addressed the molecular mechanism of enzyme inhibition using a PLB triple-mutant, N27A, N30C, L37A-PLB (PLB3), which is a potent gain-of-function PLB mutant that is cross-linkable to SERCA2a at Lys328. We observed that the protein-protein interaction between PLB3 and SERCA2a was strictly Ca2+-dependent and that several fold higher Ca2+ concentrations were required to both dissociate PLB3 from SERCA2a and to stimulate Ca2+-ATPase activity. The results suggest that PLB inhibits SERCA2a activity by competing directly for Ca2+ binding to the enzyme and that PLB must completely dissociate from SERCA2a for enzyme activation to occur. To test this hypothesis further, we co-expressed PLB3 with D351A-SERCA2a, a Ca2+ pump mutant that is inactivated at the site of ATP hydrolysis in the cytoplasm, but which retains the two high affinity Ca2+ binding sites in the membrane and maintains the thermodynamic equilibrium between E1 (high Ca2+ affinity state) and E2 (low Ca2+ affinity state). Remarkably, the affinity of D351A for Ca2+ was increased 30-fold relative to that of WT-SERCA2a, demonstrating a robust, long-range communication between the ATP hydrolysis site in the cytoplasm and the Ca2+ binding sites in the membrane. Nonetheless, PLB3 continued to bind strongly to D351A, and several fold higher Ca2+ concentrations were required to dissociate PLB3 from D351A compared to PLB molecules with normal function. Our results strongly support our model in which PLB binds to one unique conformation of SERCA2a, the Ca2+ free, E2 conformation stabilized by bound nucleotide. For both WT-SERCA2a and D351A, PLB lowers the Ca2+ binding affinity of the enzyme by stabilizing E2 thereby blocking the transition to E1.

1090-Plat

The Oligomeric Forms Of Phospholamban And Sarcolipin Physically Interact With The Sarcoplasmic Reticulum Calcium Pump

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Phospholamban and sarcolipin physically interact with the sarcoplasmic reticulum calcium pump (also known as SERCA) and regulate contractility of the heart in response to adrenergic stimuli. We have studied this interaction using electron microscopy of large two-dimensional crystals of SERCA in complex with either phospholamban or sarcolipin. The crystals are comprised of the anti-parallel dimer ribbons of SERCA molecules previously seen in helical crystals, but packed into a novel lattice with $p22_12_1$ symmetry. In previous studies, phospholamban pentamers were found interspersed between the SERCA dimer ribbons and a three-dimensional model was constructed to show potential interactions with SERCA. Herein, we have obtained two-dimensional co-crystals of SERCA and sarcolipin. Our analyses indicate that the oligomeric states of phospholamban and sarcolipin are similar in the context of the crystals and are most consistent with a pentameric arrangement. We also examined the crystallization behavior of gain-of-function mutants of phospholamban (Lys^{27} to Ala) and sarcolipin (Asn^4 to Ala) in an attempt to understand the physiological relevance of the crystal contacts. In both cases, the gain-of-function mutants enhance crystal formation, supporting the notion that the crystal contacts represent a functional interaction. This interaction occurs within the membrane and most likely involves transmembrane segment M3 of SERCA. Importantly, this transmembrane segment of SERCA bears homology with the Leu-Ile zipper found in phospholamban. The combined results suggest that SERCA reversibly dissociates the phospholamban and sarcolipin oligomers, actively influencing the pool of monomers available for the inhibitory interaction.

