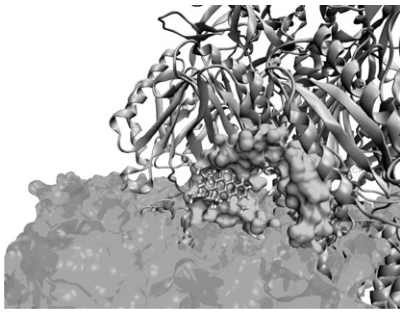
Bacteria use multidrug efflux pumps to extrude toxic substrates through their cell membranes. The RND transporters of the AcrAB-TolC (E.Coli) and MexAB-OprM (P.Aeruginosa) systems are able to export structurally and chemically different substrates, being responsible of multidrug resistance. While the energy conversion takes place in the transmembrane domain of AcrB and MexB, the energy is transducted towards the periplasmic part and used there to initiate what is believed to be a three-cyclic peristaltic pumping. Using different computational methods like adaptive bias force (ABF) and targeted molecular dynamics (TMD), we have investigated the mechanism of substrate uptake and pumping. With ABF we have studied the passage of antibiotics from the periplasm and protein-lipid interface into the inner pore of the pump, while TMD has been used to assess the effect of conformational changes on the extrusion of drugs (located into one of the proposed binding pockets). Finally, analysis of water distribution in the transmembrane region represents an important step to identify features of the energy transduction process. Comparison between the active pumps AcrB and MexB (which show different resistance patterns despite their homology) provide insights into the microscopic details of their functioning.



1963-Plat

Phenylalanine 508 Forms Intra-domain Contact Crucial To CFTR Folding And Dynamics

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Cystic fibrosis (CF) is the most prevalent genetically inherited lethal disease in the United States, with an especially high incidence rate among people of European descent and affects at least one in every 2500-4000 newborns. In 90% of CF patients, the disease is caused by the deletion of phenylalanine-508 (Phe508) from the first nucleotide-binding domain (NBD1) of the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ATP-binding cassette (ABC) transporter family. Prior experimental studies suggest that the Phe508 deletion cause the aberrant folding of NBD1 and the misassembly of various CFTR domains. To gain a fuller understanding of the role of Phe508 in channel function and of the impact of its deletion, we constructed a 3D structural model of CFTR using the high-resolution structure of another ABC transporter, Sav1866. The CFTR model predicts, and we validated experimentally, that Phe508 mediates an interaction between NBD1 and the fourth cytoplasmic loop in the second membrane-spanning domain (MSD2). This interface explains the observed sensitivity of CFTR assembly to many disease-associated mutations in CL4 as well as in NBD1. We also confirmed other intra-domain contacts between the cytoplasmic and membrane-spanning domains that were predicted by the CFTR model. Furthermore, our electrophysiological measurements and molecular dynamics simulations show that these interfaces regulate channel gating and are highly dynamic. Aside from advancing understanding of CFTR structure and function, this study identifies the region of CFTR that should be targeted in treating cystic fibrosis.

1964-Plat

Substrate-Dependent Conformational Changes of a Glutamate Transporter Homologue

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Glutamate transporters tightly control the extracellular concentration of glutamate in the brain, ensuring excitatory neurotransmission and cell integrity. These transporters utilize the energy of pre-existing ionic gradients to pump the transmitter against steep concentration gradients. Active transport is achieved by alternately exposing the ion and substrate binding sites to the extra- and the intracellular milieu and the catalytic cycle includes at least three conformational states: outward-facing, occluded and inward-facing. The crystal structures of a prokaryotic glutamate transporter homologue, GltPh, in complex with an inhibitor and a substrate showed the transporter in the outward-facing and occluded states, respectively, revealing how substrate and ions reach their binding sites from the extracellular medium. However, the conformational changes mediating the release of ligands into the cytoplasm are unknown. To gain insights into this process, we investigated ligand-dependent conformational transitions of the detergent-solubilized purified GltPh. Cysteine cross-linking experiments show that in the absence of ligands, helical hairpin 2 (HP2), which serves as an extracellular gate of the transporter, approaches TM2 bringing together residues that are more than 25 Å apart in the GltPh structures. The direction of HP2 movement is opposite of the observed in the outward-facing state, suggesting that the gate remains closed in the novel state of GltPh. We further probed the substrate-dependent solvent accessibility changes of key regions of the transporter by determining the kinetics of the fluorescence increase upon the reaction of fluorescein maleimide with single cysteine mutants. These experiments reveal a striking pattern: cytoplasmic residues become more solvent exposed and extracellular residues become less solvent-exposed upon substrate dissociation. Our results suggest that the ligand-free state is an inward-facing state of the catalytic cycle and demonstrate that the transporter can be conformationally constrained in this state by cysteine cross-linking.

