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# Principles of Selective Ion Transport in Channels and Pumps

Eric Gouaux<sup>1\*</sup> and Roderick MacKinnon<sup>2\*</sup>

The transport of ions across the membranes of cells and organelles is a prerequisite for many of life's processes. Transport often involves very precise selectivity for specific ions. Recently, atomic-resolution structures have been determined for channels or pumps that are selective for sodium, potassium, calcium, and chloride: four of the most abundant ions in biology. From these structures we can begin to understand the principles of selective ion transport in terms of the architecture and detailed chemistry of the ion conduction pathways.

The flow of ions across the cell membrane is essential to many of life's processes. Ion pumps build gradients across the membrane, which are then used as an energy source by ion channels and other transport proteins to pump nutrients into cells, generate electrical signals, regulate cell volume, and secrete electrolytes across epithelial layers (1). Life depends on the continued flow of ions into and out of cells. But the cell membrane presents a serious energy barrier to an ion crossing it (Fig. 1). This is because ions are energetically more stable in water than in the oily substance of the membrane interior: Outside the membrane, polar water molecules point their charged edges toward an oppositely charged ion, but inside the membrane such stabilizing interactions are reduced. The resulting energy difference is so large that the predominant ions in biological systems— $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$ —would essentially never cross the membrane unaided. Ion pumps, ion exchangers, and ion channels (membrane proteins that we refer to here as the ion-transport proteins) are used by the cell to transport ions across membranes. For simplicity, we group the "active" transport proteins—the ion pumps and ion exchangers—together into a single category called pumps.

Ion pumps and ion channels fulfill very different functions. The pumps transport ions against their electrochemical gradient by coupling the "uphill" transport process to an energy source such as adenosine triphosphate (ATP) hydrolysis or the "downhill" movement of another ion or substrate molecule. Ion channels by contrast are passive, simply catalyzing the downhill movement of ions, in many cases

at very high ion conduction rates. Ion pumps and ion channels share one fundamental property: an ability to transport ions in a selective manner. Ion selectivity is crucial to the operation of ion-transport proteins.

Recent x-ray crystallographic studies have revealed at least one example of a transport protein for each of the predominant ions in biology. With the variety of architectural motifs now described, we can begin to see and appreciate that nature has come up with many different solutions for overcoming the energy barrier to allow an ion to cross the membrane. Here we will describe a subset of those solutions, with particular emphasis on the chemical principles by which ions are transported selectively across the membrane.

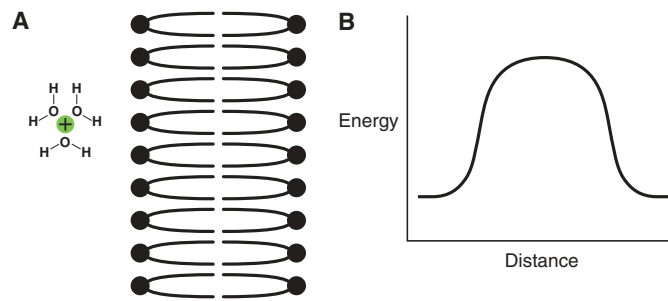
## Architecture

To understand how ion channels and ion pumps catalyze the passage of substrates across the membrane bilayer, we can begin by studying the proteins' architectures and shapes and how their shapes are related to the passageway across the membrane bilayer. Drawing from the potassium and chloride channel (ClC) families, and together with the sodium-dependent glutamate transport homolog ( $\text{Glt}_{\text{Ph}}$ ) and the  $\text{Ca}^{2+}$  ATPase, we see that in some cases the protein creates a proteinacious passageway spanning approximately the thickness of the entire membrane bilayer, whereas in other cases the protein creates aqueous cavities or vestibules, some of which reach more than halfway across the bilayer (Fig. 2), that are accessible from bulk solution. In these latter instances, ions and substrates reach selectivity filters or binding sites deep within the

membrane by simple diffusion through the aqueous solution.

