Master's Degree Project in Biochemistry and Structural Biology

Intracellular Membrane Remodeling Mechanisms Revealed by Cryo-EM

Bin/Amphiphysin/Rvs167 (BAR) proteins control various diverse intracellular membrane trafficking and remodeling events, including Golgi scission, endocytosis, endosomal fusion, autophagy, and mitophagy. BAR proteins mediate remodeling of intracellular membranes by inducing varying degrees of membrane curvature. This activity is controlled by BAR protein dimerization and assembly of crescent-shaped domains that enforce their shapes onto membranes via electrostatic interactions with negatively charged phospholipid head groups (*scaffolding*). N-BAR proteins, like the endophilin family are comprised of a N-terminal BAR domain containing two amphipathic motifs, helix 0 (H0) and an insert in helix 1 (H1i), and a C-terminal canonical SH3 domain that mediates protein-protein interactions. H0 is crucial for N-BAR protein membrane binding (*wedging*), while H1i has been suggested to contribute to BAR-domain scaffolding and curvature generation. Endophilin A1, the family member that has received most attention to date, is neuronal-specific and predominately associated with plasma membrane dynamics (2, 26-34). Endophilin A1 assembles into helical scaffolds on membranes that promote curvature and formation of constricted tubules. Our previous work shows that endophilin A1 recruits main membrane fission regulator, dynamin 1, to sites of synaptic vesicle recycling in nerve terminals. Similarly, endophilin B1 promotes recruitment of dynamin 2 to Golgi tubules, and loss of endophilin B1 blocks dynamin 2-mediated scission from Golgi and autophagosome formation in HeLa cells. This suggests that endophilins-formation of constricted membrane tubules recruit effector proteins, such as dynamins to sites of down-stream membrane remodeling events. Knockdown of endophilin B1 leads to dysregulation of mitochondrial dynamics and inhibition of apoptosis, suggesting it may coordinate membrane remodeling events during essential cell death processes and thereby serve a critical tumor suppressor role in the cell. Despite the important role of endophilin B1 in many intracellular signaling processes, its underlying mechanisms of function are largely unknown.

Our lab uses cryo-electron microscopy (EM) and functional biochemical and biophysicalmethods to elucidate exactly how endophilin B1 modulates intracellular membranes to control a multitude of critical membrane trafficking processes that contribute to regulation of carcinogenesis and neurodegeneration. This specific project aims at designing membrane templates that support endophilin B1 membrane- binding and bending for structural characterization by cryo-EM and other associated methods. *The outstanding questions you will address is: How does endophilin B1 interact with different intracellular membranes?*

Aims of the project

1. Express and purify endophilin B1 (EnB1) from E. coli
2. Design liposomes and lipid nanotubes that promote endophilin B1 membranes remodeling
3. Analyze EnB1-decorated membranes by cryo-EM

Methods

Protein production in E. coli Protein purification
Protein/lipid binding assays (lipid dot blots, gel filtration, Western blot)
EM/Cryo-EM

Contact: anna-sundborger-lunna@icm.uu.se
For more information about our research: https://www.icm.uu.se/strukturbiologi/sundborger-lunna-lab/