PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2009) **364**, 145–147
doi:10.1098/rstb.2008.0245
Published online 28 October 2008

Introduction. The blurred boundary between channels and transporters

We dedicate this volume to the memory of Peter Läuger, a pioneer of the link between channels and pumps.

Membrane transport proteins are crucial for life. They regulate the fluxes of ions, nutrients and other molecules across the membranes of all cells, and their activities underlie physiological processes as diverse as brain electrical activity, muscle contraction, water and solute transport in the kidney, hormone secretion and the immune response. Mutations in membrane transport proteins, or defects in their regulation, are responsible for many human diseases. Consequently, these proteins are targets for widely used therapeutic drugs.

Traditionally, membrane transport proteins have been divided into two groups: channels and transporters. Channels are membrane-spanning water-filled pores through which substrates passively diffuse down their electrochemical gradients whenever the regulatory gate is open. Transporters undergo a cycle of conformational changes linked to substrate binding and dissociation on opposite sides of the membrane. This conformational cycle can be coupled to energy sources like pre-existing ion gradients or ATP hydrolysis, thus allowing substrates to be moved 'uphill' against their concentration gradients, as in nutrient and ion accumulation into the cell or export from the cell of ions, drugs or xenobiotics.

All transporters must effectively have two 'gates' that control access from either side of the membrane to the substrate-binding sites as well as a conformational cycle that prevents both these gates from being open at the same time. It is obvious that if both gates were open simultaneously, the protein would then operate as a channel. And, owing to the orders-of-magnitude higher flow rates through channels than through transporters, even a fleeting moment of channel-like behaviour would render a transporter useless. To obviate any such occurrence, the conformational cycles of many transporters incorporate occluded states in which both gates are shut, enclosing the bound substrate, before one of the gates opens to release it.

However, it has been apparent for some time that such a rigid distinction between channels and transporters is no longer tenable, and a more nuanced view is called for. Before his untimely death from a climbing accident, Peter Läuger spelled out theoretically how the diffusive mechanisms of channels and the conformational mechanisms of pumps might be viewed as two manifestations of an underlying unity. He also suggested that experimentalists might eventually discover proteins in which the two mechanisms are not clearly distinguishable.

Experimentally, there have also been hints of an 'ambiguous interface' between channels and transporters for many years. For example, many sodium-coupled amino acid transporters, or neurotransmitter transporters, display a perplexing conductance to small ions in the presence of substrate. Furthermore, a single amino acid change may cause a protein to switch from transporter to channel-like function. The underlying molecular basis of these functional findings remained something of a mystery, but recent crystal structures have cast fresh light on the puzzle.

The channel–pump interface is of considerable clinical consequence. Several genetic diseases are traceable to mutations in proteins that may be viewed as having hybrid functions, including cystic fibrosis, neonatal diabetes, orthostatic intolerance and various anaemias.

This volume derives from a Royal Society Discussion meeting in 2008 that explored the boundary between channels and transporters. It focuses on a few selected examples of membrane transport proteins that most clearly reveal connections between channels and transporters. It discusses mechanistic, functional and clinical data as well as the latest structural information.

(a) *ABC proteins*

ATP-binding cassette (ABC) transporters are ubiquitous membrane proteins that couple the energy of ATP hydrolysis to translocation of diverse substrates across cell membranes. It has long been recognized that the sulphonylurea receptor SUR and the cystic fibrosis transmembrane conductance regulator CFTR are exceptional among ABC proteins in that they do not serve as pumps. Instead, they have hijacked the ATP-binding and hydrolytic activity of the nucleotide-binding domains (NBDs) to gate an intrinsic chloride channel (CFTR) or to regulate the gating of a separate inward-rectifier potassium channel (SUR).

Recent crystal structures of bacterial ABC transporters have suggested a common molecular mechanism by which binding and hydrolysis of ATP are coupled to conformational changes in the membrane-spanning domains, as discussed by Locher (2009). Muallem & Vergani (2009) consider how the structural changes occurring at the NBDs of CFTR open and close the chloride channel contained within its membrane domain. The paper by Aittoniemi *et al.* (2009) focuses on how SUR regulates the activity of the pancreatic beta-cell K_{ATP} channels and how failure of this regulation by naturally occurring mutations gives rise to human

One contribution of 16 to a Discussion Meeting Issue 'Membrane transport in flux: the ambiguous interface between channels and pumps'.

disease. Nelson *et al.* (2009) present a novel tool—a knockout/wild-type chimeric mouse—for analysing the role of the K_{ATP} channel in cardiac stress tolerance.

