Understanding ion channel selectivity and gating and their role in cellular signalling†

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Ion channels play an essential role in the communication between and within cells. Here some of the different ion channel proteins and the roles they perform are introduced, before a discussion of the mechanisms by which they discriminate between different ion types and open and close to allow the passage of ions at the appropriate times.

Introduction

Communication between and within cells is an integral part of all living organisms. Although a vast variety of mechanisms are used in this signalling process, perhaps the most fundamental and ubiquitous means by which messages are sent is through the movement of charged ions. The membrane that surrounds cells and differentiates intracellular compartments is impermeable to charged molecules and therefore provides a way to separate regions of differing ion concentrations. A number of membrane bound proteins, the ion pumps and transporters, set up such concentration and potential differences by using energy in the form of ATP or the flow of a different ion down its own concentration gradient to move an ion species across the membrane. This mechanism is relatively slow, with a typical pump moving in the order of 300 ions across the membrane each second.[1] Once such a concentration and potential difference has been set up, however, a very rapid signal can be sent by opening a pore across the membrane through which ions can passively flow down the concentration and potential gradient. Indeed, even a narrow pore can move in the order of 10^7 ions across the membrane every second. Ion channels are proteins that can form such a pore, and by doing so at the appropriate times they regulate electrical signalling within living organisms. By allowing ion permeation and thus rapidly changing the local conditions within the cell, they can initiate a range of intracellular responses. Given the importance of ion channels to cellular communication, it is perhaps not surprising to note that half the metabolic energy consumed by the human brain is used by the ion pumps that move K^+ and Na^+ in and out of nerve cells to allow the rapid nerve signalling that is regulated by ion channels.[2]

The ubiquity and versatility of ion channels can be appreciated by the variety of biological roles they perform. Electrical signalling is perhaps most visible in action potentials, the electrical wave that passes along firing neurons, that is carried by the flux of Na^+ into the cell and inactivated by a subsequent outflux of K^+. On top of this, ion channels are largely responsible for initiating intracellular responses such as muscle contraction, neurotransmitter secretion, converting sensory or chemical input into electrical signals, regulating cell volume and many other things.

There are many different types of ion channels to carry out the vast variety of functions required of them. These channels differ in their localisation within the organism, when they open and close to allow or prevent the flow of ions, the types of ions that they will pass and the rate at which they do so. Some channels allow many types of ions to pass, whereas others are much more selective, allowing just one or a few of the predominant physiological ion species, Na^+, K^+, Ca^{2+} and Cl^- passage. Also, the opening of channels can be initiated in many different ways. There are ligand-gated channels that open when one or two molecules known as neurotransmitters, secreted from nerve terminals, bind to the channel. Some channels, the so-called voltage-gated channels, open in response to step changes in the electric field, while others, mechanosensitive channels, respond to pressure on the membrane or cytoskeleton.

Although a great number of questions still remain to be answered, our understanding of how ion channels function at a molecular level has advanced considerably over the last 10 years. In large part, this has been due to the determination

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† The HTML version of this article has been enhanced with additional colour images

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of atomic resolution structures of a number of channel proteins using X-ray diffraction and other techniques. In combination with a variety of other biophysical and computational methods this has allowed the physical principles underlying the function of ion channels to be understood.

The aim of this article is to introduce in general terms the variety of roles performed by ion channels within living organisms and the assortment of ion channels that undertake these. Then, more specific details will be given about the molecular mechanisms of ion selectivity and channel gating that are essential to the functioning of these proteins, illustrating these principles with examples of ion channels for which detailed structural information has recently become available.

Roles and diversity of channels

Since there is such a variety of ion channels and they are used in a host of different ways it is difficult to accurately summarise their role in cellular signalling. In general, it is true to say that Na^+ and K^+ channels are used to control the electrical activity of cells, while Ca^{2+} channels are used to convert these electrical signals into responses. In reality, Ca^{2+} channels also participate in and help control electrical signalling, but it is the role of Ca^{2+} in initiating intracellular responses (as a so called 'second messenger') that makes it unique. Cl^- is also used to help control membrane potentials and cell volumes. Ion pumps and transporters are used to set up concentration gradients across the cell membrane as illustrated in Fig. 1. One of the most common, the Na^+–K^+ pump, moves Na^+ out of cells while at the same time moving K^+ inwards. Not only does this result in higher Na^+ concentrations outside the cell and higher K^+ concentrations in the cytoplasm, because 3 Na^+ are moved out of the cell for every 2 K^+ that come in, the electric potential inside the cell is typically about −60 mV with respect to the outside. Calcium concentrations are also kept low inside the cytoplasm of cells, but rather than being expelled into the external solution, the majority of Ca^{2+} is kept in intracellular compartments such as the sarcoplasmic reticulum. When a pore is formed across a membrane, a net current of ions will pass through it due to these concentration and potential gradients. The driving force is a result of the electrochemical potential gradients that can be estimated for any ion type from the Nernst equation

$$\Delta V = V_m - kT/q \ln(C_i/C_o),$$

in which ΔV is the electrochemical potential of the inside of the cell relative to the outside, V_m is the electric potential, C_i and C_o are the concentrations of the ion species inside and outside the cell, k is Boltzmann's constant, T is the temperature and q is the charge on the ion species. To illustrate how these concentration and potential differences are used by ion channels in cellular signalling, the operation of the neuromuscular junction and the action potential is described.

