to be ligands involved in BetP sodium binding: serine-225 and methionine-229. Both residues are found in the predicted TMD 5. The functional consequences of the S225A mutation are a slight reduction in the apparent affinity for alanine, and a dramatic reduction of the apparent sodium affinity. M229A also shows a reduced apparent affinity for alanine. These results demonstrate there is involvement of these residues in the sodium coordination and/or amino acid interaction with SNAT2. It also shows the involvement of TMD 5, which previously had not been thought to be involved with sodium binding in SNAT2.

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A Structural Model for Prestin and Related SLC26 Anion Transporters

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Mammalian hearing relies on active mechanical amplification of sound in the cochlea, which requires process termed electromotility, i.e. voltage-driven ultrafast length changes of sensory outer hair cells (OHC). The electromotility is generated by prestin (solute carrier transmembrane protein SLC26A5), an anion transporter-related, OHC-specific membrane protein. Electromechanical activity of prestin results from conformational changes that are directly triggered by changes in membrane potential and involve interaction with anions. These conformational changes are tightly coupled to substantial intramembraneous charge movement providing prestin with a unique electrical signature that can be measured as a non-linear membrane capacitance (NLC). The molecular mechanisms underlying prestin's electromotile function and anion translocation by SLC26 transporters are essentially unknown. While direct structural information on this protein family is sill lacking, different protein structures were previously suggested based on bioinformatical and mutagenesis studies. We addressed this issue by performing an extensive topological study by substituted cysteine accessibility method (SCAM). Our results indicate a structure different from those proposed earlier. Moreover, by homology modeling we succeed to establish a structural model that is in agreement with extensive experimental data and provide general insights into the molecular mechanisms of SLC26 transporters.

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Role of Mitochondrial NCX on CXCL12-Induced Chemotaxis in A20 B Lymphocytes

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Lymphocyte chemotaxis plays important roles in immunological reactions. It has been reported that mitochondria redistribute at the uropod during T lymphocyte migration triggered by a chemokine ligand (CXCL12). However, how mitochondria are involved in lymphocyte chemotaxis has been unclear. We studied roles of mitochondria Ca^{2+} handling proteins, Na-Ca exchange (NCXm) and Ca^{2+} uniporter, on CXCL12-induced chemotaxis in A20 B lymphocytes. CXCL12 (100 ng/ml) increased transwell migration of A20 cells from 4.6 $\pm$ 0.5 % (non-stimulated cells) to 12.6 $\pm$ 0.6 % ($P < 0.05$). This increase was dose-dependently inhibited by CGP-37157 (an inhibitor of NCXm), but not affected by Ru360 (an inhibitor of mitochondrial Ca^{2+} uniporter). Knock-down of a gene of NCXm (NCLX) by siRNA reduced NCLX protein expression to 52 $\pm$ 9 % and inhibited transwell migration similarly to CGP-37157. In the 8 hrs observation of cell migration under microscope, mean displacement of NCLX siRNA cells without CXCL12 was larger (21.4 $\pm$ 1.5 μm) than control siRNA cells (13.5 $\pm$ 2.2 μm, $P < 0.05$), and applying CXCL12 did not increase mean displacement and percentage of directional migration in NCLX siRNA cells. Intracellular Ca^{2+} measured by fura-2 was higher in NCLX siRNA cells (fura-2 ratio 0.49 $\pm$ 0.01) than the control siRNA cells (0.45 $\pm$ 0.01, $P < 0.05$) in the absence of CXCL12. After 2 hrs CXCL12 stimulation, the intracellular Ca^{2+} increased in control siRNA but not in NCLX siRNA cells. Treating A20 cells with BAPTA-AM (an intracellular Ca^{2+} chelator) suppressed the cell migration. Our results suggest that NCLX-mediated Ca^{2+} signaling is associated with CXCL12-induced chemotaxis in B lymphocytes.

