DOI: 10.1017/S1431927605505233
Microsc Microanal 11(Suppl 2), 2005

The Role of Dynamin in Membrane Constriction Revealed by Cryo-EM

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Dynamin, a large GTPase, is involved in numerous vesiculation events throughout the cell including receptor-mediated endocytosis, caveolae internalization and trafficking from late endosomes and Golgi, and in the nerve termini is essential for synaptic vesicle recycling. During these processes, dynamin is believed to self-assemble into small spirals at the necks of budding pits and assist in membrane fission. Previously, using an in vitro assay we have shown that dynamin-lipid tubes constrict immediately upon addition of GTP (Sweitzer and Hinshaw; 1998; Zhang and Hinshaw, 2001; Danino et al. 2004). Increasing evidence suggests that the self-assembly of dynamin is dependent on the interaction between the GTPase domain and GTPase Effector Domain (GED) of dynamin. To further explore the role of dynamin domains in self-assembly and membrane constriction we have calculated the structure of a dynamin mutant in the constricted (Zhang and Hinshaw, 2001) and non-constricted states (Chen et al., 2004). Our three-dimensional structures of delta-prd dynamin support our hypothesis that an interaction between GED and a GTPase domain from a neighboring dimer leads to the constricted state. Overall, these results clearly suggest that dynamin is a force generating "contrictase". During or following membrane constriction, other molecules may be recruited to the necks of invaginated pits to promote the final membrane fission event.

In this present study we are continuing to explore the mechanochemical properties of dynamin by time-resolved cryo-electron microscopy and 3-dimensional reconstruction methods. We are investigating the role of lipid composition in the dynamin-induced lipid-bilayer constriction. In addition we have begun to explore the role of dynamin partners during this reaction. In particular we are examining the effect of amphiphysin in our in vitro assay. Preliminary results reveal that amphiphysin enhances membrane constriction, which may lead to membrane fission in the cell. In addition to the time-resolved studies, we are pursuing higher resolution maps of dynamin in the constricted and non-constricted states. For the time-resolved studies, the specimen samples are frozen in liquid ethane, examined by a Philips (FEI) CM120 cryo-TEM at ~-180 degrees Celsius and images are recorded by a side-mount Gatan CCD camera, which provides real-time feedback on the specimen. For the high resolution studies frozen samples are examined on a FEI Polaris (FEG, 300 kV) microscope at liquid nitrogen temperatures and images are recorded on film.

References:

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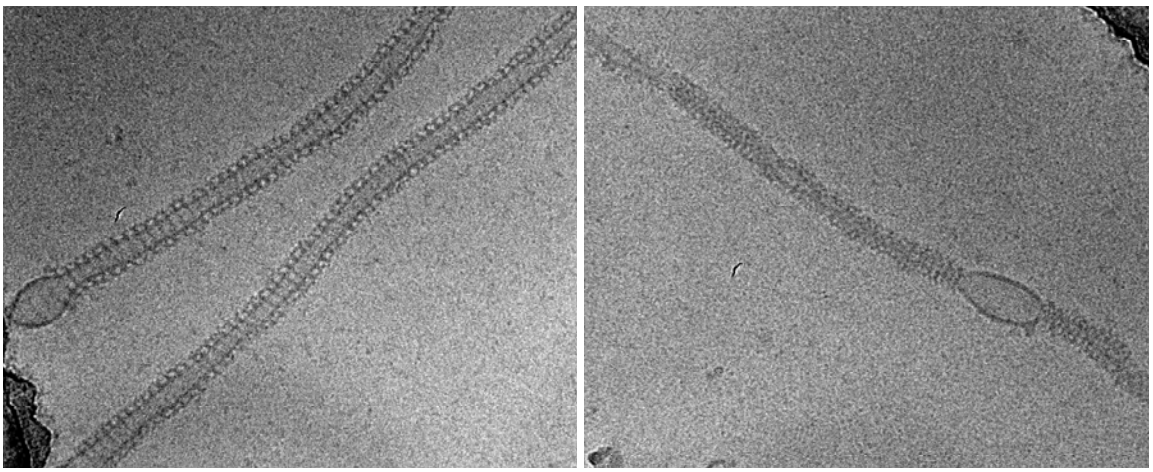

Cryo-EM images of WT dynamin-lipid Tubes.

Left panel: A typical image of dynamin-lipid tubes at time zero (before GTP treatment). Dynamin wraps around the lipid tube in a helical conformation with a diameter of 50 nm. The T-shape of the dynamin structure extends from the lipid bilayer. Right panel: 10 seconds after addition of 1 mM GTP constricted tubes with a smaller diameter (40 nm) and shorter pitch are observed. Undecorated lipid bulges are also seen at this time point.

https://doi.org/10.1017/S1431927605505233 Published online by Cambridge University Press