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Editorial

Transporters in *Channels*

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What are transporters doing in *Channels*? Simply put, the traditional distinction between channels and transporters are blurred. The two fields come together not only in technical approaches to transporter research, in which biophysical tools ordinarily associated with channels are being exploited, but also in conceptual terms where theories and mechanisms meet. One contrast may well be in the structure of these membrane proteins, channels living up to their name and transporters more elusive with regard to pathways through their structure. Yet, virtually all transporters contain ion channels in the functionally sense, from classical ATPases, such as the Na pump, to Na-dependent glutamate transporters capable of acting as bone fide Cl^- channels. But where transporter channels reside in the protein, what roles they play in normal function and disease, and whether they can be exploited in drug therapies, just as classical channels are, remain open questions. The more we learn about co-transporters, the topic of the present issue of *Channels*, the closer we come to understanding similarities and differences between channels and transporters and finding ways to use them.[1]

Co-transporters (symporters and antiporters) do not use ATP directly; rather they utilize electrochemical energy stored in Na^+, H^+, or other ion gradients established by ATP. Similarly, exchangers use the stored energy of one exchanged ion to drive the other, as in Na^+/Ca^{2+} exchange. Co-transporters employ electrochemical gradients to transport neurotransmitters (monoamines, amino acids) or other solutes against their gradient. Transport of co-ions and solutes is most often described in terms of an alternating access model. In one form of the alternating access model, solute and co-ions bind to the transporter in its outward facing conformation, an extracellular gate closes preventing back flow, an intracellular gate opens allowing solute and co-ion to enter the cytoplasm, and the empty transporter returns to the outward conformation. This widely accepted mechanism was proposed decades ago albeit in a different context.[2] The alternating access hypothesis was buoyed by the first crystal structure for any transporter by Kaback and colleagues,[3] a plethora of biochemical data,[4] and high-resolution structures by Gouaux and others on Na^+-coupled transporters.[5,6] In 2005, Yamashita et al. published the first crystal structure (PDB ID 2A65) of a bacterial homolog of Na^+-Cl^--dependent neurotransmitter transporters (LeuT) from the SLC6 family. Although not itself Cl^--dependent, the crystal structure of LeuT provided detailed insights into the structure, organization of the solute and co-ion binding pockets, and candidate helices involved in conformational changes in transporters associated with gating. Similar to other transporters in this family, LeuT is composed of 12 trans-membrane (TM) helical regions, TM1 to TM12. The TM1 and TM6 helices are highly conserved throughout the family and form most of the LeuT core. In just three years following the publication of LeuT's structure, the field has been infused with structural information from bacterial homologues in the NSS and EAAT families, suggesting experiments that point toward mechanistic explanations of co-transport.

In spite of these advances in structure and function, a parallel set of data exists that must be included for a complete picture of co-transporters: transporters have channels. This issue of Channels focuses on structural methodologies and what they tell us, as well as purely functional approaches that will demand, eventually, structural explanations. Two standard ion channel techniques employing fluorescent dyes have become powerful tools in transporter research, and electrophysiology, ammperometry, and microfluorometry[7] have helped uncover the full implications of membrane transport. Electrophysiology in particular has uncovered ion channels in co-transporters, which carry larger than expected currents during the transport process and can exhibit authentic open-closed channel properties in neurons.[8,9] This discovery, discussed in several articles in this issue, raises two fundamental questions: what possible roles do transporter channels play in cell physiology, and what structural features support the presence of channels in transporters.

Singh explores the mechanism of ion-coupled secondary transport from the broad perspective of the crystal structure of LeuT, pointing to several topics relevant to transport mechanism, inhibition, and regulation that structure/function studies of LeuT. Furthermore, Singh surveys limitations of the existing structure from a bacterial homologue for understanding its mammalian counterparts, suggesting the need for a eukaryotic transporter structure. One of the most intriguing outcomes revealed by X-ray crystallography is the existence of a completely dehydrated occluded state and its relationship to the alternating access hypothesis described above. In addition to "open-to-out" and "open-to-in" conformations, one may have to account for a thermodynamically stable occluded state, where both gates are closed and solutes and ions are locked inside. The existence of an occluded state in other members of SLC-6 family remains debatable, and it will be interesting to see whether such occluded states exist in other transporters.

