Symposium 14: Mechanisms of Exo- and Endocytosis

1733-Symp Dynamin Structure

Jenny Hinshaw

NIDDK, NIH, Bethesda, MD, USA.

The dynamin family of proteins are large GTPases involved in membrane remodeling events throughout eukaryotic cells. For example, dynamin is involved in the final stages of fission during endocytosis, and the dynamin-related protein (Drp1) is necessary for mitochondrial division. During endocytosis, dynamin is believed to wrap around the necks of coated pits and facilitate vesiculation. In support of this model, purified dynamin self-assembles into spirals (50 nm diameter) and readily form dynamin-lipid tubes, which constrict and fragment upon addition of GTP. Structural changes induced by GTP addition were characterized by solving the three-dimensional structure of dynamin in the constricted and non-constricted states using cryo-electron microscopy. Using a rigid-body Monte Carlo algorithm, the crystal structures of the GTPase and pleckstrin homology domains were fit to the cryo-EM densities. The GTPase domain is placed at the periphery of the helical array while the PH domain is in a position that allows for interactions with lipid headgroups. The placement of the crystal structures into the cryo-EM densities revealed a twisting motion of the GTPase, middle and GTPase-effector domains, which suggests a corkscrew model for dynamin constriction.

To determine if a common mechanism of action exists among the dynamin family members, we examined the structure and function of the Drp1 homologue in yeast, Dnm1. In collaboration with Dr. Jodi Nunnari (UC Davis) we have shown that Dnm1 assembles into large spirals with a diameter of 100 nm. Remarkably, the Dnm1 spirals have the same diameter as the observed mitochondrial constriction sites seen in vivo. Dnm1 also assembles onto liposomes and form Dnm1 decorated tubes that constrict significantly upon GTP addition. These results suggest that although dynamin family members share common characteristics, their structural properties are uniquely tailored to fit their function.

1734-Symp Pka Activation Bypasses The Requirement For Unc-31 In The Docking Of Dense Core Vesicles From *C.elegans* Neurons

Tao Xu[1], Ke-Ming Zhou[2], Yong-Ming Dong[2], Qian Ge[2], Dan Zhu[2], Wei Zhou[2], Xian-Guang Lin[2], Tao Liang[2], Zheng-xing Wu[2]

[1] *Institute of Biophysics, Beijing, China,*
[2] *Huazhong University of Science and Technology, Wuhan, China.*

The nematode *C. elegans* provides a powerful model system for exploring the molecular basis of synaptogenesis and neurotransmission. However, the lack of direct functional assays of release processes has largely prevented an in depth understanding of the mechanism of vesicular exocytosis and endocytosis in *C. elegans*. We address this technical limitation by developing direct electrophysiological assays, including membrane capacitance and amperometry measurements, in primary cultured *C. elegans* neurons. In addition, we have succeeded in monitoring the docking and fusion of singledense core vesicles (DCVs) employing total internal reflection fluorescence microscopy. With these approaches and mutant perturbation analysis, we provide direct evidence that UNC-31 is required for the docking of DCVs at the plasma membrane. Interestingly, the defect in DCV docking caused by-UNC-31 mutation can be fully rescued by PKA activation. We alsodemonstrate that UNC-31 is required for UNC-13-mediated augmentation of DCV exocytosis.

1735-Symp Intermediates in and Regulations of SNARE-mediated membrane fusion

Yeon-Kyun Shin

Iowa State Univ, Ames, IA, USA.

SNAREs are the central components of the intracellular membrane fusion machinery. Association of v- and t-SNAREs bridges two membranes, which facilitates the fusion. We use spin-labeling EPR to determine the structural transitions that SNAREs undergo during SNARE complex formation and membrane fusion. Additionally, we also use the newly developed single fusion assay based on wide field TIRF microscopy to dissect the individual steps along the fusion pathway. The SNARE assembly intermediates that are found with EPR and the fusion intermediates that are discovered with the single fusion assay will be discussed. We will also discuss the regulation of the SNARE-mediated membrane fusion by Ca2+, complexin, and synaptotagmin.

Platform AG: Protein Conformation

1736-Plat Probing the Cross-β Core Structure of Amyloid Fibrils by Hydrogen-Deuterium Exchange Deep UV Resonance Raman Spectroscopy

Igor K. Lednev, Ming Xu, Victor Shashilov

University at Albany, SUNY, Albany, NY, USA.

Studying the structure of amyloid fibrils is important for the detailed understanding of fibrillogenesis at a molecular level. Amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR. Several specialized techniques with less general capabilities have been developed and utilized for probing fibrillar structure. Transmission electron microscopy and scanning probe microscopy provide general information on fibril topology. The application of fiber X-ray diffraction and scattering has been limited to short peptides mimicking the core structure of the fibrils formed from amyloidogenic protein. Solid state NMR probes inter-atomic distances and torsion angles, which define local secondary structure and side-chain conformations. Deep UV resonance Raman (DUVRR) spectroscopy have been found to be a powerful tool for protein structural characterization at all stages of fibrillation (1, 2).

We report here on the first application of hydrogen-deuterium exchange DUVRR spectroscopy to probe the secondary structure of the fibril cross-β core (3). This method allowed for structural