filaments are much thinner in vitro. We can thus assume that these filaments include a set of proteins including ESCRT-III proteins and septins. Recent data have shown that the depletion of septin leads to anarchic polymerization of ESCRT-III within the midbody and ESCRT-III cannot reach the abscission site.

In the present work we propose to get more insights into the ultrastructure of the midbody using the latest available methods. We want to determine at different time points during cytokinesis how septin, ESCRTs and their partners interact and decipher their fine localization. We want to determine the nature of the filaments present in the 17 nm in diameter filament highlighted a few years ago by electron tomography (Guizetti et al; Science (2011)).

We will develop a methodology to perform correlative microscopy of **resin-embedded samples** obtained by preservative procedures: high pressure freezing and freeze substitution. On one hand, **super resolution multi-focus microscopy (MFM)** will be used on resin sections and on another hand the same sections will be transferred to an electron microscope to perform **2D imaging as well as**

electron tomography. Super resolution MFM will be carried out using the setup designed by Bassam Hajj at PCC (Institut Curie) and electron microscopy will under the supervision of A. Bertin. Ultimately, the **global organization and architecture of the ICB** we will attempt to describe its ultrastructure at the **best possible resolution**. To this end, **cryo-electron tomography** will be performed on vitrified samples followed **by sub-tomogram averaging**.

This project will be carried out in collaboration with Stéphanie Mangenot (Unv. Paris) and Bassam Hajj (PCC, I. Curie).

A few recent references from the lab:

•Francois Iv, Carla Silva Martins, Gerard Castro-Linares, Cyntia Taveneau, Pascale Barbier, Pascal Verdier-Pinard, Luc Camoin, Stéphane Audebert, Feng-Ching Tsai, Laurie Ramond, Alex Llewellyn, Mayssa Belhabib, Koyomi Nakazawa, Aurélie Di Cicco, Renaud Vincentelli, Jerome Wenger, Stéphanie Cabantous, Gijsje H. Koenderink*, Aurélie Bertin*, Manos Mavrakis*. Insights into animal septin assembly and function using recombinant human septin hetero-octamers, J Cell Sci. 2021 Aug 1;134(15):jcs258484. doi: 10.1242/jcs.258484. Epub 2021 Aug 5.

• Anthony Vial*, Cyntia Taveneau*, Luca Costa, Brieuc Chauvin, Hussein Nasrallah, Cédric Godefroy, Stéphanie Mangenot, Daniel Lévy, Aurélie Bertin*, Pierre-Emmanuel Milhiet*, Correlative AFM and fluorescence imaging demonstrates a nanoscale membrane remodeling and spontaneous ring-like and tubular structures formation by Septin, Nanoscale. 2021 Aug 7;13(29):12484-12493. doi: 10.1039/d1nr01978c. Epub 2021 Jul 6.

• Agata Szuba, Fouzia Bano, François Iv, Manos Mavrakis, Ralf P. Richter, Aurelie Bertin, Gijsje H. Koenderink, Membrane binding controls ordered self-assembly of animal septins, Elife. 2021 Apr 13;10:e63349. doi: 10.7554/eLife.63349.

• Taveneau C., Di Cicco A., Levy D., Pehau-Arnaudet G., Bertin A., Synergistic role of nucleotides and lipids for the self-assembly of Shs1 septin oligomers, 2020, Biochemical Journal, 477, 2697–2714, DOI: 10.1042/BCJ20200199.

• Aurélie Bertin, Nicola de Franceschi, Eugenio de la Mora, Sourav Maity, Nolwen Miguet, Aurélie di Cicco, Wouter Roos, Stéphanie Mangenot, Winfried Weissenhorn, Patricia Bassereau, 2020, Human ESCRT-III Polymers Assemble on Positively Curved Membranes and Induce Helical Membrane Tube Formation, biorxiv, doi: https://doi.org/10.1101/847319, Nat. Commun. 11 (1), 1-13.

• Alexandre Beber, Cyntia Taveneau, Manuela Nania, Feng-Ching Tsai, Aurelie Di Cicco, Patricia Bassereau, Daniel Lévy, João T. Cabral, Hervé Isambert, Stéphanie Mangenot, Aurélie Bertin. Membrane reshaping by micrometric curvature sensitive septin filaments, Nat Commun. 2019 Jan 24;10(1):420. doi: 10.1038/s41467-019-08344-5.

• Alexandre Beber, Maryam Alqabandi, Coline Prevost, Daniel Levy, Patricia Bassereau and Aurélie Bertin*, Stéphanie Mangenot*, Septin-based readout of PI(4,5)P2 incorporation into membranes of giant unilamellar vesicles, 2018, Cytoskeleton, https://doi.org/10.1002/cm.21480