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Functional Characterization of the KtrAB Potassium Transport System

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The Ktr transport system belongs to the large superfamily of Trk/Ktr/HKT ion transporters that share structural similarities with potassium and sodium channels. The KtrAB complex is composed by a dimer of the membrane-integrant KtrB and an octameric ring of the cytosolic KtrA protein, which is an RCK-domain. Each KtrB subunit has a K^+ selective pore. We tested several nucleotides for eluting KtrA from a high-affinity resin and found that ADP and ATP are the most potent suggesting higher affinities towards the protein. The crystal structure of the complex, recently solved by our group, revealed that the ring conformation of KtrA changes upon nucleotide binding. To determine the functional effects of these nucleotides we reconstituted the KtrAB complex from B. subtilis into liposomes and measured the transport activity reflected by uptake of 86Rb+. Our results show that ATP activates the transporter, while the flux mediated by KtrAB-ADP is similar to the flux of KtrB reconstituted without KtrA. Increasing amounts of extraliposomal K^+ or Na^+ showed that Na^+ permeates through the transporter, yet KtrAB favors K^+ over Na^+. In addition, the cytosolic ring regulates the activity of the transmembrane pore, however, it does not render selectivity towards ions as there are no selectivity differences between the KtrAB complex, either with ADP or ATP, and KtrB alone. In conclusion, our data shows that KtrA regulates the activity of KtrB and raises new ideas about the mechanism of activation by RCK domains in the superfamily. Current investigation attempts to characterize the molecular basis of activation.

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Electrophysiological Investigation of the Na^+/H^+ Antiporter NHAA from *E. Coli.*

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NhaA from E. coli serves as a prototype for Na^+/H^+ antiporters. It was proposed that the pH dependent change of the transport activity is due to the transition of the transporter from an inactive to an active conformation, which is allosterically controlled by a pH sensor.

NhaA is electrogenic. Therefore it is possible to perform electrophysiological measurements with NhaA using the solid supported membrane based electrophysiology (Mager et al., 2011). The SSM based electrophysiology permits measurements with a high time resolution and defined conditions on both sides of the membrane.

The kinetic analysis of the SSM measurements shows that the decrease of the transport activity at suboptimal pH values is due to the competitive binding of the substrates to a common binding site (Mager, et al., 2011). Based on this finding we employed a minimal kinetic model. It explains the substrate dependence of the transport activity over a large concentration range.

The changed pH dependencies of the mutants V254C NhaA and H225R NhaA were explained with the impairment of the allosteric mechanism of inactivation (Padan, 2008). However the phenomenological alterations of the transport properties can be explained by our minimal kinetic model, which does not include an allosteric mechanism of pH regulation. The altered transport properties are the result of a change of the substrate binding affinities or the rates of the conformational transitions.

1. Padan, E., Trends Biochem Sci 33, 435-443 (2008).
2. Mager, T. et al., J Biol Chem 286, 23570-23581 (2011).

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Urea and Water Permeation across the Human Red Blood Cell Membrane. New Insights into Transport Mechanisms

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The human red blood cell (hRBC) has an exceptionally high permeability for water (Pf) and urea (Purea). However, the contribution of several blood group proteins to permeability of both molecules is still a matter of debate. Here, we have taken advantage of 3 extremely rare blood group phenotypes Colton-null, Kidd-null and Gil-null characterized by total deficiency of aquaporin-1 (AQP1), urea transporter (UT-B) and aquaporin-3 (AQP3), respectively. To evaluate the possible contribution of each protein to water and urea permeation, these hRBC were analyzed using a stopped-flow spectrometer. Functional studies showed that AQP1 and UT-B proteins substantially contribute (96%) to the high water permeability of the hRBC membrane. Furthermore, molecular dynamics simulations (MDS) of water diffusion through urea transporter reveal a permeation mechanism different from that observed in AQP1. Interestingly, uptake studies using 14C-urea indicated that the rate of urea transport through UT-B pore was reduced in the absence of AQP1 (Colton-null) and can be modulated by changing the osmolarity conditions. Overall, these data provide evidence that the urea transport through UT-B was controlled by the movement of water molecules into this channel.