Ion channels versus ion pumps: the principal difference, in principle

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Abstract | The incessant traffic of ions across cell membranes is controlled by two kinds of border guards: ion channels and ion pumps. Open channels let selected ions diffuse rapidly down electrical and concentration gradients, whereas ion pumps labour tirelessly to maintain the gradients by consuming energy to slowly move ions thermodynamically uphill. Because of the diametrically opposed tasks and the divergent speeds of channels and pumps, they have traditionally been viewed as completely different entities, as alike as chalk and cheese. But new structural and mechanistic information about both of these classes of molecular machines challenges this comfortable separation and forces its re-evaluation.

Membrane potential
The difference in electrical potential between one side of a membrane and the other; usually the electrical potential inside a cell measured with respect to that of the extracellular space.

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Precisely controlled movements of ions into and out of cells and organelles are essential for life. In single cells, ion flows mediate processes as disparate as signalling, pH balance, volume regulation and the cell cycle, and in higher organisms they also underlie fertilization, immune responses, secretion, muscle contraction and all of the electrical signals in nerves, muscles and synapses. The proteins that transport ions across membranes fall into two general classes: passive conduits called ion channels, through which ions rush down gradients of concentration and electric potential, and pumps, which release energy from ATP or other sources to actively push ions against, and thereby build up, those gradients.

Ion flows through channels generate transmembrane electric currents. Currents of Na or K ions cause changes in membrane potential that act as physical signals, whereas for Ca currents the Ca ions themselves usually represent the signal, albeit a chemical one. Currents of Cl ions tend to stabilize membrane potentials, such as at inhibitory synapses and in skeletal muscle fibres, or to facilitate transmembrane salt and water movement. Because ion flows through channels dissipate the gradients that drive them, channels contain gates that are regulated to turn the ion flows on only when needed.

The term pumps encompasses all transporters capable of thermodynamically uphill transport (so-called active transport). Ion pumps that hydrolyse ATP, ATPases, are sometimes called primary pumps to distinguish them from secondary pumps. Secondary pumps exploit the energy stored in electrochemical gradients of ions (often Na) by coupling thermodynamically downhill movements of those ions to drive the uphill transport of another substrate. Secondary pumps are sometimes called co-transporters or counter-transporters (or exchangers), according to the relative directions of the coupled downhill and uphill ion flows.

The different behaviours of ion channels and pumps — passive, thermodynamically downhill and high-speed ion movement through channels, versus active, thermodynamically uphill transport, frequent incorporation of enzyme-like reaction mechanisms and low-speed ion movement through pumps — led to a separation of the efforts to understand them. Only recently have atomic resolution X-ray crystal structures and high-resolution functional measurements of examples from both classes begun to suggest that ion channels and pumps need not be as different as was once thought; they have much in common. Given this realization, now seems to be an appropriate time to reconsider their similarities and differences. This Review begins with a theoretical appraisal of what distinguishes channels from pumps, then examines clear-cut examples from each class, addresses how one might be transformed into the other, evaluates a protein family that straddles the increasingly blurred boundary between the two and looks at apparent hybrid molecules. Only a few illustrative examples from the many types of ion channels[1] and pumps (see the Transport Classification Database) are chosen for discussion, and their myriad roles in biology are not covered at all.

One gate versus two gates

The principal difference, in principle, between channels and pumps is that a channel needs only a single gate, whereas a pump needs at least two gates that should never be open at once. So what is a gate? A gate can be

considered to be the part of the protein that precludes ion movement along the translocation pathway in the prohibitive conformation, but not in the permissive conformation. Progress in determining the locations and structures of gates and the mechanisms of gating remains slow and is a major unsolved problem in the field of ion transport. Nevertheless, it is unlikely that any door-shaped gate like those in FIG. 1 will ever be found. As we will see below, examples of gates identified so far include a single amino-acid side chain in Cl ion transport proteins of the ClC family and an iris-like bundle crossing of four transmembrane helices in K ion channels.

How does the 'one gate versus two gates' principle work? As depicted in FIG. 1a, an ion channel can be viewed as a selective, hydrophilic and hence energetically favourable pathway for conducting chosen ions through the hostile hydrophobic interior of the membrane's phospholipid bilayer[1]. Passage of ions through the channel is controlled by a gate for which opening and closing is modulated by appropriate signals. The direction that ions move in depends on the sign of their charge, the magnitude and direction of their transmembrane concentration gradient and the size and sign of the transmembrane electric potential difference. Net flow of ions through a channel is always down their electrochemical potential gradient. Tens of millions of ions per second can cross the membrane through just one open ion channel, a rate that is large enough for sensitive amplifiers to record the resulting electric current in a single channel[2] (for examples, see below). Sudden starting and stopping of that current reveals the timing of individual channel opening and closing events, which occur comparatively infrequently, typically after intervals of several milliseconds. This means that the protein conformational changes that open and close the channel gate occur on the order of a hundred times per second.

Although a single gate suffices to control ion flow through a channel, many channels have more than one gate[1]. For example, voltage-gated Na, Ca or K ion channels are opened when a change of membrane potential displaces voltage sensors that are connected to a cytoplasmic-side activation gate, and they can be closed by reversal of those displacements (de-activation) in response to an opposite change of membrane voltage. But even with their activation gates in the permissive position, the ion pathways through those channels can by closed by a separate gating process called inactivation. Both gates must be in their permissive positions for the channel to conduct ions, and closure of either gate precludes ion flow.