Communication between a nerve cell and a neighbouring skeletal muscle cell occurs through the release of chemical messengers known as neurotransmitters, (in this case predominantly acetylcholine) in a region known as the synapse where the two cells are physically close together. This neurotransmitter release is prompted by the influx of calcium from the extracellular solution through presynaptic calcium channels (Fig. 2).[3] Acetylcholine that binds to specific ligand

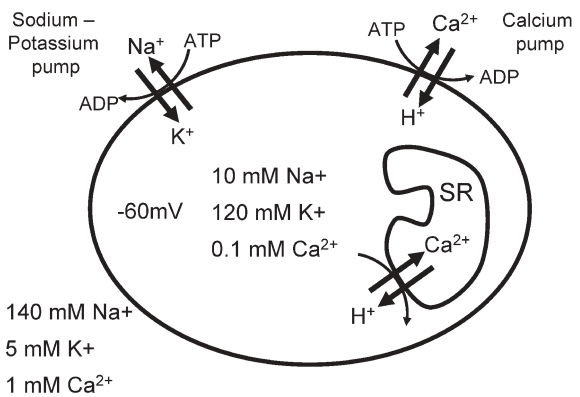

Fig. 1 Resting ion concentrations in a typical muscle cell. Concentrations of the major cations discussed in the text are indicated, along with the predominant ion pumps that control these. These pumps use the energy released in the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) to move ions against their concentration gradients into or out of cells or intracellular organelles such as the sarcoplasmic reticulum (SR).

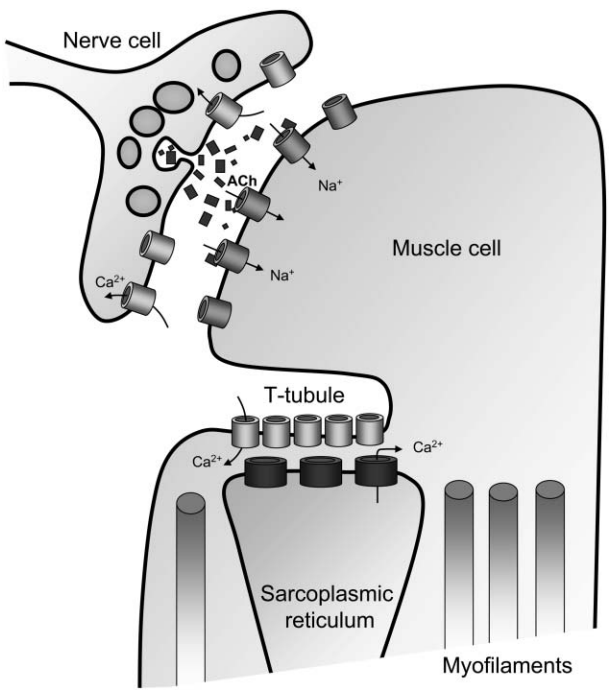

Fig. 2 Schematic depiction of ion channels at the neuromuscular junction. The influx of calcium into the nerve cell through presynaptic voltage-gated calcium channels prompts the release of acetylcholine (ACh) into the extracellular space. When this binds to receptors in the muscle cell, they allow Na^+ to flow into the cell. The changing membrane potential prompts voltage-gated calcium channels to open which in turn leads to the release of calcium from intracellular stores such as the sarcoplasmic reticulum through calcium release channels. The resulting increase in calcium concentration in the cell promotes muscle contraction. Figure inspired by ref. 24.

gated ion channels in the adjacent muscle cell, nicotinic acetylcholine receptors, prompts them to open a cation selective channel and leads to the flux of Na^+ into the cell.[4] This in turn alters the electric potential across the membrane which prompts the opening of voltage-gated Ca^{2+} channels located in folds of the plasma membrane known as T-tubules. The resulting influx of Ca^{2+} and depolarisation of the cell in turn prompts the opening of additional calcium channels in the sarcoplasmic reticulum membrane.[5] Calcium is now the switch that initiates the contraction of muscle fibres. These fibres are formed from many overlapping strands, comprising a thick filament made from myosin and a thin filament comprised of actin and tropomyosin. When Ca^{2+} is released from intracellular stores, the cytoplasmic Ca^{2+} concentration increases by up to 100 fold and Ca^{2+} binds to troponin present on the thin filaments, and alters the tropomyosin to unblock a series of myosin binding sites. The myosin can then be powered by hydrolyzing ATP to move along these binding sites causing muscle contraction until the cytoplasmic Ca^{2+} is removed (either into the sarcoplasmic reticulum or out of the cell by ion pumps and transporters) and the myosin binding sites are once more blocked.

Nerve impulses use similar principles to those involved in stimulating muscle contraction, but in this case Ca^{2+} plays a smaller role.[1] Signals are again sent between cells using neurotransmitters that bind to ligand gated ion channels on neighbouring cells. Here the resulting influx of Na^+ is used to stimulate the opening of voltage-gated Na^+ channels that let more Na^+ into the cell and further alter the membrane potential. This in turn stimulates the opening of Na^+ channels further along the cell, such that a wave of channel openings occurs and a pulse of electrostatic potential moves along the cell. Voltage-gated K^+ channels also open upon changing the membrane potential, but at a much slower rate. When this occurs K^+ flows out of the cell, reducing the membrane potential and inactivating the nerve impulse in that region of the cell.