(b) *Neurotransmitter transporters*

After neuronal electrical activity, the synaptic cleft must be cleared of released neurotransmitters, such as glutamate, noradrenaline, serotonin and GABA. This is achieved by a class of sodium-coupled cotransporters that use the energy stored in the pre-existing sodium gradient across the membrane. Many of these transporters have a parallel ion leak that is somehow gated by the transported substrate. X-ray crystal structures of prokaryotic homologues of these sodium-coupled transporters are described by Gouaux (2009) and the coupling of an intrinsic chloride leak to glutamate uptake in the EEAT transporters is covered by Holley & Kavanaugh (2009). Prasad *et al.* (2009) discuss the effects of disrupted transporter function in relation to mental disease.

In an analogous way, Ellory *et al.* (2009) describe how a human disease arises from mutations that produce an uncoupled conductance in the chloride–bicarbonate exchanger AE1 that normally does not generate any transmembrane current. The dipeptide transporter described by Meredith (2009) is another example of a transporter that similarly may contain an ion channel.

(c) *The CLC family*

The CLC proteins, originally thought to be a family of chloride channels, are now recognized to include both channels and chloride–proton antiporters: for example, of the nine CLCs in the human genome, four are channels and five are antiporters. However, both types of protein share some similar mechanistic properties. Thus Miller & Nguitragool (2009) propose a mechanism for the chloride–proton exchange, based on the structure of a bacterial antiporter, which includes some channel-like features. Conversely, Lísal & Maduke (2009) describe unusual single-channel behaviour that reflects proton transport carried out by a eukaryotic CLC chloride channel. The physiological roles of CLC antiporters and channels in plants, where nitrate is the substrate rather than chloride, are considered by De Angeli *et al.* (2009).

Finkelstein (2009) describes a system reminiscent of the CLCs, in which a protein long thought to be an ion channel, anthrax toxin, actually functions as a pump that uses a proton gradient to inject a lethal enzyme into the cytoplasm of the unfortunate target cell.

(d) *The sodium pump*

It may seem surprising for the Na,K-ATPase to show up in a meeting like this. This long-studied pump tightly couples ATP hydrolysis to sodium and potassium translocation, a function that would be undermined by any intrinsic channel-like leaks. However, a lethal marine toxin (palytoxin) reversibly converts this pump into a non-selective cation channel. Gadsby *et al.* (2009) describe how this toxin causes the gates of the pump to become uncoordinated so that they are occasionally both open, providing an ion conduction pathway through the protein. These results map nicely on to the crystal structures of the Na,K-ATPase and closely related Ca-ATPase described by Morth *et al.* (2009), which illustrate how the conformational cycles, driven by cycles of ATP-mediated pump phosphorylation and dephosphorylation, expose the ion-binding sites first to one side of the membrane and then the other.

SUMMARY

As an intrinsic part of their molecular mechanisms, transporters may harbour channels within them. By disrupting one gate or the communication between gates (so that both are sometimes open simultaneously), a transporter can be converted into a channel. It is natural to envision that this is how mutations and toxins produce channels from transporters. In addition, by eventually losing a gate through evolution, a transporter could become a channel: for example, this may be how an ABC protein like CFTR became a channel gated by ATP binding and hydrolysis. Likewise, the CLC channels may be 'broken' CLC transporters. Whether any transporters have been transmuted from channels by growing an additional gate is less certain, but one example might be the Kdp-ATPase, a bacterial K pump proposed to have evolved from a K channel.

Although it has not been reported, the examples presented in this issue make us wonder whether some proteins can flip between pump and channel mode under physiological conditions. Another question is whether proteins of intermediate function exist. Do leaky transporters occur naturally, in which the gates are uncoordinated normally or become so in response to regulatory agents, rather than as a pathological result of mutations or toxins? Such slippage could be of value to the cell, for example as a means of controlling solute gradients. We are not aware of any evidence for such a thing but it seems theoretically plausible.

The above discussion cites examples of proteins that function as either a channel or a transporter, or as something intermediate between these two ends of the spectrum. However, there are also proteins in which both channel and transporter operate at the same time. This situation has been most thoroughly studied in glutamate and other neurotransmitter transporters, but our understanding of how this feat is achieved is still quite vague.

So how do you tell whether your favourite flux is mediated by a channel or a transporter? The question is easy to pose but difficult to answer rigorously, and it has caused many headaches among membrane biophysicists. Experimentally, the unitary flux rate is most commonly used to make this distinction. Typically, channels are fast (greater than $10^6\ s^{-1}$) and transporters are slow ($1–1000\ s^{-1}$). These rates reflect the very different energy barriers of the limiting steps in the two types of substrate movement: low for diffusion (when all gates are open) and high for conformational rearrangements (alternating gating). But this distinction is not foolproof as there are low-conductance channels and there may be high-turnover transporters. Ultimately, atomic-resolution structural information, in multiple conformations, is required to understand a given

transport mechanism. After a long wait, such structures are now beginning to enrich the membrane transport field. Thus, it seems likely that during the next few years new structural insights will illuminate the ambiguous interface between channels and transporters.

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