An ion pump can similarly be viewed as an ion-selective pathway, but one to which access is controlled by two gates that open and close such that they are never both open simultaneously[3] (FIG. 1b). Instead, the gates open and close alternately, allowing the chosen ions to enter the pathway from one side of the membrane while one of the gates is open, and then to leave at the other side of the membrane while the other gate is open, after the first gate has shut[4–6]. The speed of ion movement through pumps is thus limited by the gating reactions, which, like those of ion channels, occur with frequencies of around a hundred times per second. This accounts for the several orders of magnitude slower ion transport through pumps than through channels. A practical consequence of this lower speed is that, even if a pump moves net charge across the membrane and so generates an electric current, that current will be far too small to be detectable for a single pump molecule. The vast difference in the rates of ion movements mediated by channels and pumps, together with the different directions of those ion movements — downhill, dissipating the gradients, for channels, and uphill, generating the gradients, for pumps — are the major reasons that channels and pumps have been considered unrelated.

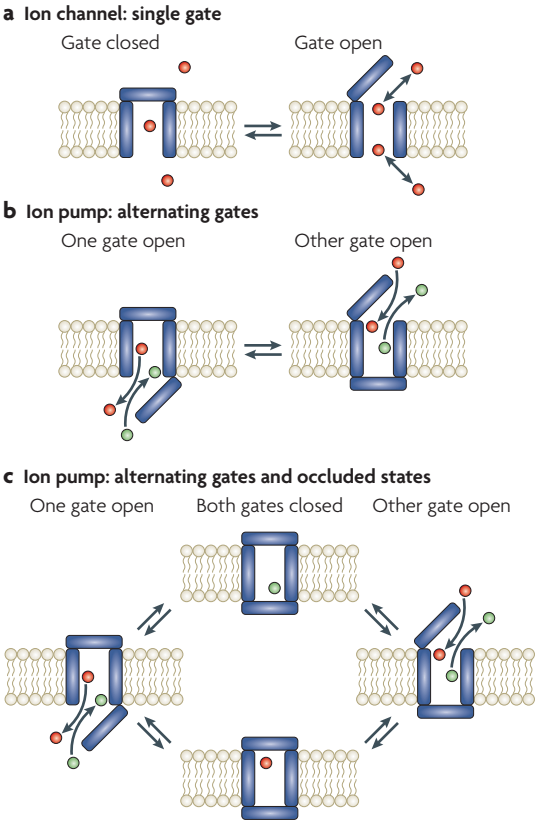


Figure 1 | **Ion channels versus ion pumps, in principle.** **a** | Schematic representation of an ion channel as a membrane-spanning pore through which ion (red spheres) movement is controlled by a single gate, represented by a hinged door. **b** | Ion pump as a membrane-spanning pore with two gates that open and close alternately. Coupling of an energy source to switch the relative binding affinity for red versus green ions between the left- and right-hand states enables active exchange of red for green ions across the membrane. **c** | Occluded states, with both gates closed around bound ions, preclude inadvertent opening of the second gate before closure of the first gate, which would allow ions to flow down their electrochemical potential gradient several orders of magnitude faster than the pump can move them against that gradient. Pumped ion movement is rate limited by the gating reactions, rather than by electrodiffusion. Scheme modified with permission from REF. 65 © (2009) The Royal Society and from REF. 84 © (2004) Macmillan Publishers Ltd. All rights reserved.

Activation gate
A gate in an ion channel that when opened initiates ion flow through the channel pore.

Gating reaction
A change in the conformation of a transmembrane transport protein that alters access to the substrate translocation pathway.