Even within the simplified descriptions provided above some of the diversity of ion channels can be appreciated. Not only do the channels described differ in the types of ions they will conduct, they also differ in the rate at which they do so. The Ca^{2+} channels in the sarcoplasmic reticulum, for example, typically conduct at much greater rates than the voltage-gated Ca^{2+} channels in the plasma membrane in order to rapidly increase cytoplasmic Ca^{2+} concentrations and initiate intracellular responses. While acetylcholine receptors are common at the neuromuscular junction, channels that respond to other chemicals such as γ-aminobutyric acid (GABA), glycine, glutamate and serotonin are more common at other synapses, such as those between adjacent nerve cells. Furthermore, some of these receptors contain a cation selective pore, some an anionic pore, some aid in the formation of nerve impulses and some inhibit them. Within the voltage-gated channels there is again much diversity, with channels responding at different rates and to different potentials. In addition there is a vast variety of channels that have not been mentioned in this description, such as the majority of chloride channels and channels that respond to mechanical stimuli, such as those involved in the sense of hearing.

Ion selectivity

To discriminate between different ion types, ion channels must find a physical property such as the size, charge or charge density that is different between the desired ion type and the remainder. While a narrow pore will block the passage of large organic ions or proteins and allow small ions to pass, to be able to differentiate between small ions with similar sizes such as K^+ and Na^+, more detailed interactions between the ions and the protein are required. The recent availability of structures determined using X-ray diffraction has meant that the protein atoms responsible for this interaction can be pinpointed.

Ion channels have to be able to rapidly transport ions, which is most easily achieved by a wide pore. But, channels also have to be able to select between ion types, which is most easily achieved with a narrow pore. The determination of the structure of a range of potassium channels has shown how this compromise can be reached.[6] This is done by utilising a short narrow section of the pore for discriminating between ions (known as the 'selectivity filter') while keeping the rest of the pore much wider to aid rapid diffusion (see Fig. 6C). Indeed this basic architecture of a narrow selectivity filter and a wider remainder of the pore appears to be common in ion channels and even many ion pumps.[7] Ion selectivity, then, takes place in a specific region of the pore and can be described as involving selective binding sites, localised regions within the conduction pathway in which the protein interacts strongly with the ions and in which they are likely to dwell for prolonged periods of time. Ions can often be seen residing in these binding sites within crystal structures of the protein, and the specific interactions between the ion and the protein can therefore be determined.

Ions of differing charge

Ions of differing charge experience different electrostatic interactions with the protein atoms, and not surprisingly most studies highlight this fact when explaining the preference of some ion channels for ions of a specific valence. All the cation selective channels that have been studied in detail have been found to have a net negative charge lining the pore that will attract cations, while repelling anions.[8] Although the effect can be subtle, it slowly became apparent when studying the small antibiotic gramicidin, the first channel for which an atomic resolution structure was determined. As illustrated in Fig. 3 this channel is atypical in that it forms a long narrow passageway barely 4 Å wide. Importantly, although the protein is neutral overall, the conducting pathway is lined by polar regions of the protein with the partially negatively charged backbone carbonyl oxygens pointing slightly inward towards the pore.[9] Studies in a variety of lipid bilayers show that monovalent cations pass through the channel at high conductance, divalent cations block the conductance of the channel, and anions neither permeate nor block the pore.[10] There was much debate as to whether the selectivity of cations over anions in this channel was a thermodynamically or kinetically controlled property.[11–13] The thermodynamic explanation stresses that binding sites within the channel are energetically more favourable to cations than anions. The

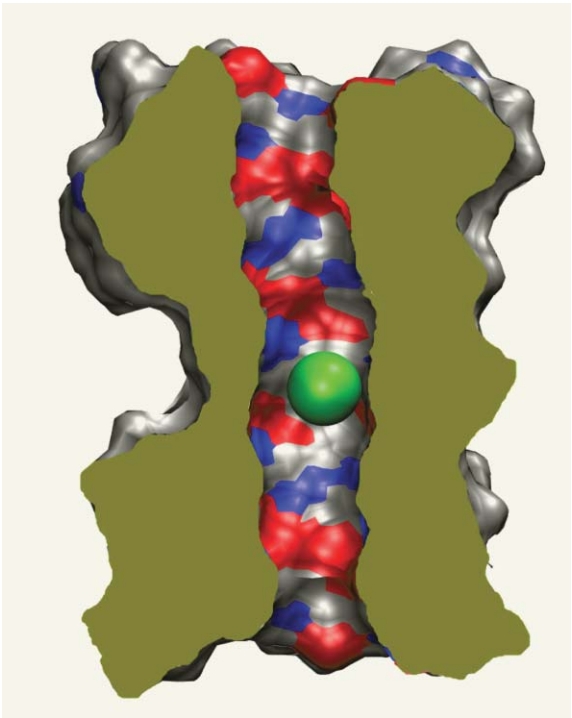

Fig. 3 Selectivity in gramicidin channels. The pore is lined by a series of oxygen (red) and nitrogen atoms (blue). The slightly negative pore lining attracts cations and repels anions.

kinetic explanation, on the other hand, asserts that there need not be a significant difference in the energy of the different ions within the channel provided there is a large energy barrier preventing anions from entering the binding site and thus slowing their rate of permeation.[12] Although a number of theoretical studies suggest that the thermodynamic principle is more likely to be in action within this pore,[8] in both explanations the specific distribution of charge amongst the protein atoms is responsible for either creating selective binding sites or energy barriers.

