The twain shall meet: channels, transporters and things between

Meeting on Membrane Transport in Flux: The Ambiguous Interface Between Channels and Pumps

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The Royal Society discussion meeting on 'Membrane transport in flux: the ambiguous interface between channels and pumps' took place on 19 and 20 May 2008 in London, UK, and was organized by F. Ashcroft, C. Miller and D. Gadsby.

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Introduction

For many years, studies of ion channels and transport proteins have bred distinct fields with divergent approaches, mindsets and methods. 'Ion channelologists' focused on gating and permeation, and mumbled about obscure kinetic schemes, whereas 'transporterologists' studied substrate coupling and transport kinetics, and mumbled about other obscure kinetic schemes. Practitioners of each field generally

held their counterparts in low regard, given the obvious superiority and importance of their own proteins: channel fans despised transporters as deplorably slow 'draught horses', whereas transporter enthusiasts scoffed at channels for being simple holes that merely squander the meticulous work accomplished by pump molecules. In recent years, however, this has all begun to change: transporters and channels are converging mechanistically as members of both groups are found to share fundamental characteristics, even to the point that several molecular families of 'mixed descent' have been discovered. In recognition of this convergence, the Royal Society recently sponsored a discussion meeting in London, UK, to explore the increasingly blurry distinction between these classes of membrane protein, and to examine some molecules that occupy the boundary between channels and transporters. This meeting provided an unusual opportunity to explore in depth what makes a channel distinct from a transporter and, perhaps more importantly, what fundamental mechanistic features they share. It is to be hoped that these two groups of scientists will continue to find common ground and will now start to mumble to each other about obscure kinetic schemes.

Here, we focus on a subset of talks that addressed the core issues of the conference: the detailed analysis of channel and transporter mechanisms. This unabashedly biased approach, by necessity, omits excellent presentations, but allows us to focus on a series of unifying themes.

Peeking into transporter open and occluded states

Several of the speakers recalled that the basic structural requirements for achieving uphill substrate transport were predicted over 40 years ago, long before the structural era. In a paper published in 1966, Oleg Jardetzky predicted that a transporter is a protein that cycles between an 'outward facing' state in which the substrate binding sites are accessible to the external medium and an 'inward facing' conformation that is open towards the cytoplasm (Jardetzky, 1966). The idea of an intermediate 'occluded state' in which the substrates are buried in the interior of the protein, and are cut off from access to either side of the membrane, was also

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postulated and demonstrated experimentally for some transporters early on (Beauge *et al*, 1979; Post *et al*, 1972). This structural design implies the presence of two gates that operate in a strictly coordinated fashion, such that they are never open at the same time (Fig 1A). Ion channels, by contrast, might be viewed as transporters with a single gate or with multiple gates that can be open simultaneously. In an ion channel, the opening of one or more gates exposes a membrane-spanning pore through the interior of the protein, which allows ions to rush down their electrochemical gradients (Fig 1C).

Because protein conformational changes occur on the millisecond timescale and each conformational cycle of a transporter results in the translocation of one or a few substrates, transport rates through pumps are typically in the order of 1×10^2 – 1×10^4 substrates s^{-1} , corresponding to the timescale of the gating process. By contrast, because equivalent ‘conformational cycles’ in channels open their aqueous pores, ions flow down through open channels at diffusion-limited rates of $\sim 1 \times 10^8$ ions s^{-1} (Morais-Cabral *et al*, 2001), which are up to five orders of magnitude faster than transporter rates. This difference sets a minimum standard for the degree of perfection a pump must attain to qualify as an uphill transporter. As pointed out by D. Gadsby (New York, NY, USA), both gates of an uphill transporter being simultaneously open for as little as 1×10^{-5} of the time would suffice to dissipate all the uphill work performed in between. When thinking about the nature of this coordination, the idea of an occluded state becomes central. This state represents a moment when both inner and outer gates are closed, which acts as a temporal buffer protecting against the ‘noxious’ simultaneously open state.

Transporter mechanisms at the current resolution

What is the scale of the transporter protein movements required to adopt inward-facing, occluded and outward-facing conformations? In his original cartoon, Jardetzky hypothesized substantial rearrangements with wide aqueous vestibules opening up towards alternating sides of the membrane, although he recognized that such rearrangements could reflect relatively small protein movements. Today, a rapidly growing structural database allows us to gradually replace cartoons with atomic-level molecular structures, and three different types of solution for implementing alternating access in transport proteins were presented at the meeting.