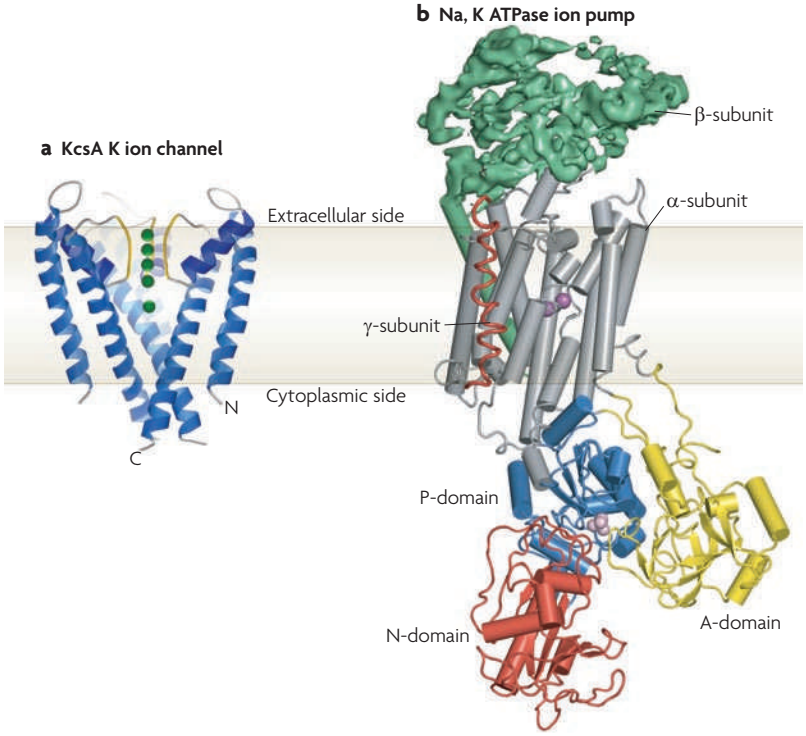


Figure 2 | **Crystal structures of a real channel and a real pump reveal clear differences. a** | The transmembrane regions of three subunits of a KcsA K ion channel tetramer (front subunit removed for clarity) show the narrow axial selectivity filter (yellow segments of α-carbon chain), containing four closely spaced sites for K ions (green spheres), towards the extracellular side of the membrane, and a fifth K ion in a large cavity half-way across the membrane. The single gate (activation gate) is near the cytoplasmic side. Horizontal lines mark approximate membrane boundaries. **b** | Na, K ATPase pump complex in a conformation related to the phosphorylated E2 state (with MgF_4^{2-} as stable phosphate mimics indicated as pink spheres in the cytoplasmic phosphorylation (P) domain), containing two Rb ions (purple spheres) occluded in the transmembrane domain. Labels indicate the catalytic α-subunit, auxiliary β-subunit (green) and regulatory γ-subunit (red helix), as well as the membrane-spanning (grey), P (blue), nucleotide-binding (N; red) and actuator (A; yellow) domains. Panel **a** is reproduced with permission from REF. 9 © (2001) Macmillan Publishers Ltd. All rights reserved. Panel **b** is modified with permission from REF. 20 © (2007) Macmillan Publishers Ltd. All rights reserved.

But the 'one gate versus two gates' formalism of FIG. 1a,b lays bare their relatedness. It emphasizes that what distinguishes a pump from a channel is the timing of the closure of the first gate with respect to the opening of the second gate. The faster downhill ion flow through channels compared with uphill flow through pumps means that any communication failure between a pump's two gates that left both momentarily open would allow channel-like dissipative ion flow that would quickly undo the pump's hard work. The briefest period of simultaneously open gates, even for less than 0.001% of the time the pump functions normally, would render the pump useless. In fact, the resulting molecule would function like ion channels that include separate gates for activation and inactivation. Because the consequences of such a communication breakdown between gates would be so catastrophic, pumps have evolved a fail-safe mechanism (FIG. 1c) in which both gates first close, occluding the bound ions inside the protein (see below), before the second gate opens to release them.

Selectivity filter
The narrow region of an ion translocation pathway in which the transport protein interacts with the ions to select among them on the basis of their physicochemical characteristics.

How well does this formalism hold?

The 'one gate versus two gates' formalism is heuristically useful, but how well does it hold up in reality? X-ray crystal structures and detailed functional measurements provide incontrovertible evidence for the existence of ion channels and pumps that fit the mould perfectly.

Bona fide ion channel exemplars. X-ray crystal structures have been obtained for several types of K ion channels, and these serve as examples of ion channels that have been exquisitely refined by evolution. These K ion channels are all tetramers, and they all contain an axial pore with conserved architecture. The pore comprises a narrow selectivity filter that spans the outer third of the distance across the membrane and contains closely spaced binding sites for a queue of K ions (FIG. 2a). The selectivity filter leads to a water-filled cavity that contains another K ion, near the middle of the membrane, and then the pore narrows again until it becomes closed to ion flow by converging helices near the intracellular surface[7].

The K ions in the selectivity filter are dehydrated, but each one sits at the centre of a cage of eight backbone carbonyl oxygens that are arranged just like the oxygens of the water molecules that contact, and solvate, the K ion in the large central cavity[8]. Evidently, the selectivity filter structure has evolved to compensate for the large energetic cost of dehydrating K ions so that they can be selectively transported across the lipid bilayer. Because the selectivity filter so closely mimics the inner hydration shell of K ions in solution, an open K ion channel can conduct hundreds of millions of K ions per second, essentially as fast as they can diffuse up to the mouth of the channel[9]. And it can do so while excluding Na ions, with a discrimination ratio of around 1,000 to 1 (REFS 1,7).

No overt gate-like structure is seen at the extracellular end of the pore near the selectivity filter (see REF. 8), and the main gate is formed near the intracellular end where the four inner transmembrane helices approach one another (FIG. 2a). In different kinds of K ion channels, the disposition of the so-called activation gate can be controlled by various devices that are connected to the cytoplasmic end of the inner or outer transmembrane helices. Those devices include ligand-binding domains and voltage sensors[7], and their movement splays the inner helices apart, thereby opening the pore.

Bona fide ion pump exemplars. P-type ATPases are quintessential ion pumps. They are exemplified by the Na, K ATPase (FIG. 2b) that maintains Na and K ion gradients across the surface membranes of animal cells, the sarcoplasmic and endoplasmic reticulum Ca ATPase (SERCA) that fills cellular Ca ion stores, the H, K ATPase that acidifies our stomachs and the H ATPase that extrudes cellular protons in plants and fungi. In these pumps, a conserved Asp residue in a central cytoplasmic domain (the P-domain; FIG. 2b) becomes phosphorylated (hence P-type) by ATP once transported ions have entered the binding pocket in the transmembrane domain through the open cytoplasmic entryway, while the exit pathway

remains closed. This autophosphorylation event shuts the cytoplasmic gate, temporarily occluding the bound ions before a large conformational change opens the gate to the extra-cytoplasmic side. The lowered affinity for the transported ions in that conformation prompts their release to the extra-cytoplasmic compartment, an event that is followed by binding of the counter-transported ions: K in the case of the Na, K and H, K ATPases, or H in the case of SERCA. Closure of the extra-cytoplasmic gate, triggered by counter-ion binding, leads to dephosphorylation of the aspartyl-phosphate and stabilization of another occluded state[10], this time enclosing counter ions (FIG. 2b). A second large rearrangement re-opens the cytoplasmic gate, restoring the conformation with lowered affinity for the counter-transported ions but higher affinity for the transported ions.