A similar asymmetric charge distribution within the protein appears to lie behind the selectivity of most channels. K^+ channels, for example, have a line of carbonyl oxygen atoms lining their selectivity filter. By presenting the negative end of the carbonyl dipole to the pore, cations will be attracted while anions will be repelled. Voltage-gated Na^+ and Ca^{2+} channels are believed to have a similar architecture to the crystalised K^+ channels, but with a number of charged amino acid side chains lining the selectivity filter rather than the carbonyl oxygens, which again create cation selectivity. In Cl^- channels the situation is reversed, with excess positive charge near the conduction pathway as can be judged from the crystal structure of a chloride transport protein that is closely related to a number of Cl^- channels.[14] In this case a number of backbone amide groups and polar side chains with the possible aid of some fully charged basic residues appear to result in anion selectivity.[15–17]

Perhaps more interesting than the discrimination between cations and anions is that between monovalent and divalent cations. If the voltage-gated potassium and calcium channels have a similar architecture, why does the first get blocked by divalent cations, when the second conducts them? The discrimination between monovalent and divalent cations cannot just rely on presence of a net negative charge as this acts to attract both kinds of ion. Rather, a number of studies suggest that it relies on the exact strength of the attraction to the permeating ions.[18–22] A divalent ion has a stronger electrostatic interaction with any charge on the protein than a monovalent ion due to its larger charge. This fact on its own appears to be enough to explain why potassium channels are blocked by divalent ions. This channel is believed to utilise a 'knock-on' mechanism of conduction in which the pore is permanently occupied by ions, and the entry of an additional ion is required to knock one of the resident ions out the other end of the channel (*via* the Coulomb repulsion of all the ions in the pore). The use of a knock-on mechanism also provides a way to avoid the apparent paradox that selectivity requires tight binding of ions within the pore, but a large conductance requires a high off-rate of the ions from any binding sites. In this case, although ions are strongly bound, the coulomb repulsion from other ions in the pore speeds the movement of ions away from the binding sites. When a divalent ion enters the potassium pore, however, it is electrostatically bound such that it is unlikely to leave, even with the aid of repulsion from nearby cations.[20] Channels that are blocked in this way are not permanently incapacitated as the divalent ion will eventually leave the channel, but the timescale over which this occurs is much greater than the timescale of ion permeation.

Voltage-gated calcium channels are known to conduct Na^+ ions in the absence of Ca^{2+}, but only allow Ca^{2+} to pass when both are present.[23] A variety of mutagenesis experiments suggest that the selectivity filter of these channels is surrounded by four negatively charged glutamate residues that attract cations into the pore rather than the carbonyl groups in K^+ channels.[24] Computational studies have shown that in the absence of Ca^{2+}, a pore surrounded by such charges can conduct Na^+ in a knock on process similar to that seen in K^+ channels. But, once a divalent ion enters the pore it is bound more strongly than would be a monovalent ion. Once there, it can only be forced out with the aid of the Coulomb repulsion from a second divalent ion, but not with the weaker repulsion from a monovalent ion as shown schematically in Fig. 4.[18–20] Thus, once Ca^{2+} enters, only divalent ions will conduct through the pore, even in a solution containing many more sodium ions.

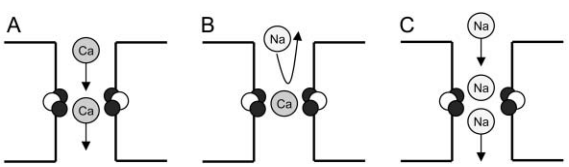

Fig. 4 Selectivity in calcium channels. The selectivity filter is surrounded by four negatively charged glutamate residues (white and black) whose charge permanently holds cations in the pore. Ca^{2+} conduction involves two ions (A) and Na^+ conduction three (C). When a Ca^{2+} ion enters the pore, however, it can only be expelled by another Ca^{2+} ion and thus prevents the passage of Na^+ (B).

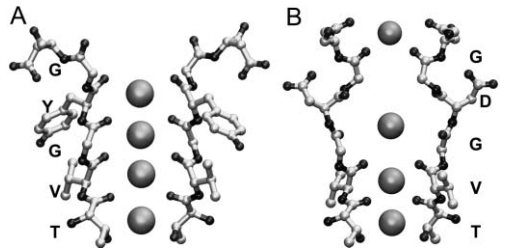

Fig. 5 Selectivity in potassium channels. (A) Two of the four protein chains forming the selectivity filter of the KcsA potassium channel[37] are shown, with backbone carbonyl oxygens forming four K^+ binding sites (spheres) in which the protein can interact intimately with passing ions. (B) The selectivity filter of the NaK channel that passes both Na^+ and K^+ has a very similar structure, with modifications in only the external two sites.[33] The amino acid sequence of each protein is shown.