1965-Plat

A Role for Topologically-Inverted Structural Repeats in Secondary Active Transport by Membrane Proteins of the LeuT Fold

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Structures of secondary active transporters often reveal the presence of large structural repeats. In the case of LeuT from the Na^+-dependent neurotransmitter symporter (NSS) family, the two repeats, each composed of five transmembrane helices, have inverted topology with respect to the plane of the membrane. An asymmetry between these two repeats in the extracellular-facing conformation of LeuT is responsible for the formation of the extracellular pathway. Structural modeling was performed in order to exchange the conformations of the two repeats. This resulted in a ~25° net rotation of a four-helix bundle around an axis in the plane of the membrane, which remarkably, created a conformation of LeuT in an intracellular-facing state. That is, the substrate binding site became exposed on the cytoplasmic side, and closed on the extracellular side. The model therefore proposes a cytoplasmic pathway for proteins with the LeuT fold. We show that accessibility of residues in this cytoplasmic pathway is consistent with a substantial number of biochemical accessibility measurements on single-cysteine mutants at the cytoplasmic face of the homologous mammalian serotonin transporter (SERT). In addition, the inward-facing structure of the related sugar transporter vSGLT shows significant similarities (Faham *et al*, Science 2008). Comparison of the two states of LeuT suggests an alternating-access mechanism in which a bundle of four transmembrane helices 'rocks' within the structure of the remaining helices. More generally, these results suggest that topologically-inverted repeats in other secondary active transporters may provide useful clues in understanding their mechanisms.

1966-Plat

Detecting Transport-related Conformational Changes In The Glutamate Transporter Homologue, Glt_{Ph}.

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Glutamate transporters (EAATs) are essential in clearing glutamate from the synapse, allowing precise control over excitatory synaptic function. Much has been learned in recent years about how these proteins function, however little is known about how the structural basis of this function; how do protein conformational changes lead to transport activity? A homolog of these proteins, Glt_{Ph} from *Pyrococcus horikoshii*, is functionally similar to the mammalian proteins; co-transporting substrate and sodium ions whilst having an uncoupled chloride conductance. Importantly, however it has been crystallized and its structure determined, making it an excellent model for understanding how structure and function are related in the EAATs. In order for transport to proceed, conformational changes must occur that allow alternating access of the substrate binding site to both the internal and external solutions. Using fluorescein maleimide labeling and protease accessibility studies on single cysteine mutants of Glt_{Ph} we have identified regions of the protein involved in this process. Changes in accessibility are seen in the presence of both aspartate and TBOA, a non-transported competitive inhibitor. Surprisingly, our accessibility studies implicate heretofore unexpected areas of the Glt_{Ph} protein in the conformational changes associated with substrate and inhibitor binding. We are also further investigating functional aspects of Glt_{Ph} transport using radioligand flux assays.

1967-Plat

Crystal Structure Of Chloride Transporter From A Cyanobaterium

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The CLC family of chloride transporting proteins is constituted of chloride channels and chloride-proton antiporters. Of the transporter subclass CLC_ec1 from E.coli has been characterized extensively structurally and functionally,

and is known to exchange two chloride ions for one proton with typical turnover of 2000 ions/s for the wildtype protein. The genome databases yield hundreds of putative chloride transporting proteins with a significant bias towards proteins with transporter like sequences. We have functionally reconstituted and determined the structure to 2.5 Å resolution of a second microbial chloride-proton antiporter from Synechocystis sp. (ORF sll0855). CLC_ss1 bears 39% sequence similarity to CLC_ec1 with the key functional residues being almost identical. Despite this high degree of conservation the observed transport rates of CLC_ss1 are several fold slower (~100 ions/s). The fold of the dimeric CLC_ss1 is almost like CLC_ec1 with an overall backbone rmsd of 1.7 Å. The residues analogous to E148 , Y445 and S107 from CLC_ec1 are situated in analogous positions, with channel lining helices displaying slight movements. No conduit through the protein is observed resulting in a more ''inward-open'' conformation. When diffraction data from crystals grown in 200 mM Br- were analyzed no evidence for ion binding was detected which correlates with the slow chloride transport displayed by CLC_ss1.

1968-Plat
Electrophysiological Investigation of the Lactose Permease from Escherichia coli on a Solid-Supported Membrane
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[1]Dept. Biophysical Chemistry, Max Planck Institute of Biophysics, Frankfurt (Main), Germany, [2]Dept. of Physiology and Microbiology, Molecular Biology Institute, University of California, Los Angeles, CA, USA.
Electrogenic events associated with the activity of the wild-type lactose permease (LacY) of *Escherichia coli* were investigated by using proteoliposomes containing purified LacY adsorbed onto a solid-supported membrane. Activation of the proteoliposomes with concentration jumps of different substrates generated transient currents. Analysis of the transient currents at different lipid to protein ratios and different pH values show that the currents represent stationary turnover of LacY. Furthermore, selective inactivation of the substrate binding by alkylation of C148 with *N*-ethyl maleimide (NEM) suppressed the transient currents, indicating that the transients correspond to the electrogenic activity of LacY. Mutant E325A LacY was used to investigate possible electrogenic steps in the transport cycle unrelated with proton translocation. In addition, electrogenic steps taking place before proton translocation were investigated with C154G LacY, which binds sugar as well as the wild-type but catalyzes very little transport activity. Both mutants show electrogenic activity after activation with different substrates. Therefore, either substrate binding or a conformational change following substrate binding is responsible for these electrical transients. As exchange (but not efflux) is almost voltage independent in wild-type LacY, it is concluded that there are least two electrogenic steps in the transport cycle.