P-type ATPases—including the Na^+/K^+ ATPase and various Ca^{2+} and H^+ ATPases—consume about one-third of our total energy, while working to set up transmembrane ionic gradients that are essential to life. P. Nissen (Aarhus, Denmark) presented a series of structures for different conformations of the P-type Ca^{2+} -ATPase (Fig 2; Olesen *et al*, 2007). These, in combination with several more that have been solved over the past few years in the Toyoshima laboratory, provide detailed three-dimensional molecular portraits of this pump in its inward-facing and outward-facing conformations, as well as in two different occluded states that harbour either two Ca^{2+} ions or two counter-transported H^+ ions. This sequence of snapshots of various states of the Ca^{2+} ATPase revealed large domain movements associated with transport. Interactions between the three subdomains (N-domain, A-domain and P-domain) of the extramembranous motor domain are markedly reshaped during each step of the enzymatic cycle, and these movements are translated into transmembrane rearrangements through the direct anchoring of the A-domain to transmembrane helices 1–4. Interpolation of the

Fig 1 | Schematic transporter and channel mechanisms. (A) Generic transporter mechanism, with gates that alternatively open to one side of the membrane or the other (far left and right). Occluded states are shown in the centre, with both gates simultaneously closed. (B) Mechanisms of transformation. Miller (left) and Gadsby (right) transformed transporters into channels using manipulations that allow both gates to be open simultaneously (mutagenesis or palytoxin). Note that for CLC-ec1 (left), the broken transporter only transports one of its substrates (Cl^-), whereas the toxin-treated ATPase (right) passes both substrates (Na^+ and K^+). (C) Generic channel mechanism. One difference from the transporter mechanism is that a channel is open when all possible gates are open, which is a state that is not tolerated in a working transporter. CLC-ec1, chloride channel *Escherichia coli* 1.

various structures resulted in a ‘real-time’ molecular movie, which would have dumbfounded any audience before the *Star Wars* age (a similar one can be seen in Supplementary Movie 1 online). Thus, the audience could follow with their own eyes as the N-domain and the P-domain of the Ca^{2+} -occluded ATPase, intimately coupled through the bound ATP, disengaged on ATP hydrolysis. This, in turn, makes room for the A-domain to swing into close contact with the newly formed phosphoaspartate in the P-domain, while exerting a large torque on transmembrane helices 1–4. Forced in this way, helices 1–4 separate from helices 6–10 to open a wide vestibule towards the ‘external’ side, allowing the release of two Ca^{2+} ions into the lumen of the sarcoplasmic reticulum.

ATP-binding cassette (ABC) ATPases couple ATP hydrolysis at two conserved nucleotide-binding domains (NBDs) to either the export or the import of a wide range of hydrophilic or hydrophobic substrates; members of this family are of great medical interest, in part because they mediate resistance to chemotherapeutics in human cancers. Although we do not have a complete understanding of the conformational cycles of these transporters, glimpses of full-length ABC protein structures presented by K. Locher (Zurich, Switzerland)

Fig 2 | Selected states from the Ca^{2+} -ATPase transport cycle. Structures of four states from the cycle are shown, with the schematic mechanism in the centre indicating states with occluded Ca^{2+} ($\text{Ca}_2\text{E1}\sim\text{P-ADP}$) or H^+ ($\text{H}_{2-3}\text{E2-P}^*\text{:ATP}$), and states allowing access of Ca^{2+} from the cytoplasm (E2/E1:ATP) or the sarcoplasmic reticulum (E2-P). Red arrows indicate the access pathways for Ca^{2+} ions. A-domain, yellow; N-domain, red; P-domain, blue. Adapted from Olesen *et al*, 2007, with permission from the author and Macmillan Publishers Ltd © 2007.

strongly suggest that similarly large protein movements alternately expose ABC transporter–translocation pathways to either side of the membrane. Locher compared the structures of a molybdate importer, ModB_2C_2 (Hollenstein *et al*, 2008), which was solved in a nucleotide-free state, with that of a multidrug exporter, Sav1866, in its ATP-bound state. In the nucleotide-free ModB_2C_2 structure, the two NBDs are separated from each other and the transmembrane domains adopt a clear inward-facing conformation. By contrast, the Sav1866 structure (Dawson & Locher, 2006) displays the canonical tight NBD dimer together with an outward-facing conformation of the transmembrane domain. Despite the different structural organization of the transmembrane domains in these two proteins, a pair of structurally conserved α -helices (the ‘coupling helices’) formed

by two cytoplasmic loops seems to have a pivotal role in coupling movements in the motor domain to movements in the pore domain that lead to substrate translocation.

