assembly with the α_1-subunit and trafficking to the plasma membrane. The β_2-subunit binds 4-5-fold more efficiently to the ER lectin chaperone, calnexin, and 2-3-fold less efficiently to the non-lectin ER chaperone, BiP, than the β_1-subunit. These results indicate that folding of the β_2- and β_1-subunits is mediated by lectin and non-lectin chaperones, respectively, consistent with the essential role of N-glycosylation for folding and trafficking of the β_2 but not of the β_1-subunit. Disruption of the α_1-β association by mutations in defined α_1-interacting regions of either β_1- or β_2-subunits results in the ER retention of unassembled mutants, indicating that α-β assembly is essential for the ER export of either β-subunit isoform. In conclusion, the ER quality control system ensures that only properly folded β-subunits assemble with the α-subunits and only assembled α-β complexes are exported to the Golgi and delivered to the plasma membrane.

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2246-Plat

Not All ABC Transporters are the Same: Correlation between Genetic, Structural, and Mechanistic Diversity

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ATP binding cassette (ABC) transporters constitute a ubiquitous super-family of integral membrane proteins that translocate a diverse array of substrates across cell membranes. Studies of several well-characterized systems suggested a mechanistic similarity between different members of this large family of transporters. However, more recent reports pointed out significant differences at the genetic and structural levels. We report here a functional comparison between several ABC transporters of different substrate specificities and find fundamental differences between them. Type I ABC transporters, exemplified by the arch typical maltose transporter, are characterized by an inherent instability of the transporter-receptor complex. In these systems, ATP binding promotes complex formation, and binding of substrate-loaded receptor accelerates the rate of ATP hydrolysis. In contrast, in type II ABC transporters (the metal-chelate transporters), the ''default'' complex is extremely stable. However, for productive transport to occur, the complex must dissociate, an event mediated by both substrate and ATP binding. Relative to type I transporters, high basal ATPase rates are measured with modest to negligible stimulation by substrate-loaded receptors. These and other findings presented here highlight significant mechanistic differences between ABC transport systems, indicating that considerable mechanistic diversity exists within this large superfamily of proteins.

2247-Plat

How Binding of the Signal Peptide Unlocks the Translocon

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In all organisms, many of the proteins newly synthesized by the ribosome are targeted to the SecY/Sec61 translocon for secretion or incorporation into the lipid membrane. Targeting of secreted proteins is generally encoded in a ~20 amino acids extension of the N-terminus of the nascent protein, denoted as the signal peptide. The translocon opens upon binding of the signal peptide and the ribosome (or the SecA motor). To understand how SecY/Sec61 opens, it is essential to know the structure and dynamics of the translocon:signal peptide complex in a hydrated lipid membrane. Molecular dynamics simulations of the SecY translocon from M. janaaschii with proOmpA signal peptide reveal that the structure and dynamics of both the translocon and the signal peptide change significantly upon formation of the complex. It appears that inside the translocon the signal peptide has a preferred location in which it interacts with water molecules and with highly conserved SecY amino acids whose mutation causes translocation defects. Binding of the signal peptide induces changes in the relative orientation of transmembrane helices of the translocon, and also affects the structure and interactions with water and the rest of the protein of the plug segment that closes the periplasmic vestibule of the translocon in the closed state. The structure and dynamics of the translocon and signal peptide are coupled: mutating the translocon induces changes in the structure and dynamics not only of the translocon, but also of the signal peptide.

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Platform AQ: Member-Organized Session: Break on through to the other side: Comparing Membrane Permeabilizers

2248-Plat

Simulation Studies of Peptide Induced Membrane Poration and Fusion

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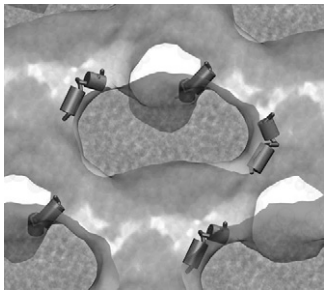

A wide range of peptides is known to modulate the behavior of lipid membranes, in particular to be able to destabilize the normal lamellar state. Here we compare the various ways in which a lipid membrane can be distorted due to the presence of such peptides, using molecular dynamics simulations. In particular we show examples of membrane poration by antimicrobial peptides, micropinocytosis by cell penetrating peptides, and the formation of cubic phases by fusion peptides (see figure).

Snapshot of a single diamond cubic phase induced by the Influenze HA fusion peptide. The helical parts of the peptides are shown as red rods, the lipid/water interface as a green surface. Lipid tail beads are shown in gray. This particular cubic phase is special as it combines both pores (the white gaps) and stalks (filled with lipid tails) in one phase. The peptides stabilize this stalk/pore structure.

2249-Plat

Cationic Lipids: from Membrane Destabilization to Cell Signaling

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Cationic liposomes have been used over the past decades with gene therapy or vaccination trials in mind, considering them primarily as the smart Trojan horse allowing to go trough the cell walls. Fusion-promoting lipids such as DOPE were thus added with some success to try to enhance the destabilizing properties of some cationic lipids, while others were intrinsically destabilizing. It is difficult to conceive that lipids that destabilize membranes would act innocently on the cell physiology. After all, lipids are not only the backbone of membranes, they also act as facilitators of membrane functions such as endocytosis, budding, curving; they regulate protein membrane activity and can even serve as signal transmitters (bioactive lipids). Rather than being considered as responsible for harmful side-effects that should be minimized, cationic lipids could be considered as a potential immunostimulating or pharmacological agents. We illustrate these aspects with diC14-amidine, which forms liposomes with a bilayer at the edge of instability. DiC14-amidine fuses easily with cell membranes, modifies cell signaling and activates immune responses through destabilization and/or activation of specific membrane receptors, like TLR4 [1,2]. Like it was shown for natural lipids, this demonstrates that cationic lipids are not only membrane destabilizing agents but might affect cell membrane components function.

1. Lonez C, Vandenbranden M, Ruysschaert JM. Prog Lipid Res. 2008. 47(5):340-7.
2. Tanaka T et al. Eur J Immunol. 2008. 38(5):1351-7.

2250-Plat

Cationic Lipid Vectors for Gene Delivery: Distinct Pathways of Endosomal Release

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Cationic liposomes (CLs) are used as non-viral gene vectors in worldwide human clinical trials of gene therapy. Because our understanding of the mechanisms of action of CL-DNA complexes remains poor and transfection efficiencies remain low compared to gene delivery with viral vectors, significant additional insights and discoveries will be required before the development of efficient chemical carriers suitable for long-term therapeutic applications. (For example, virtually all current human gene therapy protocols using lipofection as a vector contain cholesterol even though very little is understood about