Perhaps one of the most striking examples of how the architecture of a pump partially solves the problem of moving ions and charged substrates across the membrane can be found in the case of  $\text{Glt}_{\text{Ph}}$ , a prokaryotic homolog of sodium-coupled glutamate transporters (2): proteins that couple the uphill movement of glutamate to the energetically favorable movement of sodium. Here, the homotrimeric protein creates an outwardly facing aqueous basin, nearly 50 Å in diameter, the bottom of which is located approximately halfway across the membrane bilayer (Fig. 2). Because the binding sites for the substrate are located at the bottom of the basin and are accessible from the external aqueous solution, the substrate can simply diffuse through bulk solution to a binding site halfway across the membrane. The voluminous basin of these pumps effectively displaces the outer leaflet of the membrane with aqueous solution so that the protein only need catalyze the passage of the substrate and ions across one leaflet of the bilayer.

Potassium ion channel proteins, exemplified by the prokaryotic channel KcsA (3), provide a second example of how fundamental elements of architecture are intimately related to mechanisms of transmembrane transport. KcsA has a wide water-filled pore facing the cytoplasm, which traverses more than half of the membrane bilayer, ending at the selectivity filter, near the negative end charges of the four pore helices (Fig. 2). This aqueous region of the pore allows ions to rapidly diffuse across about two-thirds of the membrane bilayer be-

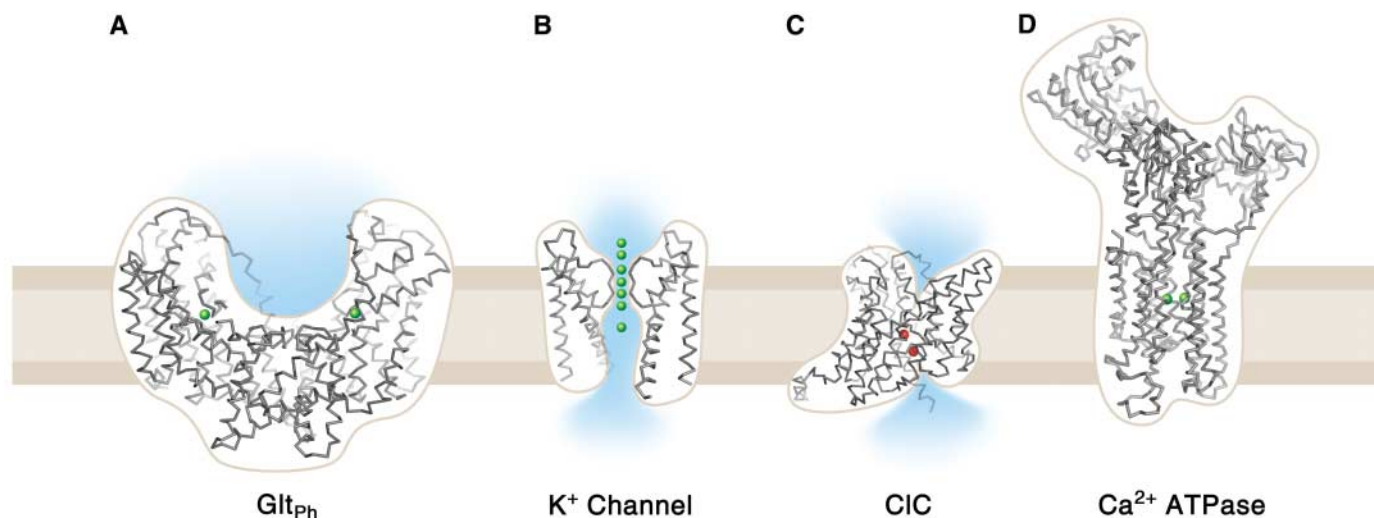


**Fig. 1.** The membrane presents an energy barrier to ion crossing. (A) Schematic of a positively charged cation (+ symbol) solvated by polar water molecules and of a membrane bilayer. (B) Simple graph showing that movement of the cation through the hydrophobic portion of the membrane bilayer is an energetically unfavorable process.

<sup>1</sup>Vollum Institute and Howard Hughes Medical Institute, Oregon Health and Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA.

<sup>2</sup>Howard Hughes Medical Institute, Laboratory of Molecular Neurobiology and Biophysics, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

\*To whom correspondence should be addressed. E-mail: gouauxe@ohsu.edu (E.G.); mackinn@rockefeller.edu (R.M.)