1091-Plat

Protein Translocation Across Membranes: Components of Outer Membrane Colicin Translocons

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Translocation of the nuclease colicins E2 and E3 across the *E. coli* outer membrane is initiated by high affinity ($K_d < 10^{-9}$ M) binding of the receptor-binding (R) domain to the vitamin B_{12} (BtuB) receptor in the *E. coli* outer membrane. Based on genetic analysis (1), and crystal structures of BtuB (2), the complex of the R-domain of colicins E2 or E3 bound to BtuB (3, 4), and of the OmpF porin containing the inserted N-terminal disordered segment of the colicin translocation (T) domain (5), a ''fishing pole'' model for the colicin translocon was inferred (3-5). The T- and C (catalytic) colicin segments must unfold before insertion into OmpF. FRET analysis was employed to study the colicin unfolding upon interaction with BtuB and OmpF (6). A rapid ($k_{1/2} < 1\ sec^{-1}$) decrease in FRET efficiency between translocation and cytotoxic domains of colicin E3 was observed upon independent and additive colicin binding *in vitro* to BtuB and OmpF. Colicin interactions with BtuB and OmpF have a major electrostatic component, provided at least partly for BtuB by R-domain Arg399. Thus, free energy for colicin unfolding is provided by binding of the R- domain to BtuB and also by binding/insertion of T-domain to OmpF. Screening the ''Keio collection'' for cytotoxicity of several group A and B colicins has shown thus far that colicin N binds to the first glucose of the LPS inner core (7).

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1092-Plat

Mechanism Of Ion-ion And Ion-substrate Coupling In Secondary Amino-acid Transporters

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The x-ray structures of LeuT and Glt, bacterial homologues of Na+/Cl–dependent amino-acid transporters, provides a great opportunity to better understand the molecular basis of monovalent cation. Both proteins possess ion-binding sites selective for Na^+ over K^+ and Li^+. Extensive QM/MM minimization combined with all-atom free energy molecular dynamics simulations of the LeuT and Glt transporters embedded in an explicit membrane are performed at different temperatures and various occupancy states of the binding sites to dissect the molecular mechanism of ion selectivity, coupling between co-transporter substrate and ions occupying binding sites. In this work, we demonstrate that there is a collective effect of multiple binding sites on a total selectivity for Na+ over Li+ both in LeuT and Glt. We also will discuss functional roles of different ion binding sites in the transport cycle. The role of local connectivity, site rigidity, atomic polarization and partial charge transfer in monovalent cation selectivity is discussed.

1093-Plat

Single Molecule Measurements on the Mechanism of Protein Import by the Toc Translocon

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In cells up to 50% of all cytosolic synthesized proteins have to traverse at least one membrane to reach their place of function. This requires the action of membrane embedded molecular machines. The Toc complex in chloroplasts is such a molecular machine (Soll, J. and Schleiff, E. Nat Rev Mol Cell Biol, 2004). Despite the identification of many components involved in the translocation process, not much is known about the mode of function of the Toc complex at the molecular level. We address this molecular mechanism with Single Molecule Fluorescence Resonance Energy Transfer (smFRET) and magnetic tweezers (MT) experiments. Specifically labelled components of the Toc complex and fluorescent labelled GTP (analogues) give insights into the stoichiometry and assembly of the Toc complex as well as its GTPase function under various conditions. The stall forces, force generating steps and translocation velocities are deduced from MT experiments. Finally, a combination of these two methods will allow to formulate a kinetic model of protein translocation, which will be an important step for the biological and systematic description of protein sorting and, subsequently, cellular function.

1094-Plat

Proton-Pumping As The Activity That Drives Living Systems

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All living membranes (as apposed to non-living myelin) pump ions. The introduction of the sources of energy upon which living cells rely, enter cells via ion, commonly proton, pumping to make ATP. These energy sources consist of either redox energy or photons. Heterotrophic cells may take in energy in the form of glucose or other covalent sources but the energy is converted into NADH or its equivalent by metabolism. The reductive product is then transduced via proton pumping to ATP production. In eukaryotes some of that ATP is used to pump protons in other, internal cellular membranes.
Thermodynamically, living cells characteristically use energy to create order. The equations that explain this apparent contradiction of the 2^{nd} Law of Thermodynamics were best derived by Ilya Prigogene and Ephraim Katchalsky who established that such activities must be near-to-equilibrium and must be local within a larger surrounding environment that increases its entropy. Prigogene