Ions of the same charge

Selectivity between ions of the same charge and similar size, such as Na^+ (radius $\sim$ 0.95 Å) and K^+ (radius $\sim$ 1.33 Å), is more difficult to explain than the cases described thus far. Much attention has recently focused on the family of K^+ channels that pass K^+ while blocking Na^+, as a significant amount of structural information is now available. As noted previously, selectivity takes place in K^+ channels within the narrow region of the selectivity filter that is surrounded by a sequence of amino acids that is virtually invariant across all K^+ channels. The backbone carbonyls that line this region form a number of sites in which the protein can interact directly with the passing ion as shown in Fig. 5A. Ions must be desolvated to enter this region of the pore as it is too narrow to fit a solvation shell of water molecules. The energy required to desolvate the ion can, however, be compensated by the interaction with the carbonyl oxygen atoms. Indeed the size of the crystalised filter is such that when a K^+ is within the pore the carbonyl oxygens make a good substitute for water with very similar ion-oxygen distances to that seen in bulk water.[6] Na^+, however, is smaller and thus it was suggested that the carbonyl oxygens would be too far apart to enable them to interact intimately with this ion.[6] In this case the energy required to remove the hydrating waters and allow Na^+ to enter the pore would not be fully compensated by the interactions with the carbonyl oxygens and it would be effectively excluded. Indeed, since it was initially proposed, the theory that ion selectivity relies on the size of the pore has become the predominant view (*e.g.* see ref. 25).

There are a number of problems with an explanation of ion selectivity that relies on the precise dimensions of the pore. This explanation requires the structure of the protein to remain fairly rigid, such that it cannot adjust to accommodate the smaller Na^+ ion. Proteins are relatively flexible structures that undergo rapid thermal fluctuations of magnitude much greater than the 0.38 Å radius difference between Na^+ and K^+.[26,27] It has been noted that the crystallographic thermal parameters of the K^+ channel structures suggest root mean square fluctuations of the carbonyl oxygens in the order of 0.75 Å,[28] while fluctuations of similar magnitude are seen in molecular dynamics simulations.[29–31] Not only is there evidence that the atoms forming the selectivity filter vibrate about their mean positions, it also appears that the protein backbone is mobile. A crystal structure obtained at low K^+ concentration shows a large conformation change in the filter adapting to the lower occupancy of ions in the pore.[32] Large structural alterations are also seen in molecular simulations in the absence of ions and when K^+ is replaced with ions of smaller or larger radius.[29–31] It is hardly surprising that the dimensions of the selectivity filter match those of K^+ in the crystal structures, given that they were crystalised in the presence of K^+. Furthermore, it has recently been proposed that rather than relying on the specific dimensions of the pore, K^+ selectivity is a consequence of having the ion coordinated by dipoles of appropriate strength that prevent each other coming too close to a small ion due to their Coulomb repulsion while undergoing large thermal fluctuations.[28] Thus, whether selectivity relies on the specific size of the pore as suggested on initial inspection of the crystal structure, on the limitations on the flexibility of the pore that allow it to coordinate K^+ but not Na^+ as suggested more recently by Gouaux and MacKinnon,[7] or on the size of the thermal fluctuations of the protein remains to be determined.

An interesting addendum to this story has arisen since the recent publication of the structure of a Na^+ and K^+ conducting channel that is closely related to the family of K^+ channels.[33] As illustrated in Fig. 5B the selectivity filter of this channel has an identical sequence to the K^+ channels, except for the replacement of one tyrosine residue with aspartate. This has the effect of replacing two of the four binding sites in the K^+ channel structure with a single weaker site located further to the extracellular end of the pore. The presence of a structure that is in many ways so similar to the K^+ channel, including 2 chemically identical binding sites, but very different in that it conducts both Na^+ and K^+ provides a tantalising test of any theories of ion channel selectivity. It is not clear how any of the explanations described above can account for why the selectivity of this so called NaK channel is different from that in K^+ channels.

Channel gating

For a channel to be able to open and close in response to a signal two components, in addition to the basic pore and selectivity filter, are needed. The first of these is a 'sensor', something that can sense the change in voltage, pH or pressure or bind a ligand in order to activate channel opening. The second is a channel gate, a region of the pore responsible for blocking the passage of ions in the closed state and allowing them to pass when open. Understanding ion channel gating, therefore, involves determining the regions of the protein responsible for each of these roles, the mechanisms by which they function and the way these two components are coupled. Here I describe gating in three different ion channels for which some knowledge of the structural basis of channel gating is known: voltage-gated potassium channels, nicotinic acetylcholine receptors and ClC Cl^- channels.

Voltage activation

The family of voltage-gated potassium channels provides a topical example of channel gating as a number of structures

exist for potassium channels in different functional states, but the mechanisms of gating are still contentious. In these K^+ channels, the pore is lined by 2 transmembrane helices as well as the joining loop that forms the selectivity filter as shown in Fig. 6A. The voltage sensor is known to be comprised of a series of basic amino acid residues (lysine or arginine) in a different transmembrane region, each of which is separated from its neighbour by two non-polar residues. If some of these residues carry a positive charge, then they will feel a force when the electric potential across the membrane changes, which can be harnessed by the protein to create the structural changes necessary to open and close the pore. That this occurs in voltage gated potassium channels has been elegantly demonstrated *via* both neutralisation of these charges, and by measurements of a transient 'gating current' caused by the movement of these charges across the electric field of the membrane.[34–36] Different channels require differing degrees of depolarisation of the cell membrane in order to open. Utilising a number of charged residues in the voltage sensor, however, is likely to be important for enabling many of these channels to be sensitive to changes as small as 20 mV in the membrane potential.