**Fig. 2.** Architectures of ion channel and ion pump proteins showing approximate locations of solvent-accessible or solvent-filled regions of the proteins (blue shading) and the relative position of the membrane (horizontal beige bands). (A) The bottom of the aqueous basin of the  $\text{Glt}_{\text{Ph}}$  pump lies near the middle of the bilayer and allows direct access, from bulk solution, to substrate (green spheres) and ion binding sites [Protein Data Bank (PDB) code 1XFH]. (B) An open-channel model of the  $\text{KcsA}$   $\text{K}^+$  channel, based on the structure of the  $\text{MthK}$  channel (PDB code 1LNQ), possesses a water-filled pore in the middle of the transmembrane portion of the channel

and a selectivity filter near the outside occupied by  $\text{K}^+$  ions (green spheres) (PDB code 1K4C). (C) Funnel-shaped structures are present in the CIC Cl transport proteins and allow ions (red spheres) to reach the selectivity filter, located at the midpoint of the bilayer (PDB code 1OTS). (D) A structure of the calcium-bound form of the  $\text{Ca}^{2+}$  ATPase shows that this transport protein does not have large solvent-filled cavities (PDB code 1SU4). Rather, the two bound ions (green spheres) occupy solvent-occluded sites within the transmembrane domains of the pump. In (A) to (C), the intracellular surfaces are “down,” whereas in (D) the intracellular surface is “up.”

fore reaching the selectivity filter. The selectivity filter, only  $\sim 12$  Å in length, is the locus of ion discrimination and forms the remainder of the passageway across the membrane bilayer. In potassium channels, a long aqueous passageway connecting to a short selectivity filter is probably a key element of the channel architecture that contributes to the remarkably high flux rates of  $\sim 10^8$  ions per second.

Not all ion-transport proteins have large aqueous vestibules within the transmembrane-spanning portion of the protein, however. The CIC chloride channels and chloride proton exchangers (CIC Cl transport proteins), in contrast to potassium channels, harbor double hourglass-like funnels at the extracellular and cytoplasmic surfaces, leading to a narrow constriction—the anion selectivity filter—located at the middle of the bilayer (4, 5). When the channel is viewed parallel to the membrane (Fig. 2), one can see that the two funnels are related by a pseudo twofold axis, and indeed the protein folds of the amino and carboxy terminal halves of each subunit are related by a twofold axis. A roughly similar shape is also seen in the glycerol (6) and water channels (7, 8), which suggests that double-funnel architecture may be a relatively common shape in channels and pumps. If this is the case, what purposes(s) might such architecture serve? On the one hand, the double-funnel architecture, together with the twofold axis parallel to the membrane, may facilitate the formation of ion binding sites and selectivity filters at the midpoint of the membrane bilayer that are at least partially composed of reentrant

helices and loops. On the other hand, this architecture may also be rooted in evolution; perhaps the primordial ancestors of CIC proteins, as just one example, were about half the size of their modern-day counterparts and were bona fide dimers. Over time, gene fusion and mutation gave rise to the present-day proteins.

Knowing the structure of an ion channel or ion pump does not always illuminate how the ion or substrate reaches binding sites within transmembrane domains, and it appears that at least some pumps (the  $\text{Ca}^{2+}$  ATPase being an example) certainly do not possess tell-tale vestibules or channels and may have particularly cryptic pathways for substrate entrance and egress. Manifold studies of the  $\text{Ca}^{2+}$  ATPase, a paradigm of so-called P-type ATPases, still have not clearly revealed the route(s) by which calcium binds to and leaves from occluded transmembrane binding sites, even though there are crystal structures of nearly every intermediate along the transport pathway (9). All we know at present is that calcium may reach the intramembrane binding sites, from the cytoplasm, via a narrow pathway lined with acidic residues. Surprisingly, studies have not yet uncovered a convincing pathway from the occluded binding sites to the lumen, and thus conformational states not yet observed must exist to allow calcium to reach the lumen.