A common thread connecting the P-type transporters and the ABC transporters is their use of ATP hydrolysis to drive transport. These two transporter types share a common structural feature in that the transmembrane substrate–translocation pathway and the ‘motor domain’, which burns the fuel ATP, are physically separated. The cytosolic motor domain is formed from three subdomains (N-domain, A-domain and P-domain) in P-type ATPases and from two homologous NBDs in ABC proteins. In both cases, hydrolysis of an ATP sandwiched as molecular glue between two domains leads to the dissociation of interdomain interactions, which in turn

triggers the conformational changes of the transmembrane region that are responsible for substrate pumping—a direct conversion of chemical free energy into mechanical work.

Smaller movements are implied by the structures of two bacterial Na⁺-coupled amino-acid transporters, LeuT (Yamashita *et al*, 2005) and Glt_{ph} (Yernool *et al*, 2004), as discussed by E. Gouaux (Portland, OR, USA). These proteins are close homologues of mammalian transporters that remove neurotransmitters from the synaptic cleft, with LeuT as a model for the NaCl-coupled transporters for dopamine, serotonin and γ -aminobutyric acid, and Glt_{ph} as a model for the excitatory amino-acid transporter (EAAT) family of glutamate transporters. The structures of LeuT point to a mechanism for alternating access in which parts of a pair of closely associated transmembrane α -helices rotate around a central 'joint' formed by a short unwound region, which is the binding site for transported substrates. In several structures, bound substrate is present in an occluded state, blocked from access to aqueous solution on either side of the membrane. Gouaux speculated that the external and internal gates might be provided by the external and internal halves, respectively, of this helix pair. A 'membrane normal' position would correspond to a closed gate, whereas a tilt relative to this axis would open the central substrate-binding site towards the respective membrane surface. Gouaux suggested that movements on a similar scale might be associated with transport by the glutamate transporters, as represented by Glt_{ph}.

By contrast, some transporters seem to make do with smaller protein movements. C. Miller (Waltham, MA, USA) noted that conformational changes in CLC antiporters might be relatively subtle, with individual amino-acid side chains swinging to and fro, into and out of the ion pathway. Can one raise the bid further? Is it possible to implement strictly coupled transport with even less protein movement? A natural reaction would be to look for a pump with a tiny substrate. It comes as a surprise, then, that A. Finkelstein (New York, NY, USA) won this contest with his presentation on the translocation machinery of the 90 kDa subunit of anthrax toxin protein, known as 'lethal factor'. The 'transporter' in this case is a β -barrel, which is a transmembrane projection of a heptamer formed from the 'protective antigen' of the toxin (Nassi *et al*, 2002). It is through this β -barrel that the lethal factor protein, accompanied by six hydrogen ions, is released from acidic endocytic vesicles into the cytoplasm of the cell (Zhang *et al*, 2004). One would be tempted to invoke the famous drawing by Saint-Exupéry in *The Little Prince* of the snake gobbling up the elephant—but *that* snake had to undergo a non-trivial conformational change to accommodate its prey (Fig 3). Quite by contrast, the β -barrel snake of the protective antigen of the toxin watches with a motionless face while the elephant—in this case, lethal factor—humbly unfolds into a worm that eventually threads itself all the way through the pore, driven by a combination of pH gradient and membrane voltage (Fig 3). All of this occurs with no evident conformational change in the channel protein itself.

The channel-transporter twilight zone

Consideration of these emerging details of transporter mechanisms begins to bring into focus the 'ambiguous interface' between these proteins and ion channels. Where a transporter shows alternating access, perhaps with an occluded state, a channel must fully open to establish a continuous aqueous pore, ruling out an occluded state as a transport intermediate. Therefore, it might be possible

Fig 3 | Mechanism of anthrax toxin transport. The snake in *The Little Prince* underwent a notable conformational change in order to swallow an elephant. By contrast, in the translocation of lethal factor (LF) by PA₆₃, the swallowed protein undergoes a large conformational change, unfolding to thread its way through the PA₆₃ channel, driven by a combination of proton and voltage gradients.

