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source adenosine triphosphate (ATP) to carry out this biological process, which is evolutionarily preserved across all organisms. By combining biochemical experiments with structural biology, we seek to understand how the conversion of ATP to ADP controls the conformational changes of an ABC importer, which in turn allows substrates to enter the cell. Since the current mechanism of membrane transport for importers is incomplete, understanding the transport process requires a comprehensive structural analysis of one full length ABC transporter trapped in different conformations along the transport process. This research program has set out to close critical gaps in the understanding of the fundamentals of the transport mechanism in bacterial pathogens. The results will yield insights into how type II transporters utilize ATP hydrolysis to coordinate transport across all organisms, crucial for cell viability.

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Movement of the Nucleotide Binding Domains in the Reconstituted ABC Transporter MsbA During the ATP-Hydrolysis Cycle

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ATP-binding cassette (ABC) transporters move substrates across membranes, including nutrients, toxins, peptides and small inorganic ions. MsbA is a homodimeric bacterial lipid flippase homolog of P-glycoprotein, a transporter involved in multidrug resistance. ABC transporters are formed by two transmembrane domains and two highly conserved nucleotide binding domains (NBDs) that bind and hydrolyze ATP. Crystal structures have shown widely separated NBDs (open conformation; nucleotide-free state) and NBDs forming a dimer with nucleotide trapped at the interface (closed conformation, nucleotide-bound), leading to the proposal of a switch model, where NBDs associate/dissociate during the ATP hydrolysis cycle. Other proposed mechanisms suggest instead that the NBDs are always in contact. Recent studies using Luminescence Resonance Energy Transfer (LRET) in detergent-solubilized MsbA have shown transitions between completely separated and dimeric NBD, in agreement with the switch-model (Cooper & Altenberg, 2013. JBC 287:14994). Here, we used LRET to determine if complete NBD separation also occurs when MsbA is reconstituted in a membrane. Basically, the single cysteine MsbA (T561C) was labeled with LRET donor and acceptor probes, reconstituted in nanodiscs, and the donor-acceptor distance was determined at different steps during the ATP hydrolysis cycle. The reconstituted protein displayed higher ATPase activity than MsbA in detergent. Donor-acceptor distances in nucleotide-free and nucleotide-bound states, as well as during hydrolysis conditions (MgATP), indicated small distance changes, consistent with a partial opening of the NBD dimers. There was no evidence for the longer distance (>50 Å) observed in the open conformation and in detergent. These results show a dramatic effect of the lipid bilayer on the molecular mechanism of MsbA, and suggest that the NBDs remain in close proximity during the hydrolysis cycle under more "physiological" conditions. This work was supported by CPRIT grant RP101073.

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Asymmetry and Conformational Changes of the E. Coli Ribose ABC Transporter

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The ABC transporter superfamily of proteins is diverse, ubiquitous, and related to a number of genetic diseases as well as multidrug resistance in bacteria and cancer. The ribose transport complex may serve as a model for understanding how this clinically relevant protein family functions. An open question in the field is how ATP hydrolysis is linked to transport, and the stoichiometry of this process. Typical ABC transport systems possess two equivalent sites of ATP hydrolysis, while the ribose transporter possesses an intact active site and a degenerate site. To address how transport is fulfilled by asymmetric ATP hydrolysis, different ribose transport complexes associated with sequential stages of the transport cycle were isolated in the presence of different substrates, and these complexes were studied using Electron Paramagnetic Resonance (EPR) spectroscopy to observe how substrate variation affected their formation and nature. The results show ribose disrupts the interaction of the transmembrane domain (TMD), RbsC, with ribose-binding protein, RbsB. Additionally, apo-RbsB interacts with RbsC to yield the outward-facing conformation of the TMD. In turn, the association of RbsB and RbsC disrupts interactions with the nucleotide-binding domain (NBD), RbsA. This suggests a transport model whereby the apo state of the NBD has a weak interaction with the outward-facing TMD, non-canonical behavior for an ABC system. Mg-ATP loaded RbsA then binds the complex, and is required to return RbsC to an inward-facing state. Subsequent ATP hydrolysis destabilizes the interaction between RbsC and RbsB, completing the transport cycle.