In spite of the remarkably variable architectures and shapes of channel and pump proteins, they can teach us some useful lessons. First, ion-channel proteins, whose business is typically to catalyze the selective passage of ions at a high rate, have wide regions of their

pores that allow ions to remain solvated by water as long as possible before reaching constrictions that confer selectivity; these regions are elements of protein structure that may span only a fraction of the bilayer thickness. Second, although some pumps such as  $\text{Glt}_{\text{Ph}}$  have large basins that reach to substrate binding sites deep within the membrane, other pumps, such as the  $\text{Ca}^{2+}$  ATPase, do not. Furthermore, for  $\text{Glt}_{\text{Ph}}$  and the  $\text{Ca}^{2+}$  ATPase, there are no apparent pathways for ions and substrate to pass from occluded binding sites to the cytoplasm and lumen, respectively, and thus additional, as yet uncharacterized, conformational states must exist. Although the flux rates of channels and pumps vary by as much as  $10^6$ -fold and they have completely different structures, both classes of proteins exhibit selectivity for their cognate substrate, and in the next section we examine the physical and chemical principles underlying ion selectivity in channels and pumps.

### Ion Selectivity

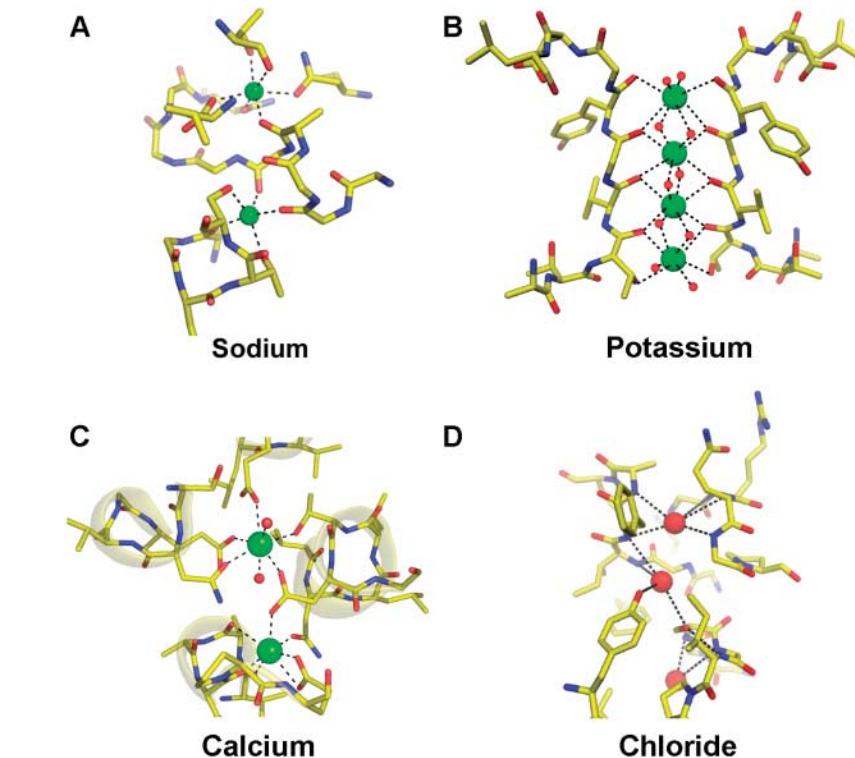
Most transport proteins need to get certain ions across the membrane while at the same time excluding others. Such ion selectivity can be extremely precise, for instance, between ions as similar as  $\text{Na}^+$  and  $\text{K}^+$  (1). Ion selectivity between such similar ions requires the ion pathway to have specific binding sites over at least part of its length. These sites allow a transport protein to “feel” ions to ensure that only the right ones can pass. Of course, for an ion to be felt, it has to be dehydrated (at least partially if not completely),

and dehydration costs energy. Binding sites therefore have to compensate for the energetic cost of dehydration by providing favorable compensatory interactions with the ion. Selectivity results when this energetic compensation is more favorable for one type of ion than for another, relative to the energy of dehydration. Studies over the past 50 years on synthetic and naturally occurring ion-binding small molecules (host/guest chemistry with ions) have established the basic rules of ion selectivity within small molecules (10). Two major factors contribute to ion binding site selectivity: the atomic composition and the stereochemistry (e.g., size) of the binding site. Recently, protein structures have begun to show us for the first time how ion selectivity for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$  ions is accomplished in membrane transport proteins.