For SERCA, X-ray crystal structures have been obtained in all four of these principal conformations[11–19]. The structures reveal that, in P-type pumps, gates correspond to closely apposed segments of transmembrane helices that form 10–20-Å barriers on either side of the binding pocket[11,12,18]. The two major conformations (called E1 and E2) correspond to the left- and right-hand cartoons in FIG. 1b,c, and the two occluded states correspond to the upper and lower cartoons depicting trapped ions in FIG. 1c. In the crystal structure of Na, K ATPase (FIG. 2b) containing two occluded Rb ions[20] (as K ion surrogates), as in the structures of SERCA with two occluded Ca ions[11,15,19], the occluded ions sit almost side by side, more than a third of the way across the membrane from the cytoplasm.

Lessons from the exemplars. These examples illustrate three distinctions between highly evolved ion channels and perfected pumps. First, in contrast to the K ion channel structure, but as expected for an occluded-ion conformation of a pump, a space-filling representation of the Na, K ATPase structure of FIG. 2b confirms the absence of any pathway through which the trapped ions could escape from the binding pocket[20]. Second, the side-by-side arrangement of bound K, Rb or Ca ions in the pumps (FIG. 2b) stands in stark contrast to the single-file arrangement along the fourfold axis of symmetry, of ions in K channels (FIG. 2a); the high-throughput capability of the ion channel arrangement[9] provides no advantage when pumped ion flow is rate-limited by large conformational changes. Third, the structures involved in ion selectivity and activation gating are separate in the channels, permitting various gating modules to control the conserved pore. But these features are intertwined in the pumps, in which the binding pocket becomes remodelled during the gating conformational changes that switch access from one side of the membrane to the other and alter relative ion-binding affinity[14].

Straddling the pump–channel divide

These structural distinctions served to reinforce the division between those who studied pumps and those who worked on channels. They also relegated the understanding that, in principle, subtle timing differences in the opening and closing of two gates could separate a pump from a channel[3–6] to an intellectual curiosity. But the recent discovery[21] that a single family of Cl transport proteins (the ClC family) includes examples of Cl ion pumps as well as of Cl ion channels was a startling reminder that small structural differences can underlie extremely different functions.

The first member of the family, ClC-0, was identified in electric fish over a quarter of a century ago as a double-barrelled channel with independent fast gates that open and close each barrel on a timescale of tens of milliseconds (FIG. 3a,b) and a common slow gate that operates on a timescale of seconds[22] (not shown). The double-barrelled behaviour was later explained by the homodimeric structure of ClC proteins, in which each subunit contains its own ion transport pathway[23–25]. Comparable mammalian Cl ion channels that belong to the same ClC family were also found; in humans, there are nine ClC family members[26]. The crystal structure (FIG. 3c) of a homodimeric prokaryotic ClC protein, ClC-ec1, was a breakthrough[25]. The structure revealed that the protein had a narrow pathway with multiple transported anions (Cl or Br) in a row[25] (FIG. 3c,d); not a straight row like that in K ion channels, but a row nonetheless. Whether there were two or three anions depended on the position of the side chain of a Glu residue (E148); the side chain of E148 was found to occupy the extracellular end of the pathway (FIG. 3d) in the space where the third anion was found after neutralization of the side chain by mutating the Glu residue to Gln[27] (FIG. 3e). As neutralization of the equivalent Glu residue in ClC-0 channels essentially abolished their fast gating (FIG. 3b), the Glu side chain was identified as the fast gate and was proposed to swing out of the ion pathway upon protonation, so opening the pore to Cl ion flow[27]. The structures of ClC-ec1 and their analysis were equally successful in rationalizing other results from examinations of structure and mechanism in ClC-0 and mammalian ClC-1 and ClC-2 channels[28–30].

It came as a shock a year later, therefore, when ClC-ec1 was found not to be a Cl ion channel, but rather to be a Cl/H exchange pump that couples downhill movement of one proton to uphill transport of two Cl ions in the opposite direction, or vice versa[21]. The unavoidable conclusion is that the structure of a ClC pump must so closely resemble that of a ClC channel that they cannot yet be told apart, even at near-atomic resolution. Subsequent work has confirmed that some of the human ClCs are indeed channels, but at least as many are Cl/H exchange pumps[31].

This outcome violated the comfortable notion that these two classes of ion transport protein ought to be visibly dissimilar. But does it violate the 'one gate versus two gates' formalism? Not really, because the largely conserved Glu side chain appears to fulfil the role of a fast gate in ClC channels[27], and one of the two required gates in the ClC pumps[21,25,32,33].

So what constitutes the second gate in ClC pumps? A plausible candidate is a conserved Tyr residue (Y445 in FIG. 3d) that coordinates the central Cl ion, as ever smaller side chains at this position in ClC-ec1 allow Cl ion flow that is increasingly uncoupled from proton transport[34]. And, when both the Glu residue at the external site and the Tyr residue at the central site are replaced with the

