APols (3, 4), a particularly successful one is folding MPs from an unfolded or denatured state to a functional one, or expressing them in vitro (5-9). Not only are these approaches of great practical interest, but they also provide a stringent test of how ''membrane-mimetic'' an environment must be in order to allow MPs to reach their native state. Their applications and implications will be discussed.

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New Insights into the Molecular Mechanism of Beta Barrel Outer Membrane Protein Folding

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Inspired by the seminal work of Anfinsen, investigations of the folding of small, water-soluble proteins have culminated in detailed insights into how these molecules attain and stabilise their native folds. In contrast, despite their overwhelming importance in biology, progress in understanding the folding and stability of membrane proteins remains relatively limited. Focusing on the β-barrel outer membrane protein, PagP, we have been using mutational analysis to determine how this protein folds from its urea denatured state into lipid vesicles and how this process is facilitated by molecular chaperones. In this lecture I will describe our recent experiments that have investigated the initial interactions of PagP with a bilayer, its mechanism of insertion into lipid, and how this process is facilitated by the molecular chaperones skp and surA. The work is at an early stage compared with the plethora of knowledge about the folding of water soluble proteins and how this is assisted by chaperones. Nonetheless the folding of this membrane protein is revealing new insights, new challenges and fascinating synergies with the folding mechanisms of water soluble counterparts.

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Periplasmic Chaperones and their Function in the Folding of Outer Membrane Protein A into Lipid Membranes

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In Gram-negative bacteria, outer membrane proteins (OMPs) are translocated across the cytoplasmic membrane in unfolded form via the Sec translocon. Prior to insertion and folding into the outer membrane, the OMPs have to traverse the periplasm. Molecular chaperones prevent OMP aggregation before the OMPs reach the outer membrane. OMPs like outer membrane protein A (OmpA) have been shown to interact with chaperones, like Skp (1-3) and SurA (4-6).

OmpA folds spontaneously into lipid bilayers from a urea-unfolded form when the denaturant urea is strongly diluted. We have examined the kinetics of folding of OmpA and how it is affected by the chaperones Skp, SurA, FkpA. In all experiments, either in the absence or in the presence of any of these chaperones, the kinetics are well-described by two parallel kinetic processes of OmpA folding and insertion into lipid bilayers. Both processes were of first order. All examined chaperones increased the contribution of the faster folding process. However, only SurA caused an increase of the rate constant of the fast folding process. The temperature dependence of OmpA folding into lipid bilayers indicated that SurA lowers the activation energy of folding for the faster process.

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Symposium: The Synapse

1001-Symp

Reconstituting Basic Steps of Synaptic Vesicle Fusion

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Neurotransmitter release depends critically on: the SNAREs syntaxin-1, synaptobrevin and SNAP-25, which form SNARE complexes that bridge the vesicle and plasma membranes; NSF/SNAPs, which disassemble SNARE complexes; Munc18-1, which binds to syntaxin-1 and, together with Munc13, orchestrates SNARE-complex assembly; and the Ca^{2+} sensor synaptotagmin-1. Previous attempts to reconstitute neurotransmitter release revealed efficient fusion of syntaxin-1/SNAP-25-liposomes with synaptobrevin-liposomes in the presence synaptotagmin-1/Ca^{2+}, in stark contrast with physiological data showing that Munc18-1 and Munc13 are essential for neurotransmitter release. We now solve this paradox, showing that Munc18-1 displaces SNAP-25 from syntaxin-1 and that syntaxin-1/Munc18-1-liposomes fuse efficiently with synaptobrevin-liposomes in a manner that requires SNAP-25, Munc13-1 and synaptotagmin-1/Ca^{2+}. Moreover, when starting with syntaxin-1/SNAP-25-liposomes, NSF/a-SNAP disassemble the syntaxin-1/SNAP-25 heterodimers, thus inhibiting fusion, and fusion then requires Munc18-1 and Munc13-1. These results suggest that, for the first time, our experiments reconstitute synaptic vesicle fusion with the eight major components of the release machinery. We propose a model whereby the pathway to synaptic vesicle fusion does not proceed through syntaxin-1/SNAP-25 heterodimers and starts at the syntaxin-1/Munc18-1 complex; Munc18-1 and Munc13 then orchestrate membrane fusion together with the SNAREs, synaptotagmin-1 and Ca^{2+} in a manner that is not inhibited by NSF/SNAPs.

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Reconstitution of Calcium-Triggered Synaptic Vesicle Fusion

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The highly conserved SNARE protein family mediates membrane fusion in eukaryotic cells. We recently developed an assay to study calcium triggered synaptic vesicle fusion using single vesicle-vesicle optical microscopy. Prior to calcium injection, the system starts from a metastable state of single interacting pairs of donor and acceptor vesicles. Upon calcium injection, the system monitors content mixing (exchange or release of content) as well as lipid mixing (exchange of membrane components). Our system differentiates between vesicle docking, hemifusion, and complete fusion. Events are monitored on a hundred-millisecond time scale. We found that our system with reconstituted neuronal SNAREs, synaptotagmin-1, and complexin qualitatively mimics effects of calcium-triggered fast synchronous release. New insights into the mechanism of action of calcium-triggered synaptic vesicle fusion will be discussed.

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1003-Symp

Ultra-High Resolution Imaging Reveals Formation and Preponderance of Neuronal SNARE/Munc18 Complexes In Situ

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Membrane fusion is mediated by complexes formed by SNAP-receptor (SNARE) and Sec1/Munc18-like (SM) proteins, but it is unclear when and how these complexes assemble inside the cell. Here we describe an improved fluorescence nanoscopy technique that can achieve effective resolutions of up to 7.5 nm FWHM (3.2 nm localization precision), limited only by stochastic