Symposium 15: Awards Symposium

1969-Symp
TBD
Robert Stroud.
University of California, San Francisco, San Francisco, CA, USA.

1970-Symp
Lollipops and Membranes
Stephen H. White.
University of California at Irvine, Irvine, CA, USA.

1971-Symp
Combining Patch-clamp and Fluorescence to Study Structural Dynamics of Ion Channels
Teresa Giraldez.
University of La Laguna, La Laguna-Tenerife, Spain.

1972-Symp
The Biophysics of Neural Computation
Adrienne Fairhall.
University of Washington, Seattle, WA, USA.

1973-Symp
Biophysical Tools Meet Biochemistry in Living Systems: A Tale of Protein Kinases and Second Messengers
Jin Zhang.
University of Maryland School of Medicine, Baltimore, MD, USA.

1974-Symp
Structures and Functions of Large Molecular Assemblies
Keiichi Namba.
Osaka City Univ, Osaka, Japan.

1975-Symp
Connecting Speckles – Relations between Cortical Actin Dynamics and Endocytosis
Gaudenz Danuser.
The Scripps Research Institute, La Jolla, CA, USA.

1976-Symp
Chemistry under Force
Julio Fernandez.
Columbia Univ, New York, NY, USA.

Minisymposium 3: Protein-Ligand Interactions in Cellular Signaling

1977-MiniSymp
Resolving Cadherin Interactions at the Single Molecule Level
Sanjeevi Sivasankar[1], Yunxiang Zhang[2], W. James Nelson[2], Steven Chu[3].
[1]Iowa State University, Ames, IA, USA, [2]Stanford University, Stanford, CA, USA, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
The cadherin family of Calcium-dependent cell adhesion proteins are critical for the morphogenesis and functional organization of tissues in multicellular organisms, but the molecular interactions that are at the core of cadherin binding are poorly understood. The generally accepted model is that cadherins adhere in three stages. First, the functional unit of cadherin adhesion is a cis-dimer formed by the binding of the extracellular regions of two cadherins on the same cell surface. Second, formation of low affinity trans interactions between cadherin cis-dimers on opposing cell surfaces initiates cell-cell adhesion, and third lateral clustering of cadherins cooperatively strengthens intercellular adhesion. Direct molecular proof of these cadherin binding states during adhesion is, however, contradictory, and evidence for cooperativity is lacking. We used single molecule structural (Fluorescence Resonance Energy Transfer) and functional (Atomic Force Microscopy) assays to demonstrate directly that cadherin monomers interact via their outermost domain to form trans adhesive complexes. We could not detect the formation of cadherin cis-dimers, but found that increasing the density of cadherin monomers cooperatively increased the probability of trans-adhesive binding. We also resolved the role of Tryptophan-2 a key amino acid in cadherin trans-binding. These results resolve conflicting data on trans- and cis-cadherin binding states, and provide quantitative evidence for cooperativity in trans-cadherin adhesion.

1978-MiniSymp
A Fluorescence Spectroscopic Approach To Quantify The Binding Characteristics Of The Intracellular Domain Of Crumbs With Pdz Domain-containing Proteins In Drosophila Melanogaster
Heike Hornen[1], Christian Schwarz[1], Paul J. Rothwell[1], Elisabeth Knust[2], Claus A.M. Seidel[1].
[1]Heinrich-Heine Universitaet Duesseldorf, Duesseldorf, Germany.
[2]MPI of Molecular Cell Biology and Genetics, Dresden, Germany.
Formation of multiprotein complexes is a common strategy to pattern a cell, thereby generating spatially and functionally distinct entities at specialized regions. Central components of these complexes are scaffold proteins, which contain several protein-protein interaction domains and provide a platform to recruit a variety of additional components. There is increasing evidence that protein complexes are dynamic structures and that their components can undergo various interactions depending on the cellular context and/or the developmental status. The large transmembrane protein Crumbs is required for the establishment and maintenance of apico-basal polarity in Drosophila embryonic epithelia. The short intracellular domain of Crumbs localizes an evolutionary conserved protein scaffold via its interaction with the single PDZ-domain of Stardust. The Crumbs/Stardust/DPATJ complex coexists in Drosophila epithelial cells with another apical protein complex consisting of Bazooka, DmPar-6 and DaPKC. The degree of spatial overlap between components of these two complexes found in the subapical regions of many epithelia is striking and several in silico, qualitative in vitro and in vivo interaction experiments performed with constituents of these complexes have all pointed to a direct interaction between Crumbs and DmPar-6. To investigate and compare the interaction of the intracellular domain of Crumbs with the PDZ domains of Stardust and DmPar-6 we labeled the (putative) interaction partners with fluorescent dyes. This enables us to quantify the respective binding characteristics and complex properties with single molecule and ensemble FRET- and anisotropy- as well as with stopped flow-measurements.