Elvington and Maduke probe the fundamental relationship between protein structure and protein dynamics. They review

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comprehensively biochemical, spectroscopic, and computational methods for examining conformational changes of transporters, and focus on techniques to examine smaller, subtler movements that may be essential to transporter function.

Bette et al. investigate the Cl^- dependence of the Na^+- and K^+-dependent amino acid transporters KAAT1 and CAATCH1 by expressing them in Xenopus laevis oocytes and measuring amino acid uptake. These insect members of the SCL6 family are only weakly Cl^- dependent, and putative residues for Cl^- binding suggest that intrinsic negative charges can be partially supplied by glutamate 338 in KAAT1 and CAATCH1. Negative charge due to non-conserved aspartate in position 338 presented in both transporters helps stabilize cation binding, theoretically diminishing the dependence on Cl^- ions.

Diallinas and Gournas focus on purine and purine/pyrimidine carriers from bacteria, fungi, and protozoa as well as plants and mammals. The nucleobase transporters include the prokaryotic proton symporters, but the NAT/NCS2 family also includes the mammalian L-ascorbate/Na^+ transporters. The SLC2A9 transporter gene, presumable coding for a fructose transporter but expressed in *Xenopus laevis* oocytes, facilitates uric acid transport. Sequence variants of SLC2A9 are associated with hyperuricemia, gout, cardiovascular disease and diabetes. High affinity uric acid transport is apparently a recent gain-of-function modification, and perhaps the structure of ascorbic acid led to evolutionary pressure for structural changes in primate NATs. Because uric acid and ascorbate have similar antioxidant roles, this suggests that ascorbate transporters evolved from uric acid permeases as an adaptation of antioxidant strategies.

Andrini et al. provide a detailed review of uncoupled currents and permeation through different members of SLC34 family Na^+-coupled inorganic phosphate transporters. Uncoupled currents depart from the fixed stoichiometry implied by the alternating access model. Uncoupled currents can be observed in the presence of solute (solute-induced currents), but also in the absence of solute (so-called leak currents). Transport proteins can exhibit either or both modes of uncoupled currents. The structural mechanism and physiological relevance of this phenomenon remains unknown, but the existence itself warrants a revision of the canonical model. Andrini et al. explain that electrogenic (NaPi-IIa/b) or electroneutral (NaPi-IIc) inorganic phosphate (Pi) transporters have different transport stoichiometry. When NaPi-IIa or NaPi-IIb are expressed in *Xenopus* oocytes, the specific transport inhibitor phosphonoformic acid (PFA) in the absence of Pi, blocks an inherent leak current. They proposed a uniport mode of transport based on activation energies; however, removal of external Cl^- ions alters the leak reversal potential, implying that the leak current is more complex. After modification by methanethiosulfonate reagents, only the leak mode operates, supporting a kinetic scheme in which the leak and co-transport modes are mutually exclusive.

Bonar and Casey survey the anion exchangers of the SLC4a (sodium-independent) and SLC26a (sodium-dependent) gene families, which are important for bicarbonate transport and pH regulation. Mutations in several of these genes are associated with human disease (e.g., hereditary endothelial dystrophy, Pendred syndrome) and SL26a6 is thought to be involved in cystic fibrosis through a direct interaction with the CFTR protein. In the absence of available crystal structures little is known with certainty about the structure of these anion exchangers, and in many cases even the number of TM's is unclear and under current scrutiny. Furthermore, Bonar and Casey review the uncertainty/controversy regarding substrate specificity and transport stoichiometry of many of the SLC26a transporters. In the context of the above discussion on uncoupled leak currents and uniporter transport it is noteworthy that SLC26a7 has been reported to be a pH-regulated Cl^- channel.