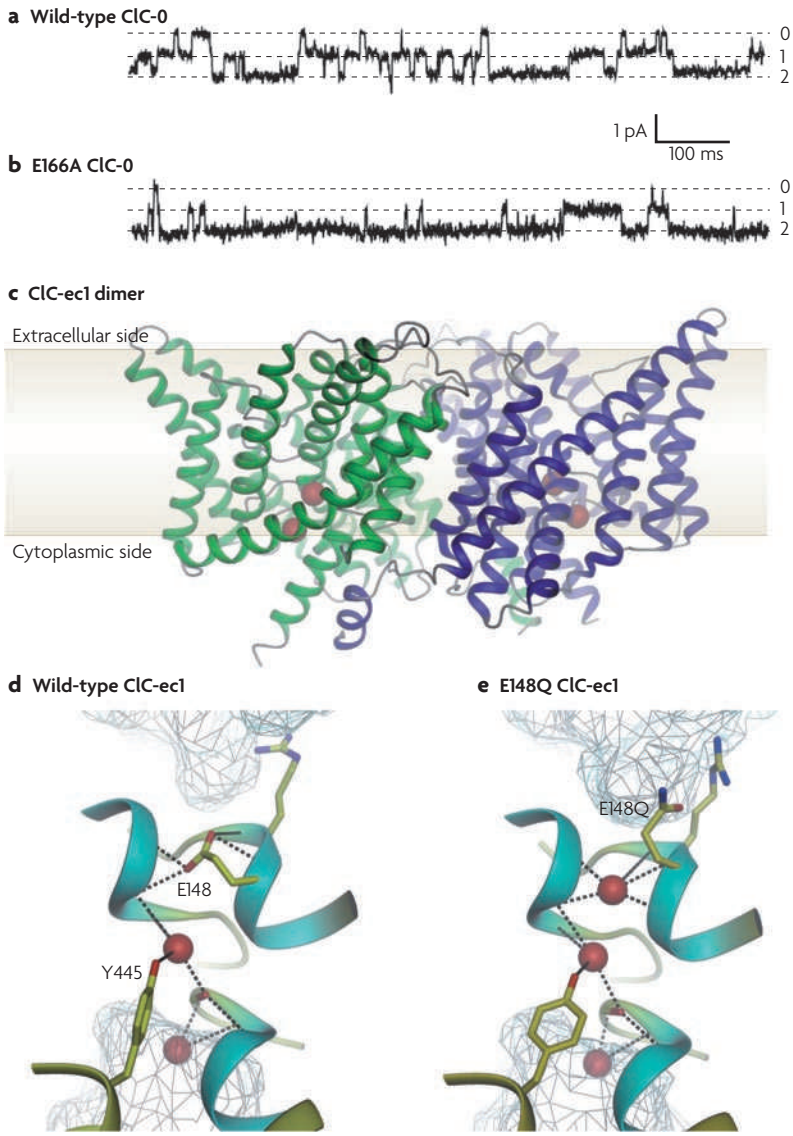


Figure 3 | **A Cl ion channel evolved from a Cl/H exchanger. a**,**b** | Current recordings showing independent opening and closing of the two pores in a wild-type ClC-0 Cl ion channel (**a**), and the mostly open behaviour of a mutant E166A ClC-0 channel (**b**). Labels 0, 1 and 2 indicate the number of open pores in each dimeric channel. **c** | The transmembrane region of a wild-type ClC-ec1 dimer viewed from within the membrane (membrane boundaries indicated). The ion pathway in each monomer (green, blue) contains two anions (red spheres). **d**,**e** | Pore region connecting extra- and intracellular aqueous vestibules (mesh) in wild-type (panel **d**) and mutant E148Q (panel **e**) ClC-ec1. The wild-type contains two Cl ions (red spheres), whereas the mutant contains three. The third Cl ion occupies the space that is vacated by the swung-out side chain of the external gate residue, 148 (E148 of ClC-ec1 is equivalent to E166 of ClC-0). Panels **a** and **b** are modified with permission from REF. 27 © (2003) American Association for the Advancement of Science. Panel **c** is modified with permission from REF. 85 © (2006) Macmillan Publishers Ltd. All rights reserved. Panels **d**,**e** are modified with permission from REF. 86 © (2007) Elsevier.

smallest amino acids, the passive Cl ion flow is accelerated to ~4×10^4 ions per second, consistent with the expected consequences of shrunken gates[33]. But, even in these double-gate mutants, something else must restrain Cl ion movement, because this flow rate is still more than a hundred times slower than Cl ion transit through human ClC-1 channels[35] and a thousand times slower than through fish ClC-0 channels[22]; this is despite the fact that both ClC-1 and ClC-0 contain the conserved Tyr residue, which is retained in the channels as well as in the pumps.

Ion channels evolved from perturbed pumps?

We have seen that there are pumps that look like pumps, channels that reek of channels and a family of proteins that includes some pumps and some channels. We have also seen that, in theory, disruption of the coordinated timing between a pump's two gates, or of the structure of one of the gates, can convert a pump into a channel. Are any natural examples of such conversion known? Indeed, there are examples of ion channels, and of ion channel-like proteins, that belong to families of transporters, their close genetic relationship implying that the channels arose from pumps following evolutionary degradation of a gate[32,36–41].

Even ClC channels transport protons! The channel–pump dichotomy in the ClC family, together with the non-equilibrium nature of ClC channel gating[42] and its characteristic dependence[28] on pH, raise the question of whether the ClC channels evolved from an ancestral ClC Cl/H pump in which one of the gates became incompetent to hold back Cl ions[32,36]. Recent direct measurement of pH changes close to the extracellular surface of ClC-1 channels during gating[43], and the demonstration that non-equilibrium gating of ClC-0 channels depends on the proton electrochemical potential gradient[36], imply that gating of the Cl ion pore in ClC channels involves proton transport. This supports a view of ClC channels as broken ClC Cl/H exchange pumps[32,36].