### Sodium and Potassium

The alkali metal ions  $\text{Na}^+$  and  $\text{K}^+$  are the most abundant cations in biological systems.  $\text{Na}^+$  ions are most often present at high concentrations outside the cell, and  $\text{K}^+$  is present at high concentrations inside. Gradients for these ions across the cell membrane provide the energy source for action potentials generated by opening  $\text{Na}^+$  and  $\text{K}^+$  channels, for synthesizing ATP in some organisms (11, 12), and for moving solutes and other ions across the cell membrane via coupled transporters. The  $\text{Na}^+$ -selective binding sites in the  $\text{Na}^+$ -dependent leucine transporter LeuT (13) and the  $\text{K}^+$ -selective binding sites in the  $\text{K}^+$  channel (14) provide a direct comparison of selectivity among these ions in transport proteins at high resolution.

LeuT transports leucine and  $\text{Na}^+$  in the same direction across the cell membrane (13). By coupling the transport of leucine to  $\text{Na}^+$ , LeuT uses the energy of the  $\text{Na}^+$  gradient to pump leucine into the cell. The atomic structure shows a leucine and two  $\text{Na}^+$  ions bound deep inside the protein, partway across the membrane (Fig. 3A). The  $\text{Na}^+$  ions are completely dehydrated. One site contains six oxygen atoms in direct contact with the ion. Five of these oxygen atoms bear only a partial negative charge (the main-chain carbonyl, side-chain hydroxyl, and side-chain amide atoms) and one bears a full negative charge on a carboxylate group. The second site contains five oxygen atoms surrounding the  $\text{Na}^+$  ion. In this case, all the oxygen atoms bear only a partial negative charge (the main-chain carbonyl and side-chain hydroxyl atoms). Two important features of these binding sites are evident. First, the binding sites consist of oxygen atoms in direct contact with  $\text{Na}^+$ ; a formal negative charge can occur (site 1) but is not essential (site 2). Second, the size of the binding site cavity formed by the oxygen atoms is a good match to the  $\text{Na}^+$  ion, with a mean  $\text{Na}^+\text{-O}$  distance for both sites combined of 2.28 Å.



**Fig. 3.**  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$ -selective binding sites in transport proteins. (A) Two  $\text{Na}^+$  binding sites in the LeuT  $\text{Na}^+$ -dependent pump (PDB code 2A65). (B) Four  $\text{K}^+$  binding sites in the KcsA  $\text{K}^+$  channel (PDB code 1K4C). (C) Two  $\text{Ca}^{2+}$  binding sites in the  $\text{Ca}^{2+}$  ATPase pump (PDB code 1EUL). (D) Two central  $\text{Cl}^-$  binding sites in a mutant ClC  $\text{Cl}^-/\text{H}^+$  exchanger (PDB code 1OTU).

$\text{K}^+$  channels conduct  $\text{K}^+$  ions selectively across the cell membrane, down the electrochemical gradient. The  $\text{K}^+$  channel contains four  $\text{K}^+$  binding sites in a row, forming a selectivity filter (Fig. 3B) (3, 14). In each of these sites, a  $\text{K}^+$  ion is dehydrated and interacts with eight partial-charge-bearing oxygen atoms (main-chain carbonyl or side-chain hydroxyl atoms). The size of the cavity formed by the selectivity filter sites is a good match to the  $\text{K}^+$  ion, with a mean  $\text{K}^+\text{-O}$  distance of 2.84 Å. The greater number of oxygen atoms forming the  $\text{K}^+$  binding sites (eight oxygen atoms) compared to that of the  $\text{Na}^+$  sites (five or six oxygen atoms) is a simple geometric consequence of the larger radius of  $\text{K}^+$ , which allows a greater number of oxygen atoms to surround the ion (10, 15, 16).

