2023-12-19

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Ultrastructural architecture of the intercellular bridge by cryo-electron microscopy

General information

Call	2024
Reference	2024-01-BERTIN
Keyword(s)	Cytokinesis; septins; ESCRTs; cryo-electron microscopy; super resolution microscopy

Director(s) and team

Thesis director(s)	Aurélie Bertin
Research team	Molecular Microscopy of Membranes (MMM)
Research department	UMR 168 – Physical Chemistry Curie

Description of the PhD thesis project

Abstract

Towards the end of cell division, in mammals, cells remain connected via a tubular structure, called the Intercellular Bridge (ICB) before they eventually split in two. We aim at describing the architecture of the intercellular bridge, localize and decipher the role of key proteins in cytokinesis. The ICB is a tubular structure of about 1 µm in diameter and up to 10 µm long, with a central midbody domain. Throughout this process, septins appear early on both sides of the midbody before ESCRTs are recruited and polymerize within the midbody. We want to determine at different time points during cytokinesis how septin, ESCRTs and their partners interact and decipher their fine localization. In particular, we want to determine the nature of the 17 nm in diameter filaments discovered at the abscission site a few years ago by electron tomography. Besides, we aim at comparing and confronting our observations from current experiments we are pursuing using in vitro reconstituted systems.

This project will be carried out in collaboration with the lab of Arnaud Echard, a cytokinesis specialist (I. Pasteur). To this end, we will combine super resolution fluorescence microscopy with cryo-electron tomography using Hela cells. The proteins of interest will be localized using MFM (Multifocus Microscopy) in collaboration with Bassam Hajj, a super resolution expert (I. Curie). Cryo-lamellae of the region of interest will be generated by cryo-FIB SEM and subjected to cryo-electron tomography.

Background

Septins are ubiquitous eukaryotic cytoskeletal proteins that interact with the inner plasma membrane and play a fundamental role in constraining the diffusion of membrane bound proteins. Septins assemble into high order filamentous networks (bundles, filaments, rings and gauzes) from hexameric or octameric palindromic rod-like complexes. Like Septins, ESCRT proteins assemble into intracellular filamentous

structures and are involved in a number of membrane remodeling processes. ESCRT-III (13 proteins in Human) assemble into spiral filaments and actively fission membranes through poorly understood mechanisms. Both Septins and ESCRTs are essential for cytokinesis, the last step of cell division that physically splits cells into two. The final scission (abscission) requires the concentration of ESCRT-III filaments at the abscission site, close to the midbody, a structure located at the center of the intercellular bridge (ICB) that connects the two daughter cells. The ultrastructure of the ICB revealed that ESCRT-dependent 17 nm-thick filaments form a conical structure from the midbody to the abscission site. However, the nature of these filaments, remains poorly understood, since individual ESCRT-III filaments are much thinner in vitro (about 4 nm diameter). Our central hypothesis is thus that the direct interplay between septins and ESCRTs and their resulting structural organization is key for the achievement of cytokinesis.

Objectives

We aim at describing in vivo the structure-function relationship between septins and ESCRTs by characterizing the architecture of these filaments within the ICB at different stages of cytokinesis, using cellular cryo-electron tomography. For the first time, we hope to reveal the cytokinetic intercellular bridge at different maturation stages at the nanometer or subnanometer resolution. In addition, some of the major factors governing the cytokinetic process will be finely localized within the architecture by correlative microscopy. Analyzing frozen hydrated specimens should generate unprecedented insights, in terms of ultrastructure and resolution on the septin/ESCRTs architecture in a crucial cellular function.

Experimental approaches

The project proposes to use the latest imaging methods both in fluorescence microscopy and cryo-EM. First, correlative microscopy of resin-embedded samples obtained by preservative procedures: high pressure freezing and freeze substitution will be performed.

- Aim 1. Super resolution multi-focus microscopy (MFM, Bassam Hajj set up) will be used on resin sections that will be transferred to electron microscopes to perform 2D imaging as well as electron tomography (EM facility I. Curie). We will obtain sufficient resolutions to decipher whether given septins and/or ESCRTs are present at the midbody or within the abscission site and determine whether they are localized within the 17 nm filament. Proteins of interest will be genetically tagged with TdEOS fluorescent probes suited for super-resolution microscopy.
- Aim 2. On another hand, to describe the ICB at the best possible resolutions, we will analyze frozen hydrated unfixed and unstained Hela cells (A. Echard) by generating cryo-lamellae by cryo-FIB SEM using the platform at I. Pasteur. Cryo-electron microscopy and tomography will be performed either at I. Curie equipped recently with a 200kV Glacios microscope or at I. Pasteur equipped with both 200 and 300kV microscopes. Ultimately, sub-tomogram averaging will be carried out to reach the best possible resolutions on the cryo-tomograms.

International, interdisciplinary & intersectoral aspects of the project

This is a multi-disciplinary proposal, gathering structural biologists, cell biologists and biophysicists. Cell biology will be carried out in collaboration with experts in cytokinesis who have been generating appropriate cells lines for the achievement of the project. We will collaborate with an expert in super resolution microscopy which set up is directly available in the unit. We will benefit from our expertise in Cryo-tomography and subsequent image processing methods for structural biology. We believe that this unique combination of expertise will help overcoming scientific issues. The context at PCC is inter-disciplinary which facilitates any collaborative work will overcome bottlenecks to unravel novel insights into the conjoint role of septins and ESCRTs.

Recent publications

1. Wei Mao, Lars D. Renner, Charlène Cornilleau, Ines Li de la Sierra-Gallay, Sarah Benlamara, Yoan Ah-Seng, Herman Van Tilbeurgh, Sylvie Nessler*, **Aurélie Bertin***, Arnaud Chastanet*, Rut Carballido-López*; On the role of nucleotides and lipids in the polymerization of the actin homolog MreB from a Gram-positive bacterium, eLife, 2023 Oct 11:12:e84505. doi: 10.7554/eLife.84505.

2. Koyomi Nakazawa, Gaurav Kumar, Brieuc Chauvin, Aurélie Di Cicco, Luca Pellegrino, Michael Trichet, Bassam Hajj, João Cabral, Anirban Sain, Stéphanie Mangenot, **Aurélie Bertin**, A human septin octamer complex sensitive to membrane curvature drives membrane deformation with a specific mesh-like organization, JOCES, 2023, 136(11): jcs260813, doi: 10.1242/jcs.260813.

3. Julien Maufront, Bérengère Guichard, Lu-Yan Cao, Aurélie Di Cicco, Antoine Jégou, Guillaume Romet-Lemonne, **Aurélie Bertin**, Direct observation of the conformational states of formin mDia1 at actin filament barbed ends and along the filament, Mol Biol Cell. 2023 Jan 1;34(1):ar2. doi: 10.1091/mbc.E22-10-0472.

4. 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.

5. 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.

Expected profile of the candidate

We expect a candidate with an interest in cryo-electron microscopy from the sample preparation to image processing. Possibly, a former experience in cryo-electron microscopy methods would be best. The candidate should adapt to novel methodologies and expect to perform challenging experiments. Besides, the project requires to be comfortable with computation and image processing methodologies.
The candidates' initial training could range from physics, chemistry or cell biology but with a motivation and interest for other fields.
The candidate will have to communicate with collaborators and be comfortable in interacting with different scientists.