A Cl ion channel evolved from an ABC transporter. Another example of a family of transport proteins begetting an ion channel child is the CFTR (cystic fibrosis transmembrane conductance regulator) Cl ion channel[44] (FIG. 4a). CFTR is the only one of thousands of ABC (ATP-binding cassette) proteins known to function as an ion channel. Most of the others are thought to be ATP-driven transporters, each with its own designated cargo. A few, including the SURs (sulphonylurea receptors)[45], appear to use ATP-induced conformational changes to signal to other proteins[45–47]. ABC proteins bind and hydrolyse ATP in composite catalytic sites that are formed in the interface between paired cytoplasmic nucleotide-binding domains (FIG. 4a), which are drawn together by bound ATP and later separated by ATP hydrolysis[45,48,49]. These movements are transmitted to the transmembrane domains to alternately open and close gates to the substrate-binding site, and so effect substrate transport. Transmission occurs via links that extend from the transmembrane domains into the cytoplasm. In CFTR, ATP binding opens the ion channel pore, and hydrolysis of that ATP triggers channel closure (reviewed in REF. 37). Thus, CFTR channels remain closed until exposed to ATP (FIG. 4b,c), and mutation of a key catalytic residue to prevent ATP hydrolysis makes channel closure upon ATP withdrawal nearly a 1,000 fold slower (FIG. 4c) than normal (FIG. 4b).

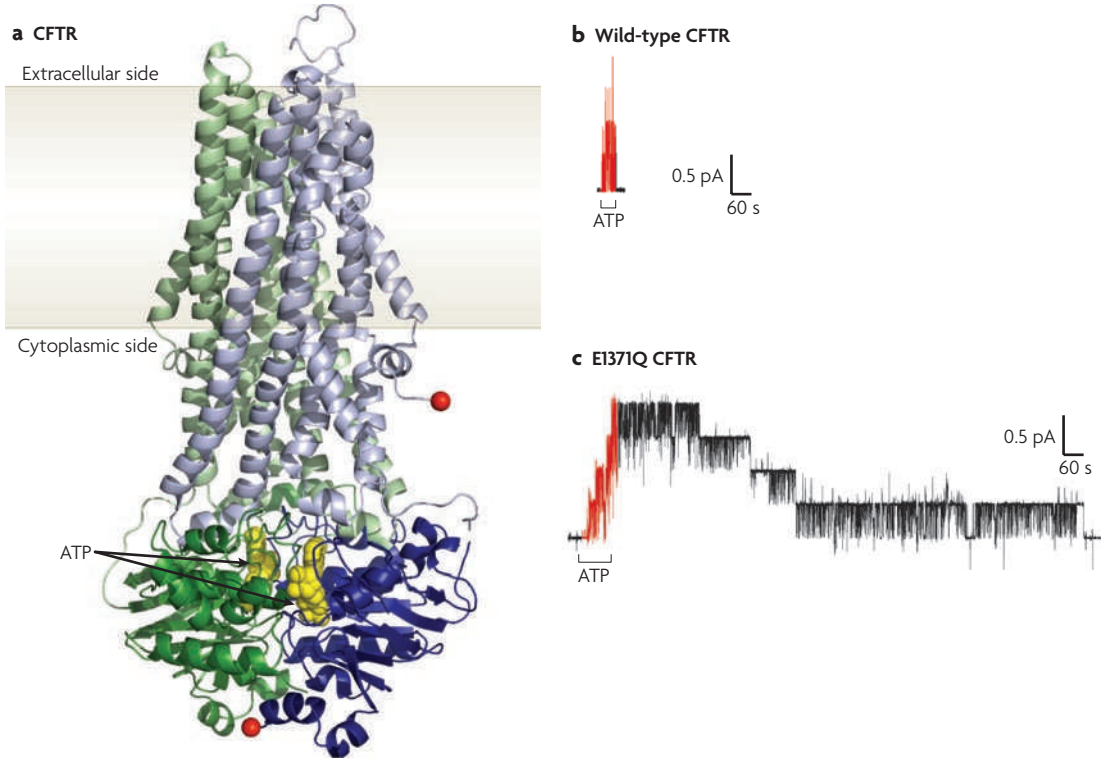


Figure 4 | **A Cl ion channel evolved from an ABC transporter. a** | Model of CFTR (cystic fibrosis transmembrane conductance regulator; amino-terminal half shown in green, carboxy-terminal half shown in blue) based largely on homology with the prokaryotic ABC transporter Sav1866 (REFS 50,51). The transmembrane domains (lines mark membrane boundaries) and cytoplasmic linkers are lighter in colour, and the dimerized cytoplasmic nucleotide-binding domains containing two bound ATPs (yellow spheres) are darker. The ~200-residue regulatory domain of unknown structure that somehow links the N- and C-terminal halves by connecting the two red spheres is absent. **b**,**c** | Recordings of CFTR channel currents in excised inside-out membrane patches. Exposure to cytoplasmic ATP (red segments) allows opening and closing of wild-type CFTR channels (**b**) and of mutant E1371Q CFTR channels that bear a point mutation that prevents ATP hydrolysis (**c**). Each 0.4-pA current step reflects opening or closing of a single CFTR channel. The greatly delayed closing of all four E1371Q channels (**c**), compared to the four wild-type CFTR channels (**b**), after ATP removal shows that ATP hydrolysis initiates normal channel closing, and that the channel open-burst state corresponds to a conformation with ATP bound in the dimerized nucleotide-binding domains (see panel **a**). Panel **a** is modified with permission from REF. 87 © (2008) Springer. Panels **b** and **c** are reproduced with permission from REF. 37 © (2006) Macmillan Publishers Ltd. All rights reserved.

As yet, there are no X-ray crystal structures of eukaryotic ABC transporters, but crystal structures of ABC transporter relatives from prokaryotes have revealed that in the nucleotide-bound conformation the extracellular-side gate in the transmembrane domains is open while the cytoplasmic-side gate is closed[50–53]. Since around 10^7 Cl ions per second flow through an ATP-bound open CFTR channel (from the size of single-channel currents; FIG 4b,c) in which, by analogy, the extracellular-side gate ought to be open, it seems reasonable to surmise that the cytoplasmic-side gate of CFTR has become atrophied, or uncoupled from the outer gate. Thus, CFTR can be considered to be a broken ABC transporter[38,39].