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Structural Model of the Human Sodium-Phosphate Cotransporter NaPi-II

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Homeostasis of inorganic phosphate (Pi) in vertebrates is maintained by control of intestinal absorption, storage and release in bones, and renal excretion. These processes depend on tightly regulated expression of Na^+-coupled Pi transporters of the SLC34 solute carrier family (NaPi-II). Their crucial role in Pi homeostasis is underscored by pathologies resulting from naturally occurring SLC34 mutations and SLC34 knock-out animals. SLC34 isoforms have been extensively studied with respect to transport mechanism and structure-function relationships; however, the 3-dimensional structure is unknown. All SLC34 transporters share a duplicated motif comprising a glutamine followed by a stretch of threonine or serine residues, suggesting the presence of structural repeats as found in other transporter families. Nevertheless, standard bioinformatic approaches fail to clearly identify a suitable template for molecular modeling. Here, we used hydrophobicity profiles and hidden Markov Models to first define a structural repeat common to all SLC34 isoforms. Similar approaches identify a relationship with the core regions in a crystal structure of Vibrio cholerae Na^+-dicarboxylate transporter VcINDY, from which we generated a homology model of human NaPi-IIa. The aforementioned SLC34 motifs in each repeat localize to the center of the model, and were predicted to form Na^+ and Pi coordination sites. Functional relevance of key amino acids was confirmed by biochemical and electrophysiological analysis of expressed, mutated transporters. Moreover, the validity of the predicted architecture is corroborated by extensive published structure-function studies. The NaPi-IIa model provides a firm foundation for a molecular understanding of Na^+-coupled Pi uptake by SLC34 transporters.

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Correlating Charge Movements with Local Conformational Changes of a Na-Coupled Cotransporter

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To gain insight into the steady-state and dynamic characteristics of structural rearrangements of an electrogenic secondary-active cotransporter during its transport cycle, two measures of conformational change (presteady-state current relaxations and intensity of fluorescence emitted from reporter fluorophores) were investigated as a function of membrane potential and external substrate. Cysteines were substituted at externally accessible sites in the Na^+-coupled inorganic cotransporter (SLC34A2) and the mutants expressed in Xenopus oocytes. Fluorophore labeling at one site resulted in complete suppression of transport activity, whereas Cys-substitution and labeling at 6 other sites had marginal effect on kinetics. For these 6 mutants, the properties of the presteady-state charge relaxations (mid-point potential, apparent valence) were similar, whereas fluorescence intensity changes (delta-F) differed significantly depending on the labeling site. By using a 5-state kinetic model, we simulated the measured delta-F and determined the contributions from each state as a function of membrane voltage to obtain a unique set of apparent quantum yields for each mutant. At one site, delta-F originated from the fluorophore sensing inward and outward conformations, whereas for the other sites delta-F was associated principally with one or the other orientation. In response to step changes in voltage, the presteady-state current relaxation and the time course of change in fluorescence intensity were described by single exponentials. For one mutant, the time constants matched well with and without external Na^+, providing direct evidence that the fluorophore at this site reported conformational changes accompanying intrinsic charge movement and cation interactions in response to voltage steps. For other mutants, correlations were found only in the presence of Na^+ and $V>0$. Additional evidence for the movement of parts of the protein into a more aqueous environment was obtained using iodide as a collisional quencher.

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The Role of Sodium Sites in LeuT Conformational Changes

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We used LeuT, a bacterial homologue of neurotransmitter:sodium symporter family (NSS transporters), as a model system for studying the role of the Na^+ ions in NSS mediated transport. LeuT has previously been crystalized