Proposition de stage de Master 2
MASTER SBCP

Characterization of the mutual interaction and organization of septin and ESCRT proteins

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Project description :

Septins are ubiquitous in eukaryotes, essential for numerous cellular processes (cytokinesis, neuro-morphogenesis, …) and are involved in membrane remodeling. Septins are filamentous proteins interacting with the inner plasma membrane. They localize at the cleavage furrow during cell division. They are implicated in neuro degenerative diseases like Parkinson's and Alzheimer, and are also implicated in a variety of cancer.

Like Septins, ESCRT proteins assemble into intracellular filamentous structures and are involved in a variety of membrane remodeling processes (like virus budding, cell division and MVB formation). ESCRT proteins are made of five different complexes: ESCRT-0; I; II; III and the ATPase VPS4. ESCRT-0, I and II serve as a platform to recruit ESCRT-III which further assemble into an active filament perform abcission of the membrane during cytokinesis.

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 (Figure 1).

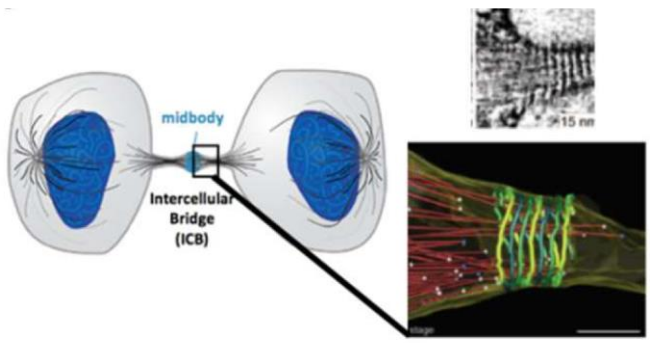

Figure 1: Intercellular Bridge between the mother and daughter cell. A 17 nm proteins filament is observed by electron microscopy (Top right image). Bottom right: 3D reconstruction of the abscission structure

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 understand the mutual interplay between the septin cytoskeleton and the ESCRT-proteins.

We will first characterize the interaction of septin filament with ESCRT-III proteins in solution using classical biochemical strategies. We will then characterize the interaction of these proteins on lipidic membrane of various sizes and geometries (Supported lipid bilayer, small and large unilamellar vesicles and Giant vesicles). The ultra-structure of proteins will be visualized using fluorescent microscopy (confocal and super resolution microscopy) and cryo-electron microscopy.

S. Mangenot is an expert of in vitro lipidic systems. In her lab she has all the equipments to produce the in vitro system. The optical imaging will be performed either directly in the lab or at the platform located rue des St Pères. A. Bertin is an expert of septin proteins with a long knowledge of electron microscopy imaging. Electron microscopy imaging will be performed either in Curie or at a Platform at Institut Pasteur. Some protein complexes will be expresses and purified in the lab where all facilities are available. Both supervisors already published articles on the structural organization of septins or ESCRT-III alone (Septins: Beber et al Nature com 2019; ESCRT-III: Bertin et al Nature com 2021).

This **work could be follow by a PhD.** S. Mangenot and A. Bertin already secure a 3 years PhD **funding by the ANR** for this project.