What does comparison of LeuT and the  $\text{K}^+$  channel teach us about alkali metal ion selectivity in transport proteins? The  $\text{Na}^+$  and  $\text{K}^+$  sites both contain oxygen atoms, mostly the kind with partial negative charges. This agrees well with the rules learned from host/guest chemistry with ions. There is a tendency for  $\text{Na}^+$  sites to contain one formal charge, undoubtedly owing to this ion's smaller radius and higher charge density, but a formal charge is not essential for a  $\text{Na}^+$ -selective site (10, 15). A very important factor distinguishing  $\text{Na}^+$  and  $\text{K}^+$  sites is the size of the cavity formed by the binding site. This also

agrees with the small-molecule chemical literature. Chemists have created molecules of a given class with selectivity favoring  $\text{Li}^+$  (radius 0.60 Å),  $\text{Na}^+$  (radius 0.95 Å),  $\text{K}^+$  (radius 1.33 Å), or  $\text{Rb}^+$  (radius 1.48 Å) by simply adjusting the cavity size to match the ion (10). LeuT and the  $\text{K}^+$  channel suggest that the essence of alkali metal cation selectivity is similar to that in ion-binding small molecules: The protein selects for a particular ion,  $\text{Na}^+$  or  $\text{K}^+$ , by providing an oxygen-lined binding site of the appropriate cavity size.

Size selectivity does not mean that an ion binding site has to be rigid. A rigid, preformed, binding-site cavity can increase its affinity for an ion through entropic terms in the free energy (10), but it is not a requirement for size selectivity. Size selectivity comes about through the potential of a molecular structure to conform more favorably to an ion of a particular size. Beautiful examples of this are provided by nonactin and valinomycin, which are molecular cages that bind  $\text{K}^+$  selectively over  $\text{Na}^+$  (17, 18). The structure of these molecules changes depending on whether the binding site is empty or occupied by  $\text{K}^+$  or  $\text{Na}^+$  (17, 18). These molecules are not rigid, but we can nevertheless understand their selectivity in terms of the energetic stability of the  $\text{K}^+$  complexes because of the shapes and sizes they are able to adopt. Nonactin and valinomycin more com-