to understand these mechanisms further by probing the nature of the occluded state. Because it contains both channels and transporters within the same small protein lineage, the family of CLC Cl⁻-conducting channels and transporters provides an ideal setting for such explorations. Miller reported on his attempts to turn a CLC transporter into a channel. In X-ray structures of CLC-ec1, which is a homologue from *Escherichia coli*, the transporter is in an occluded state, with bound Cl⁻ blocked from accessing either side of the membrane by three amino-acid side chains that literally occlude its path (Dutzler *et al*, 2002). Miller showed that with simultaneous mutations of two of these residues, which have been implicated as components of the internal and external gates, the transporter can dramatically increase its turnover. The rates of the doubly mutated transporters are faster than those of any known transporter, but are still too slow for single-channel recordings—in fact, Miller constructed a 'chansporter'. These studies highlight

the broad boundary between channels and transporters as such intermediate rates do not allow conclusive assignment of a mechanism. Miller argued, however, that these alterations make CIC-ec1 into a channel because they establish a continuous aqueous pathway through the protein, and because changing the size of the side chain at both of these positions affects the throughput, which is an outcome not expected for a transporter. Miller essentially ablated the occluded state in CIC-ec1 by mutagenically opening both gates (Fig 1B, left). Gadsby also converted a transporter (Na^+/K^+ ATPase) to a channel, this time by the application of a marine toxin; this toxin seems to decouple the inner and outer gates of the ATPase, again eliminating the occluded state of the protein and allowing it to act as a channel, conducting its natural substrates Na^+ and K^+ (Fig 1B, right). This conversion allowed Gadsby to use methods of channel biophysics to map the access pathways of ions into their binding sites deep inside the ATPase (Reyes & Gadsby, 2006).

In addition to these 'crowbars', which were used to pry open transporters in order to morph them into channels, more subtle traces of channel and/or transporter identity crisis were on display. M. Kavanaugh (Missoula, MN, USA) discussed the members of the EAAT family of glutamate transporters. In these proteins, substrate transport is coupled to that of Na^+ , H^+ and K^+ . However, Na^+ and glutamate can also activate a thermodynamically uncoupled Cl^- permeability through the EAAT proteins (Zerangue & Kavanaugh, 1996). Although it is difficult to understand mechanistically, this Cl^- movement has many of the features that are expected of an ion channel, as it allows the anion to move down its electrochemical gradient without coupling to the other transported ions.

Similarly, traces of transporters were also found in several ion channels. M. Maduke (Stanford, CA, USA) presented elegant data that reveal a vestigial transport function in the CLC-type ion channel, CLC-0. Each time the channel cycles among gating states, it transports an H^+ across the membrane. This loose coupling (1 H^+ per $\sim 1 \times 10^8$ Cl^- ions) is the evolutionary relic of the strict 1 H^+ per 2 Cl^- stoichiometry observed in the sibling CLC antiporters. P. Vergani (London, UK) discussed vestigial transporter behaviour in the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel. This descendent of ABC transporters couples the gating of its pore to the same irreversible cycle of ATP hydrolysis that drives substrate transport in its cousins (Vergani *et al*, 2005). For a different form of channel–transporter association, F. Ashcroft (Oxford, UK) discussed disease-causing mutations of the K_{ATP} channel, which is a complex of a traditional ion channel, Kir 6.2, with a member of the ABC transporter family, sulphonylurea receptor (SUR). Although without a known transport function itself, SUR confers several key ABC-transporter-like properties on the complex, including drug (sulphonylurea) binding and regulation by magnesium nucleotides. Finally, the Finkelstein anthrax toxin channel transports a protein across the membrane with all of its acidic groups protonated, taking six protons for each protein transported. Therefore, this peptide-transporting 'hole' is an authentic protein–proton symporter (Krantz *et al*, 2006).

Specificity and coupling in co-transporters

One class of uphill transporters achieves its goal through coupled downhill transport of a second type of substrate. Binding and transport of the two preferred substrates frequently show positive cooperativity, with increasing concentrations of either substrate