The region of the protein that forms the channel gate is now relatively well understood, in large part due to the fact that crystal structures exist for K^+ channels from a variety of organisms in both the open and closed states.[6,37,38] Although not all of these channels are voltage gated, they can be easily compared because K^+ channels have a clear separation between the sensing and pore forming domains of the protein. In all of these channels, the conduction pathway is occluded at the internal end by a ring of hydrophobic residues on the innermost helix. In contrast, a calcium activated K^+ channel was crystalised at high Ca^{2+} concentration when the channel would normally be in an open state,[39] and rather than being occluded, the internal end of the pore is very wide (Fig. 6C). The main difference between this structure and the previous ones is that the internal end of the inner helices bend outwards.[40] Although other subtle effects, such as a realignment of the pore dipoles and alterations in the selectivity filter, may also contribute to channel gating, the simple alterations at the intracellular end of the pore provide a mechanism of gating that can easily be transferred across different K^+ channel types by attaching different sensing domains to the internal end of the transmembrane pore.[40] Furthermore, much of the variety of K^+ channel conductance properties observed in different members of the family can arise from small differences in the width of the intracellular gate in these channels.[41]

Even though the location of the channel gate and voltage sensing domains are known, the way in which changing the membrane potential affects the voltage sensor, and how this is coupled to the gate is still a controversial issue. When the first crystal structure of a voltage gated K^+ channel KvAP was published in 2003,[37] a controversy erupted over the structure of the voltage sensor and the mechanism of its function. Earlier models had predicted that the charges in the sensor would be insulated from the hydrophobic core of the membrane by the remainder of the protein or aqueous pockets, and that gating would only require small physical movements of the sensor.[36,42,43] One 'transporter' model suggested that

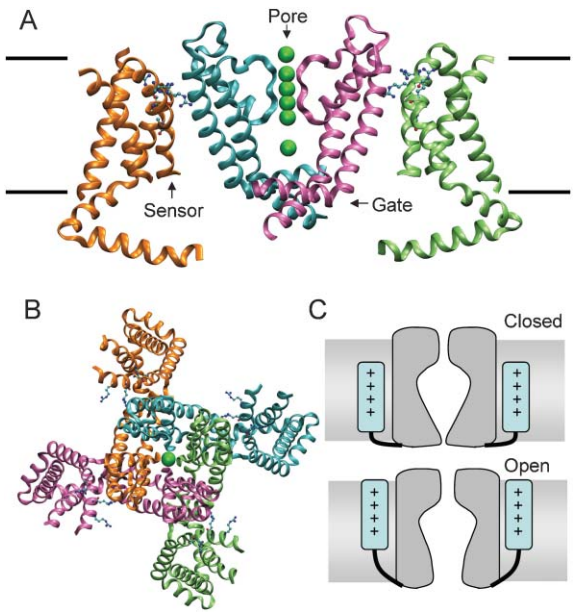

Fig. 6 Structure of a voltage-gated potassium channel (PDB 2A79). (A) Side view showing two of the pore forming domains (cyan and purple) with the associated ion binding sites in the filter (green spheres) and two of the voltage sensing domains (orange and green). The four arginine residues responsible for voltage sensing are shown in ball and stick representation. The approximate location of the membrane is shown by black lines. Voltage sensor and pore forming domains are shown from different subunits so no connection between the two is illustrated. (B) Top view of the complete transmembrane protein structure with the four subunits indicated in different colours. The voltage sensor from one subunit sits adjacent to the pore-forming domain of the next subunit. (C) Schematic depiction of voltage gating. In the closed conformation the intracellular end of the pore is occluded. Upon membrane depolarisation the voltage sensor moves slightly or undergoes a conformational change such that the intracellular end of the pore is opened.

rather than the sensor moving, the electric field could move across the sensor by opening a water filled crevice accessible alternately from one side of the membrane or the other. The crystal structure, however, showed the voltage sensing domain extending into the lipid in a so called 'paddle' like structure. In response to a change in membrane potential, it was believed that the paddle and its cargo of positive charge would move a large distance across the membrane, perhaps as much as 15–20 Å as suggested by examining the accessibility of biotin labelled sensors from each side of the membrane.[44] However, questions were raised about the X-ray structure, in particular whether the antibodies that had been used to help crystalise the protein had influenced its structure and were responsible for placing the voltage sensor so far from the pore.

Since the X-ray structure was unveiled, numerous experiments have been conducted in an attempt to understand the movements of the voltage sensor. New experiments again suggest only limited vertical movement of the voltage sensor upon membrane depolarisation.[45–47] More recently a crystal structure of an open-state mammalian voltage-gated K^+ channel has been determined without the use of antibodies which draws some of these theories together, but still leaves

many questions unanswered.[48,49] In this structure, the voltage sensing regions still form a compact structure that is largely independent from the pore forming protein, similar to the previous structure and in contrast to many of the earlier models. The voltage sensor, however, is packed more closely toward the remainder of the protein (Fig. 6A,B), and although one of the four arginines thought to constitute the gating charge is exposed to the lipid core, another could interact with the polar lipid headgroups, and the last two form salt bridges with the neighbouring protein subunit. How this voltage sensing region moves in response to changes in the membrane potential, and how to reconcile experiments that suggest a large amount of movement and those that suggest only limited movement is still not clear.