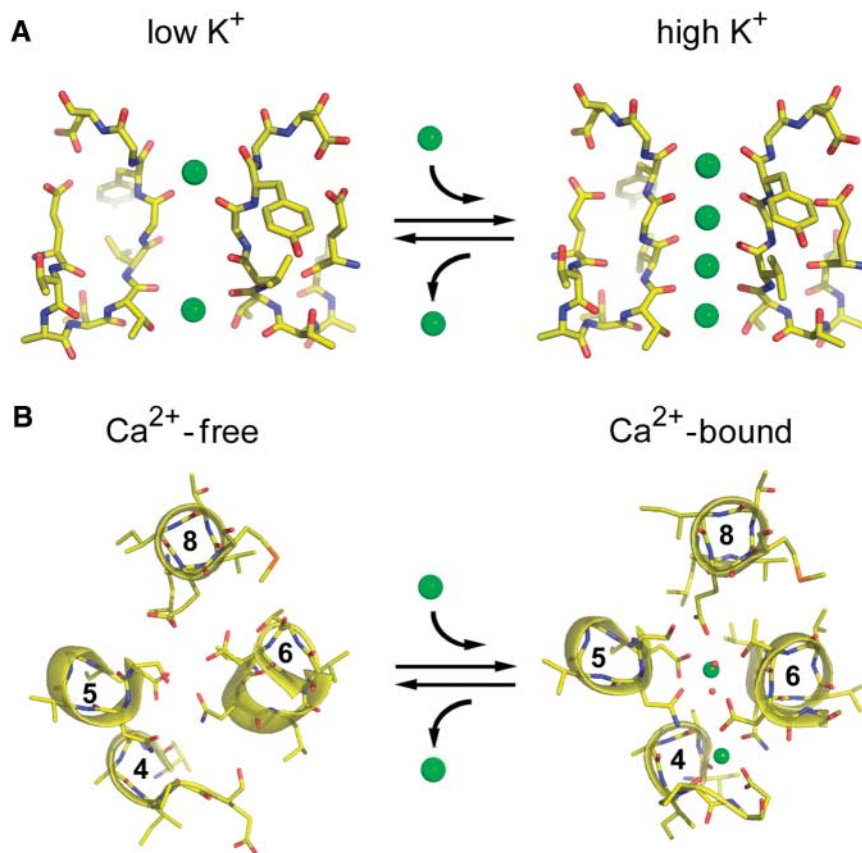


fortably (that is, without strain) fit around a  $K^+$  ion and so more effectively compensate for the energy cost of  $K^+$  ion dehydration. Indeed, the  $K^+$  channel in this respect is quite similar to, but perhaps even more extreme than, the small  $K^+$ -selective molecules. In crystal structures, when  $Na^+$  replaces  $K^+$  in solution, the selectivity filter of the  $K^+$  channel undergoes a conformational change and collapses shut, occluding two of the four binding sites (Fig. 4A) (14, 19). In other words, the selectivity filter sites in a  $K^+$  channel comfortably fit around  $K^+$  ions and not  $Na^+$  ions when the filter adopts what we recognize as a conductive conformation. This is an elegant example of size selectivity, and it has little to do with the rigidity of the ion binding sites. In fact, one could say that the selectivity for conducting  $K^+$  ions is enabled by the ability of the protein to undergo particular conformational changes and thus form the correct coordination sphere for  $K^+$  in response to the presence of  $K^+$ .

The importance of a connection between protein conformation and ion binding site selectivity (and affinity) is even more obvious in the case of ion pumps, in which the properties of ion binding are coupled to conformational states of the protein in an obligatory manner in order to move ions against their electrochemical gradient (the  $Ca^{2+}$  ATPase), or to allow ion gradients to move another substrate against its gradient (LeuT).

### Calcium

The  $Ca^{2+}$  ATPase pump has to discriminate between  $Ca^{2+}$  and the other dominant cations in biology:  $Na^+$ ,  $K^+$ , and  $Mg^{2+}$  (20). Atomic structures in numerous conformations have revealed how this pump creates high-affinity  $Ca^{2+}$  binding sites in one conformation and then reorganizes the sites in another to change their  $Ca^{2+}$  affinity (21, 22). In the high-affinity conformation,  $Ca^{2+}$  ions bind at two sites wedged between  $\alpha$  helices inside the membrane (Fig. 3C). One site is formed out of oxygen atoms from side chains and water molecules, and the other is formed out of side- and main-chain oxygen atoms. The main-chain oxygen atoms are made available because one of the  $\alpha$  helices is disrupted inside the membrane, freeing the carbonyl oxygen atoms from their usual hydrogen-bonding interactions. Seven oxygen atoms surround the  $Ca^{2+}$  ion in both sites. An obvious difference between the  $Ca^{2+}$  binding sites and the  $Na^+$  and  $K^+$  sites discussed above is the greater importance of fully charged oxygen atoms contributed by glutamate and aspartate side chains: A higher charge density is apparently required to compensate for the dehydration of a divalent cation. Because  $Ca^{2+}$  is such an important regulatory ion inside cells, there are many examples of proteins with  $Ca^{2+}$  binding sites (23). A coordination number of seven is



**Fig. 4.** Ion binding correlated with protein conformational changes in a  $K^+$  channel and a  $Ca^{2+}$  pump. (A) The selectivity filter of a  $K^+$  channel in the presence of low  $K^+$  concentrations (high  $Na^+$ ) undergoes a conformational change that occludes its two innermost binding sites (left, PDB code 1K4D).  $K^+$  entry at high  $K^+$  concentrations induces the conductive filter conformation, which contains four sequential  $K^+$  binding sites (right, PDB code 1K4C). (B) Ion binding loci in a  $Ca^{2+}$ -free conformation of the  $Ca^{2+}$  ATPase (left, PDB code 1IWO) undergo conformational changes to form a  $Ca^{2+}$ -bound conformation (right, PDB code 1EUL). Helix numbers are shown.

very common, just as is observed in the  $Ca^{2+}$  ATPase sites, and we can understand selectivity for  $Ca^{2+}$  over  $Mg^{2+}$  at least in part because  $Mg^{2+}$  prefers a coordination number of six (24).