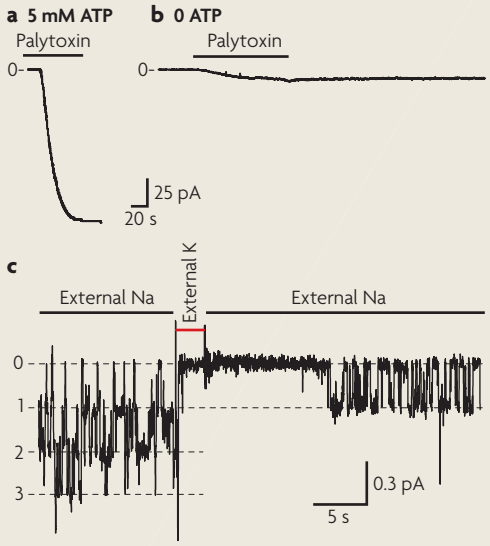
Although CFTR is unquestionably an anion channel, it might also be able to transport something, perhaps glutathione[54,55]. Regardless, mutant cycle analysis based on multiple sequence alignment[40] suggests that the ATP-dependent cycle of dynamic rearrangement of the nucleotide-binding domains that drives opening and closing of the channel gates in CFTR is the same as that which powers substrate transport in most ABC proteins.

Light-activated pump–channels. Channelrhodopsins[41] may also be examples of ion channels that evolved, by loss or uncoupling of a gate, from microbial pumps such as bacteriorhodopsin (a proton pump) and halorhodopsin (a Cl ion pump). The two channelrhodopsins are light-activated channels that are selective for protons (channelrhodopsin 1) or are permeable to a range of cations (channelrhodopsin 2)[41]. However, preliminary estimates[41] suggest that only ~ 3×10^4 cations per second flow through channelrhodopsin 2, a rate that, like that of double-gate mutant ClC-ec1 pumps[33], puts channelrhodopsins squarely in the grey zone between pumps and channels (see below).

Acute transformation of a pump into a channel

Nature has also found a means to pharmacologically subvert the fail-safe mechanism of the Na, K ATPase pump, in which strict coupling between its two gates ensures that each gate opening is followed by an occluded state. The sophisticated marine toxin, palytoxin, binds specifically to extracellularly exposed parts of the Na, K pump

Box 1 | **Pharmacological transformation of pumps into channels**



Each Na, K ATPase pump in a cell membrane exports three Na ions and imports two K ions for each ATP hydrolysed[77] and can repeat this up to a hundred times a second[78]. The net charge movement generates a current that can reach ~20 aA for a single Na, K pump, which is too tiny for present recording equipment. But the >20 pA that are generated by the millions of Na, K pumps in an entire cell can be measured[79]. If all K ions are removed, leaving only Na ions, Na, K pumping is precluded and steady current through the pump becomes zero, regardless of the membrane potential[80]. This means that there is no channel-like electrodiffusive flow of Na ions through the stalled Na, K pumps, confirming that the two gates remain tightly coupled. This is the condition (0 current at –40-mV membrane potential) at the beginning of the recordings of current flow through excised outside-out patches of cell membrane, each containing thousands of Na, K pumps (see figure panels **a**,**b**). However, in the presence of 5 mM ATP, a saturating concentration (100 nM) of palytoxin quickly elicits a relatively large current that reflects rapid Na ion flow through Na, K pumps after palytoxin has transformed them all into cation channels[60,61,81] (see figure panel **a**).

The gates are uncoupled, but still functional

Without ATP, a much weaker current is activated by the same palytoxin concentration (see figure panel **b**) in a comparable patch, with presumably similar numbers of palytoxin-bound Na, K pump–channels. The current is weaker because individual palytoxin-bound pump–channels spend a much greater fraction of their time closed in the absence of ATP than in its presence[61]. This channel-opening effect of ATP is mimicked by the ATP analogue AMP-PNP and by ADP, and reflects the known action of nucleotides on unmodified Na, K pumps to open the cytoplasmic-side gate[60,61,82,83].

External cations also modulate the probability of palytoxin-bound pump–channels being open. Just as external K ions enter unmodified Na, K pumps and become occluded in their binding sites after closure of the extracellular-side gate (FIG. 2b), brief replacement of external Na ions with K ions temporarily shuts palytoxin-bound pump–channels[61] (see figure panel **c**; labels 0, 1, 2 and 3 indicate the number of simultaneously open pump–channels). Evidently, palytoxin transforms Na, K pumps into channels in which the two gates still respond to their physiological ligands but are no longer in synch: palytoxin breaks the fundamental rule of the 'one gate versus two gates' formalism, which is that a pump's two gates should never be open at the same time. Panels **a** and **b** are modified with permission from REF. 81 © (2002) The New York Academy of Sciences. Panel **c** is modified with permission from REF. 65 © (2009) The Royal Society.

and thereby disrupts the tight communication between the gates, allowing both to sometimes be open at the same time, so transforming the pump into a cation channel[56–59] (BOX 1). The channels are not highly selective; they distinguish poorly among small monovalent cations and they even slowly conduct organic cations that are up to three times larger than a Na ion[60].

The fact that the gates of palytoxin-bound Na, K pump–channels continue to respond to extracellular K ions and to cytoplasmic ATP bolsters the interpretation that the two gates still work, just no longer in synch[61] (BOX 1). The size of the current through single palytoxin-bound pump–channels (BOX 1) indicates that, when both gates are open, more than a million Na ions flow through the pump per second. So, these transformed pumps provide a sobering illustration of the orders of magnitude increase in ion throughput, albeit dissipative, that are posited above to result from a breakdown in communication between a pump's two gates.

