61-Subg
Molecular Mechanism of Synaptic Vesicle Exo-Endocytosis
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Fast neurotransmission is crucial for brain function and depends on the exo-endocytic cycling of synaptic vesicles (SVs). Malfunction or loss of select SV proteins is associated with impaired viability, defective neurotransmitter release and with neurodegeneration. In my talk I will focus on two aspects of this process: First, I will describe our recent studies regarding the mechanism by which fast exocytic vesicle fusion at active zones is coupled to the endocytic retrieval of SV membranes. Second, I will discuss the pathways by which SV proteins are sorted and regenerated through multiple rounds of SV cycling at synapses. We have recently identified molecular scaffolds that may link SV exocytosis and endocytosis as well as endocytic adaptors for the specific sorting of select SV membrane proteins.
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62-Subg
Role of Intracellular Calcium in Release from Nerve Terminals
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Oxytocin (OT) and vasopressin (AVP) are released from terminals in the neurohypophysis (NH). These neuropeptides (NP) are contained in large dense core vesicles (LDCV). Ryanodine receptors (RyR) in NH terminals can induce spontaneous focal Ca^{2+} transients (DeCrescenzo, *et al*., 2004 *J. Neurosci.***24**:1226-1235), making them ideal for studying the role of intraterminal Ca^{2+} in NP release.
Fluorescent immunolabeling and immunogold micrographs of NH terminals show that RyR are localized specifically to LDCV. Furthermore, a large conductance non-specific cation channel, previously identified in the LDCV membrane with properties similar to that of a RyR (Lee, *et al.*, 1992 *Neuron* **8**:335-342*)*, is pharmacologically affected in the same characteristic manner as a RyR.
We found that individual Ca^{2+} release events alone are not enough to drive peptide release (McNally, *et al.*, 2009 *J. Neurosci.* **29**:14120-14126). However, these RyR sensitive events could potentially play a role in modulating NP release. To test this hypothesis, the association of LDCV within an area 0.45 µm of the plasma membrane was assessed using immunolabeling of Neurophysins I (OT) and II (AVP) in isolated NH terminals. We found that the amount of membrane associated NP-immunoreactivity varies significantly between terminal types and that this distribution pattern can be enhanced by agonist concentrations of ryanodine, but only in OT terminals.
Importantly, Ca^{2+}-evoked NP release from permeabilized-terminals was increased by agonist concentrations of ryanodine and decreased by antagonist concentrations of this drug. Amperometric recording of spontaneous release events from artificial transmitter-loaded terminals corroborated these ryanodine effects. Agonist concentrations of ryanodine were also able to increase the asynchronous phase of low frequency electrically-stimulated capacitance increases in NH terminals. Thus, the ryanodine-sensitive mobilization of LDCV seems to have a functional role in modulating secretion of NP from NH terminals. [Support: NS29470, NS40966, P60037094900000].

63-Subg
Control of Membrane Fusion in Exocytosis
James Rothman.
Yale University, CT, USA.
"Molecular Mechanism of Synchronous Neurotransmitter Release"
Synchronous transmitter release enables neural circuits to keep pace, and results when docked synaptic vesicles are rapidly triggered to fuse with the presynaptic plasma membrane by calcium ions entering the nerve terminal. Membrane fusion in the nerve terminal and elsewhere is mediated by SNARE proteins which assemble between the vesicle and plasma membrane. Biochemical studies have recently established that only two additional synapse-specific proteins - synaptotagmin and complexin - are needed to synchronize release by SNAREs and to add calcium dependence. Complexin cross-links assembling SNAREs into a highly co-operative array, freezing the frame of vesicle fusion to synchronize the readily-releasable pool. Upon binding calcium, Synaptotagmin releases the frozen SNAREs enabling them to rapidly complete assembly and release transmitter at the right time and place.

Subgroup: Permeation & Transport

64-Subg
Water Mediated Membrane Transport: From Channels to Proton Pumps
Gerhard Hummer[1], Ville R.I. Kaila[1], Fangqiang Zhu[2], Kei-ichi Okazaki[1], Mårten Wikström[3].
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Molecular simulations provide a powerful tool to study the energetics, dynamics, and mechanisms of biological membrane transport. We have used molecular dynamics simulations and modeling to study the gated transport of ions through transmembrane channels in signal transduction and the uphill pumping of protons in biological energy transduction. Remarkably, water consistently emerges as a critical player in the function of these molecular machines. In my talk I will examine how proteins exploit the unique properties of water at the nanoscale to achieve both high efficiency and high fidelity in a range of membrane transport phenomena.

65-Subg
Structure and Mechanism of the Tripartite CusCBA Heavy-Metal Efflux Complex
Edward Yu.
Iowa State University, Ames, IA, USA.
Gram-negative bacteria frequently expel toxic chemicals through tripartite efflux pumps that span both the inner and outer membranes. The three parts are the inner membrane, substrate-binding transporter (or pump); a periplasmic membrane fusion protein (or adaptor); and an outer membrane-anchored channel. The fusion protein connects the transporter to the channel within the periplasmic space. One such efflux system CusCBA is responsible for extruding biocidal Cu(I) and Ag(I) ions. We previously described the crystal structures of both the inner membrane transporter CusA and the membrane fusion protein CusB of *E. coli*. We also determined the co-crystal structure of the CusBA adaptor-transporter efflux complex, showing that the transporter CusA, which is present as a trimer, interacts with six CusB protomers and that the periplasmic domain of CusA is involved in these interactions. Here, we summarize the structural information of these efflux proteins, and present the accumulated evidence that this efflux system utilizes methionine residues to bind and export Cu(I) and Ag(I). Genetic and structural analyses suggest that the CusA pump is capable of picking up the metal ions from both the periplasm and cytoplasm. We propose a stepwise shuttle mechanism for this pump to export metal ions from the cell.