In the  $Ca^{2+}$ -free conformation, the  $\alpha$  helices within the membrane undergo structural rearrangements that disrupt the  $Ca^{2+}$  binding sites by shifting the positions of the coordinating oxygen atoms (Fig. 4B) (22). The  $Ca^{2+}$  ATPase provides a very good example of what must be a general property of transport proteins: the ability to form selective ion binding sites in a protein conformation-dependent manner.

### Chloride

The  $Cl^-$  ion is the only halogen ion used in abundance in biological systems.  $Br^-$  and  $I^-$  are used in specialized circumstances, but their abundance is low. Thus,  $Cl^-$  transport proteins seem to be faced with the modest challenge of selecting  $Cl^-$  over phosphate, sulfate, bicarbonate, and anionic proteins. Crystal structures of two prokaryotic members of the CIC  $Cl^-$  channel/ $Cl^-$ -H $^+$  exchanger family provide

a first view of the chemistry underlying  $Cl^-$  selectivity in transport proteins (4, 5). These proteins blur the distinction between channels and pumps: Some members of the CIC family are channels (such as CIC-0 from the torpedo fish) (25) whereas others are  $Cl^-/H^+$  exchangers (such as CIC from *Escherichia coli*) (26), and yet their related amino acid sequences indicate that they have essentially the same protein structure. Apparently, subtle properties of the ion pathway distinguish the channel and pump members of this family.

The CIC transport proteins have a  $Cl^-$  selectivity filter located at the neck of an hourglass-shaped ion pathway that forms a row of  $Cl^-$  ion binding sites near the center of the membrane (Fig. 3D) (4, 5). The resolution of the CIC structures is not as high as for the  $Na^+$  and  $K^+$  transport proteins, but the basic chemistry of the binding sites is defined. In the narrowest region of the filter, chemical groups that surround two  $Cl^-$  ions share their proton to stabilize the ion's negative charge: The hydroxyl from a tyrosine side chain readily shares its proton because the aromatic ring stabilizes the excess negative charge left

on the oxygen atom; the serine hydroxyl, because it is hydrogen-bonded through its lone electron pair to a main-chain amide, also shares its proton readily; and finally, main-chain amide groups direct their proton toward  $\text{Cl}^-$ . This selectivity filter does not distinguish between  $\text{Cl}^-$  and  $\text{Br}^-$  with high fidelity (27), but in nature such discrimination is not required. Selectivity in the ClC transport proteins can thus be understood in simple terms: The anion is stabilized by partial positive charges, and the filter is wide enough to permit  $\text{Cl}^-$  but not larger competing anions.

### The Relationship Between Channels and Pumps

In our considerations of ion selectivity, we have grouped channels and pumps as a single collection of transport proteins because the chemical principles of ion discrimination are likely to be the same in both. But in our consideration of some of the architectural principles of transport proteins, we highlighted several unique structural aspects—protein shapes—that are related to the need for channels to conduct ions rapidly and for pumps to move ions against an electrochemical gradient. These different requirements of channels and pumps must influence certain aspects of the ion-binding sites, in particular their number and disposition in the transport protein. We observe in the  $\text{K}^+$  channel a queue of four  $\text{K}^+$  binding sites spanning a distance of 12 Å, which then opens into wide aqueous regions on both ends. This arrangement of ion-binding

sites makes sense for rapid conduction because it provides the shortest path for diffusion across the membrane and for repulsion between adjacent ions in the queue. We observe in the LeuT and  $\text{Ca}^{2+}$  ATPase pump proteins something very different: ions embedded deep inside the protein with no unobstructed pathway to the aqueous surfaces. This arrangement makes sense for proteins that can move an ion against its concentration gradient (using the energy of ATP hydrolysis or a chemical gradient for leucine), because what is most important for a pump is not speed, but the property that it will never open its ion pathway to both sides of the membrane at the same time. A crystal structure of a pump should thus under most circumstances show buried ions. The ClC proteins, on the other hand, seem to be an intermediate class of transport proteins in between pumps and channels. Structurally, they exhibit channel-like features, including vestibules leading to a short selectivity filter with a queue of ions. Functionally, some are true channels whereas others are pumps driven by the countertransport of protons. Perhaps as the collection of ion-transport proteins grows, we might expect to see a continuum ranging from the channels to the pumps.

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