Ligand activation

The Ca^{2+} activated K^+ channel described previously is an example of a ligand-gated ion channel as the binding of Ca^{2+} is coupled to the opening of the intracellular gate. Another ligand-gated channel for which detailed structural information has recently come to light is the nicotinic acetylcholine receptor (nAChR) that has a very different architecture to the K^+ channels but shares the feature of having distinct ligand binding (sensor) and gating domains.

Cryo-electron microscopy has recently been used to determine near atomic resolution pictures of receptors from the *Torpedo* electric ray[50,51] as well as a lower resolution picture of the channel in the open state.[52] The channel is formed by five similar, but not necessarily identical, subunits that surround a central pore. The transmembrane domain is formed from 4 α helices, 3 of which contact the surrounding lipid and one of which lines the pore. Attached to this is a large extracellular domain responsible for ligand binding as shown in Fig. 7, which is closely related to a soluble ligand binding protein AChBP.[53]

The atomic resolution images of the protein were obtained in the absence of acetylcholine, meaning that they should be in a closed state. However, both these 4 Å resolution structures show a continuous non-occluded pore passing across the membrane.[54,55] This supports a hypothesis that a physical blockage of the pore is not required to prevent ions from passing through the channel. Rather, it has been suggested that a narrow pore lined by hydrophobic residues can prevent ion permeation by providing an unfavourable home for ions and water and thus forcing an ion to lose some of its hydration shell in order to cross the pore.[54,56] Indeed, computational studies have shown that such a desolvation barrier can be sufficient to prevent ions from passing through model pores,[57] and that a series of hydrophobic residues midway through the membrane can have the same effect in the nAChR.[58]

If the gate is formed by hydrophobic residues that do not occlude the pore, one may ask what kind of conformational change is required to make the channel conductive. Comparison of the open and closed images shows significant changes in both the ligand binding and transmembrane regions. In the transmembrane domain, the outer 3 helices do not appear to move significantly, while the pore lining helix appears to undergo a 10–15° rotation about an axis

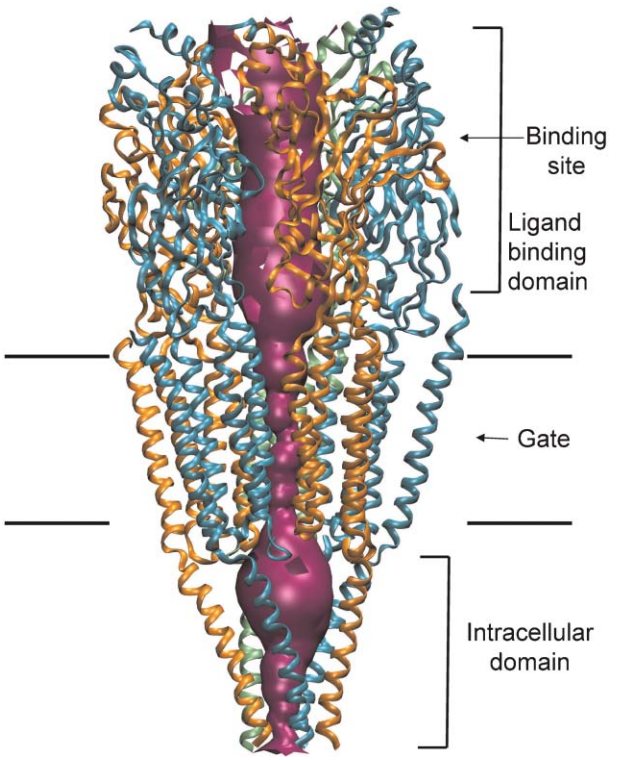

Fig. 7 Structure of the ligand gated nicotinic acetylcholine receptor.[55] The protein contains an extracellular ligand binding domain, a transmembrane domain containing the channel gate and a smaller intracellular domain. The dimensions of the pore are indicated by the purple surface, and illustrate that the narrowest portion of the pore lies in the membrane spanning region.

perpendicular to the membrane passing through a disulfide bridge in the extracellular domain. It is unlikely that such a small rotation would present different residues to the pore, and a recent mutagenesis study suggests that the same side chains line the pore in both the closed and open states.[59] If the chemical environment of the pore does not change upon channel opening, then this implies that the pore must widen slightly. A computational investigation also illustrated that the same hydrophobic residue line the pore after such a such a 15° rotation, but that this did widen the pore by 1–2 Å which was sufficient to remove the desolvation barrier to ion permeation.[58]

The ligand binding sites lie over 50 Å from the channel gate in the distant extracellular domain, yet the kinetics of channel activation after the application of acetylcholine show that ligand binding is conveyed to the channel gate within tens of microseconds.[60,61] This requires a remarkable structural organisation of the protein such that conformational changes in one region can be rapidly passed to another. Such changes must begin locally to the binding site and probably involve one or two protein loops that appear to be in different positions in the ligand free nAChR[55] and ligand bound AChBP structures[62] and these loops also shift position in simulation studies.[63] From here, the structural changes are believed to propagate as a wave probably consisting of a number of rigid body movements.[64] This must then be coupled to the transmembrane domain, most likely *via* a direct interaction

between the top of the inner transmembrane helix with two loops of the extracellular domain. Although a vast variety of mutagenesis and simulation data exists (*e.g.* see ref. 65–67) to help answer how ligand binding is propagated into the transmembrane pore, the exact mechanism of coupling and the residues involved is yet to be firmly established.