increasing the affinity of the transporter for the other (for example, see Hoffmann *et al*, 1976). How is this cooperativity achieved on a molecular level? Although there are numerous examples of proteins that achieve positive cooperativity through classical allosteric mechanisms involving long-range communication between separated substrate binding sites, several talks at the meeting revealed a much simpler way in which this can be brought about. The structures of the two bacterial Na^+ -coupled amino-acid transporters LeuT and Glt_{ph}, discussed by Gouaux, provided clear pictures at a molecular level of both types of substrate occluded at the same time in the interior of the protein. Although unrelated in sequence, the structural organization of the Na^+ binding sites has similar features in both proteins: the occluded dehydrated Na^+ ions are coordinated by main-chain carbonyl oxygen atoms of broken α -helices, which are partly unwound to allow interaction of backbone carbonyl groups with the bound ions. In the LeuT structure, co-crystallized with its substrates leucine and Na^+ , one of the two transported Na^+ ions is coordinated by the negatively charged carboxylate group of co-transported leucine. Therefore, Na^+ senses the presence of leucine, because each substrate forms part of the binding site of the other. This is, indeed, a simple way to produce thermodynamic coupling, but how is the necessary specificity achieved? In the case of LeuT K^+ , the most obvious competitor of the substrate Na^+ is simply too big to fit into the binding site. As pointed out by Gouaux, "the Na^+ ions are like the last piece in a jigsaw puzzle—trying to force in a piece which is too big will disrupt the entire structure." Although LeuT is a bacterial protein, a potentially similar mechanism operates in its mammalian homologues, which remove neurotransmitters from the synaptic cleft, such as the glutamate transporter EAAT3 studied by Kavanaugh. A non-related mammalian peptide transporter introduced by R. Boyd (Oxford, UK), PepT1, absorbs dipeptides and tripeptides from the gut. Remarkably, the co-transported single H^+ directly protonates the free carboxylate of the transported peptide—yet another example of direct involvement by the two substrates in forming the binding site of the other.

Summary

Our understanding of transporter mechanisms has recently leapt forward, stimulated in large part by progress made in determining the structures of transport proteins and by the mechanistic insights gleaned from these structures. As we progress, we become more aware of the common threads that tie together all membrane transport mechanisms, including both channels and transporters. This meeting provided an opportunity to explore the overlap between channel and transporter mechanisms in detail; it turns out that probing this boundary can yield valuable insights into both. With luck, this new common ground will produce not only mumblings about obscure kinetics schemes, but also real continued progress in understanding membrane transport in general.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>)

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REFERENCES

- Beauge LA, Cook RH, Glynn IM, Smith W (1979) A rapid ion-exchange technique used to detect the occlusion of ions within the sodium pump. *J Physiol* **295**: 4P
- Dawson RJP, Locher KP (2006) Structure of a bacterial multidrug ABC transporter. *Nature* **443**: 180–185
- Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R (2002) X-ray structure of a CIC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity. *Nature* **415**: 287–294
- Hoffmann N, Thees M, Kinne R (1976) Phosphate transport by isolated renal brush border vesicles. *Pflügers Arch* **362**: 147–156
- Hollenstein K, Frei DC, Locher KP (2008) Structure of an ABC transporter in complex with its binding protein. *Nature* **446**: 213–216
- Jardetzky O (1966) Simple allosteric model for membrane pumps. *Nature* **211**: 969–970
- Krantz BA, Finkelstein A, Collier RJ (2006) Protein translocation through the anthrax toxin transmembrane pore is driven by a proton gradient. *J Mol Biol* **355**: 968–979
- Morais-Cabral JH, Zhou Y, MacKinnon R (2001) Energetic optimization of ion conduction rate by the K⁺ selectivity filter. *Nature* **414**: 37–42
- Nassi S, Collier RJ, Finkelstein A (2002) PA₆₃ channel of anthrax toxin: an extended beta-barrel. *Biochemistry* **41**: 1445–1450
- Olesen C, Picard M, Winther A-ML, Gyrop C, Morth JP, Oxvig C, Møller JV, Nissen P (2007) The structural basis of calcium transport by the calcium pump. *Nature* **450**: 1036–1042
- Post RL, Hegyváry C, Kume S (1972) Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. *J Biol Chem* **247**: 6530–6540
- Reyes N, Gadsby DC (2006) Ion permeation through the Na⁺,K⁺-ATPase. *Nature* **443**: 470–474
- Vergani P, Lockless SW, Nairn AC, Gadsby DC (2005) CFTR channel opening by ATP-driven tight dimerization of its nucleotide-binding domains. *Nature* **433**: 876–880
- Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005) Crystal structure of a bacterial homologue of Na⁺/Cl⁻-dependent neurotransmitter transporters. *Nature* **437**: 215–223
- Yernool D, Boudker O, Jin Y, Gouaux E (2004) Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. *Nature* **431**: 811–818
- Zerangue N, Kavanaugh MP (1996) Flux coupling in a neuronal glutamate transporter. *Nature* **383**: 634–637
- Zhang S, Udho E, Wu Z, Collier RJ, Finkelstein A (2004) Protein translocation through anthrax toxin channels formed in planar lipid bilayers. *Biophys J* **87**: 3842–3849

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