The next two contributions as well as a review published a few issues back concern sodium-dependent counter-transporters, the SLC9a Na/H exchangers (NHE), the SLC8a Na/Ca exchangers (NCX) and SLC24a Na/Ca-K exchangers (NCKX). On page 329 of this issue, Kemp et al. start with a brief overview of the tissue distribution and physiology of NHE1-9. Most NHE proteins are thought to be plasma membrane proteins and regulate cytosolic pH, except for NHE7-9 which are less well studied and located in intracellular compartments presumably regulating their pH. The major part of their review is devoted to an in depth discussion of our current knowledge of NHE1 topology and structure. Starting with hydropathy analysis which suggests that NHE1 is a polytopic membrane protein containing 12 TM's, Kemp et al. next describe the first experimentally tested model of NEH1 topology based on accessibility of inserted cysteine residues (cysteine scanning mutagenesis) and compare this model with a new model based on bioinformatics and fold alignment algorithms, made possible when the crystal structure of the *E. coli* Na/H exchanger NhaA was published in 2005. NhaA and NHE1 only share 10% sequence identity and have significantly different functional properties. The detailed discussion presented here illustrates some of the experimental and theoretical strategies used and the challenges and complexities that accompany structural studies of most human transporters which are typically polytopic membrane proteins with 10–12 TM's.

Reeves and Condrescu review ionic regulation of the cardiac NCX1 Na/Ca exchanger, allosteric regulation by cytosolic Ca^{2+} and Na^+-dependent inactivation. In terms of regulation of transport, NCX1 is probably the best studied and understood transporter, in large part due the ability to measure NCX-mediated currents in inside-out giant patches, a method developed by Hilgemann.[10] More recently, structural insight is gained in domains responsible for Ca^{2+} regulation when both NMR and crystal structures became available of parts of the large cytosolic loop of NCX1 that impart this allosteric regulation by cytosolic Ca^{2+}. The occurrence of an inactivated state of NCX1 may be taken as a further illustration of the similarities between channels and transporters; moreover, it adds to the complexity of the canonical alternate access model of transporters as discussed above. Reeves and Condrescu lead us through what is learned from these structures and conclude with a brief discussion of physiological implications and the potential therapeutic use of NCX inhibitors.

In an earlier issue of Channels. Altimimi and Schnetkamp[11] discussed the SLC24a family of Na/Ca-K exchangers, members of which use both the inward Na^+ gradient and outward K^+ gradient for Ca^{2+} extrusion. NCKX proteins, first identified in the outer segments of vertebrate retinal rod photoreceptors, share with NCX exquisite ion selectivity for Na^+ as no other alkali cation (nor protons) can substitute for Na^+ in mediating counter transport of Ca^{2+}. Although the SLC8 and SLC24 are clearly related, surprisingly, very

little sequence identity exists between NCX and NCKX proteins. Apart from their role in visual transduction in rod (NCKX1) and cone (NCKX2) photoreceptors, NCXK2 is distributed throughout the brain, while NCXK3 and four transcripts have a relatively wide distribution but their physiology remains to be established. An unusual and interesting role was recently described for NCKX5. NCKX5 has been reported to be located in intracellular membranes rather than the plasma membrane, as is the case for NCKX1-4, and is critically important in melanin synthesis and/or melanogenesis in melanocytes and in the retinal pigment epithelium.[12,13] Furthermore, a non-synonymous SNP in the NCKX5 gene is found on both alleles of people from European descent and strongly associates with light skin. Thus, SLC24a5 is a key gene in the natural variation in skin pigmentation found in human populations.[14,15]

Like channels, many transporters are important for pathophysiology and therefore important targets for drug therapy. Like channels, transporters are most often low abundance proteins and screening transporters for candidate drugs is challenging due to the absence of a common and well established methodology, like patch clamp electrophysiology in the case of ion channels. As a result, different methodologies have been developed to study transporters, and Weinglass et al. present an overview of the progress that has been made towards high throughput screening of many different transporters using a variety of analytical methodologies including electrophysiology, radio-isotopes, atomic absorption spectrophotometry, and, of course, membrane potential-sensitive and ion-indicating fluorescent probes.

What are transporters doing in *Channels*? Read on.

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