An ion pump from an embellished channel?

Although it is conceptually simpler for a transporter to lose function of a gate during evolution and to thereby become a channel, an ion pump could conceivably evolve from a channel by gaining a second gate. For instance, Kdp, a P-type ATPase that pumps K ions into bacteria[62], appears to have evolved by association of an ATPase component with a K ion channel polypeptide that has similarities in sequence to the tetrameric KcsA pore that is shown in FIG. 2a. However, neither the translocation route of the K ions transported by the Kdp complex nor the mechanism of transport is known yet[62]. And, as the ATPase component itself retains seven transmembrane helices that are homologous to those which in other P-type ATPases contain the ion-binding pocket and ion-translocation pathway[11,13,17–19,63–65], it seems premature to classify Kdp as a channel that has simply adopted a second gate.

Pump–channels with hybrid behaviour

Thus far, we have discussed examples of channels, of pumps, and of pumps converted to channels by evolutionary degradation of a gate (or inter-gate communication) or by pharmacological subversion of the tight coupling between a pump's two gates. But we have not explicitly considered molecules that can simultaneously display both pump and channel function. Given the observation that downhill ion flow through a typical channel is orders of magnitude faster than uphill flow through a pump, a single molecule combining those functions for the same ion travelling the same pathway makes no sense. But proton transport apparently conserved throughout evolution to gate electrodiffusive Cl currents in ClC channels[36,43], in part by protonating the external gate Glu residue[27,32], is a striking example of hybrid behaviour, involving different ions, in a single molecule. And, at least in the ClC Cl/H exchange pumps, the protons and Cl ions travel some of the way along separate paths[66]. The full route taken by the protons transported by ClC channels, however, and whether their transport serves any metabolic, or other, function is unknown.

Cl ion channels in neurotransmitter pumps. There are also other examples of hybrid pump–channels. Released excitatory and inhibitory transmitters and biogenic amines are cleared from synaptic spaces by two families of Na-coupled neurotransmitter pumps[67,68], and prokaryotic homologues of these pumps have begun to be structurally characterized[69]. Despite the distinct architectures of these pumps, binding of extracellular Na ions plus substrate triggers their occlusion in both families, and then a large conformational rearrangement releases the ions to the cytoplasm[69]. The inward-directed electrochemical gradient for Na ions thus powers, and ensures, net uptake of neurotransmitter.

Both in vertebrate glutamate pumps and in their purified, reconstituted, prokaryotic homologues, Na-dependent transport is accompanied by a thermodynamically uncoupled electrodiffusive flow of Cl ions[67,70–74]. The Cl current is not required for substrate transport, because that persists when the current is diminished by Cl replacement with impermeant anions[70,73] or by mutation of the pump[73,75]. The latter finding, the reconstitution of both transport and Cl current by purified protein[73], and impairment of both by inhibitors[73,76], all demonstrate that glutamate transporters are hybrid molecules that unite a stoichiometric pump with a separate channel-like pathway. Exactly how channel-like this pathway is remains unclear, as estimates of the Cl current size[71,72] indicate flows of only 10^4–10^5 Cl ions per second, which is again, like that of double-gate mutant ClC-ec1 pumps[33], too small for single-molecule recordings. Nevertheless, correlations between changes in stoichiometric transport and in Cl current suggest that only a subset of the pump conformations visited during the transport cycle can conduct Cl ions[71,72,74,76].

The 'one gate versus two gates' perspective

It is now certain that integral-membrane ion-transport proteins span a broad and continuous spectrum, from clear-cut, highly evolved, exquisitely-selective ion channels at one extreme to prototypical, primary, stoichiometric ion pumps at the other. And the evidence that emerges from the ClC protein family mandates a blurry region near the middle of this continuum. There we will find exceedingly fast pumps in which the gates are small and so can be moved rapidly, as well as extraordinarily slow ion channels in which the ion pathway embraces, and is deformed by, the conducted ions, thereby slowing their diffusion. We should not be surprised if the fastest pumps turn out to be capable of higher ion transport rates than the slowest open ion channels.

But there is still no reason to abandon the 'one gate versus two gates' formalism to distinguish pumps from channels. It is worth re-emphasizing that, more specifically, the distinction boils down to the requirement of strict coordination between a pump's two gates (whatever their physicochemical form). The ultimate functional distinction between a pump and a channel will always rest on whether the membrane protein is capable of thermodynamically uphill transport, against the electrochemical potential gradient. Those that meet this test are pumps, and may be further subdivided into primary or secondary active transporters, according to the coupled energy source. Let us call all the others channels, regardless of how many gates they have, or of how tightly they grip the ions they conduct. And as we ponder the sloth of the slowest ion channels, the growing structural understanding of membrane-spanning ion transport proteins should help us avoid the obfuscation and semantic diversion of applying to them outmoded terms such as carrier, uniporter and facilitated diffusion. Across the full spectrum of ion transport proteins there is much to learn about the features that define them — that is to say, about the structures of their binding pockets and gates, and about the mechanisms of gating and of gating coordination — even if we now have a clearer appreciation of the significance of those features.

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DATABASES

UniProtKB: http://ca.expasy.org/sprot
CFTR | channelrhodopsin 1 | channelrhodopsin 2 | ClC-1 | ClC-2 | ClC-ec1 | KcsA

FURTHER INFORMATION

David C. Gadsby's homepage: http://www.rockefeller.edu/labheads/gadsby/gadsby_home.html
Transport Classification Database: http://www.tcdb.org

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