Identical sensor and gate

The last example I wish to mention involves the family of ClC Cl^- channels and a little more speculation. This example, however, appears to be one in which the sensor and gate are actually the same thing and the movement of only a few atoms is required to open and close the pore. ClC proteins are a family of anion channels and closely related transporters present in every living organism that perform a diverse range of functions from stabilising membrane potentials to controlling cell volumes.[68] These channels form two-pore dimers that are controlled by two distinct gating mechanisms as typified in the most well studied channel of this family, ClC-0, from the *Torpedo* electric ray. One gate, termed the 'slow' gate, operates on both pores simultaneously with bursts of activity lasting 10–100 s. The other gate controls each pore individually opening them upon depolarisation, and operates at a much faster time scale and is therefore called the 'fast' gate.[69] The opening of this fast gate is also facilitated by the presence of external Cl^- and decreasing pH.

A possible mechanism for the operation of this gate has been suggested since the structures of two related ClC transporter proteins were determined.[14,70] This is slightly speculative, of course, as the principles of channel gating are being based upon the structure of a transporter, and although they have similar amino acid sequences there may be important structural differences between them. In the crystal structures, the ion-transport pathway is blocked by the side chain of a glutamate residue. Mutating this single residue is sufficient to remove the fast gating behaviour of the related Cl^- channels, and structures of such mutant transporters show the corresponding side chain to be in a different conformation to that of the glutamate. On this basis, Dutzler *et al.*[70] postulated that motion of this side chain is responsible for fast gating in the ClC channels. A possible mechanism of action of this gate has been suggested from a computational study.[71] Here it was suggested that when the side chain extends into the pore and slightly toward the external end, it electrostatically blocks Cl^- conduction as shown in Fig. 8A. However, the presence of Cl^- on the external side of this residue, whose likelihood is increased with both increasing concentration or potential, can push this side chain downward into the pore, where it tucks into the protein and allows Cl^- conduction in either direction (Fig. 8B). Alternatively, low pH in the external solution can lead to protonation of the side chain when it is in the closed conformation, removing the electrostatic barrier to ion permeation (Fig. 8D). Whether or not the detail of this mechanism is correct, it demonstrates that a single residue can act as both a sensor and gate, and how only minimal conformational changes can be responsible for some types of channel gating.

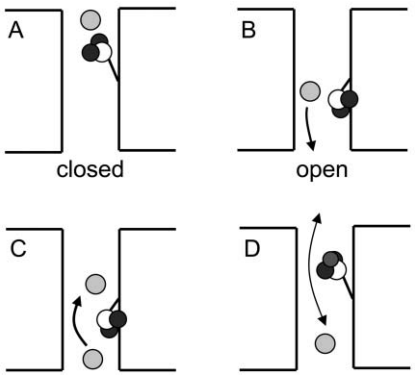

Fig. 8 Schematic model of gating in ClC Cl^- channels. (A) A central glutamate residue is believed to be the 'fast' gate. In the upward position it provides an electrostatic barrier to ion permeation. (B) The entrance of Cl^- on the external side of the gate (whose likelihood increases with membrane potential and concentration) promotes opening of the pore. (C) A Cl^- ion present near the glutamate residue holds open the gate to allow permeation in either direction. (D) Low external pH or mutations of the glutamate residue can neutralise its charge in the closed position, allowing conduction in either direction.

Conclusions

Ion channels are proteins that regulate the electrical activity of cells by controlling a transmembrane pore. In order to perform their functions most channels must be able to discriminate between different ion types and open and close their pore in response to various stimuli. To do this, channels contain a number of functional elements. The first is a narrow region of the pore known as the selectivity filter, that enables the protein to interact intimately with passing ions. Large ions can be rejected based on size, differently charged ions can be differentiated by their electrostatic interactions with the partially charged protein atoms, while discrimination between more similar ions most likely involves subtle differences in the ability of the protein to coordinate the ions. In all the cases discussed it is the thermodynamics of ions in the binding site that appears most important in determining cation versus anion selectivity of the channel. Thermodynamics is also important in determining the selectivity between monovalent and divalent ions (as well as between Na^+ and K^+ ions), but the presence of multiple ions in the channel and the use of a 'knock-on' conduction mechanism means that it is not necessarily the ion that binds most strongly in the channel that will be allowed to permeate. In this case it may be easier to think in terms of an electrostatic explanation of ion selectivity. Pinpointing the mechanisms of selectivity used to distinguish between Na^+ and K^+ ions remains a difficult task that will no doubt be the focus of future work.

To respond to stimuli, channels use a sensor region that can bind a ligand or sense a change in membrane potential and this is coupled to a gate that controls the flow of ions through the pore. In many cases the sensor and gate have been identified as separate regions of the protein, while in others these may actually be the same region of the protein. But, even when these structural components have been identified, as in all three examples described here, much further work is required to find out exactly how the sensor responds to a stimulus and

how this is translated into a structural change at the channel gate. Although our understanding of the molecular basis of ion channel function has progressed enormously over the last ten years, in particular due to the recent availability of atomic resolution structural information, the details of how most channels function still remains to be elucidated.

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