during regeneration in differentiated newt cells, but not in their mammalian counterparts. There has been some progress along these lines but not at the level of pinpointing the genetic differences. It is important to remember that there are examples, such as the axolotl lens, where a particular tissue does not regenerate even though the animal is capable of regenerating other structures. Such a context may be more favourable experimentally to identify what makes a tissue regeneration competent.

What about newts in literature?
There is the book 'War with the newts' by the Czech author Karel Capek, but granted a little phylogenetic licence we prefer 'Axolotl', one of the incomparable short stories by the Argentinian writer Julio Cortazar. You are visiting the Jardin des Plantes in Paris, and standing in front of a tank of strange beasts…

Where can I learn more?
For 'Axolotl', see www.cis.vt.edu/modernworld/d/axolotl.html
For much interesting information about amphibians see www.amphibiaweb.org
For information about regeneration in different systems see Nature Encyclopedia of Life Sciences at www.els.net
Brockes, J.P. and Kumar, A. (2002). Plasticity and reprogramming of differentiated cells in amphibian regeneration. Nat. Rev. Mol. Cell Biol. *3*, 566–574
For Miller's work, see the article by Felice Frankel at www.americanscientist.org/template/IssueTOC/issue/406

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Primer

Voltage-gated ion channels

Zara Sands, Alessandro Grottesi, and Mark S.P. Sansom

Voltage-gated ion channels are integral membrane proteins that enable the passage of selected inorganic ions across cell membranes. They open and close in response to changes in transmembrane voltage, and play a key role in electrical signaling by excitable cells such as neurons. Voltage-gated K^+, Na^+ and Ca^{2+} channels are thought to have similar overall architectures. X-ray crystallographic studies of bacterial homologs have provided considerable insights into the relationship between channel structure and function in various classes of K^+ channels, including the voltage-gated (Kv) ones. But despite these advances, the exact structure of the Kv voltage sensor, and how the Kv channel structure changes in response to changes in transmembrane voltage, remain elusive.

Potassium channel architecture
When the cell membrane is polarized, so that the interior of the cell is at a negative voltage relative to the exterior, Kv channels remain closed. When the membrane is depolarized, these channels open rapidly (<1 ms), allowing ions to flow passively down their electrochemical gradients, at near diffusion rates (~10^{-8} ions sec^{-1}). Kv channels thus have two principal functions: ion conduction, and voltage sensing. Corresponding to these two functions, Kv channel subunits contain two distinct, but functionally coupled transmembrane domains (Figure 1A). The pore domain is responsible for the ion selectivity and conduction, and also for channel gating *per se*, whereas the voltage-sensing domain triggers a change in conformation of the pore domain in response to changes in transmembrane voltage.

Kv channels comprise four subunits that encircle a central ion conduction pathway. Each subunit consists of six α helices (S1–S6) with both amino and carboxyl termini on the intracellular side of the membrane. The first four transmembrane helices (S1–S4) form the voltage-sensing domain (Figure 1B), whereas the last two transmembrane helices (S5–S6), along with an intervening re-entrant P loop, form the pore domain (Figure 1C). The re-entrant loop contains a short pore helix and an extended region of polypeptide chain that contains the characteristic sequence motif TVGYG and forms the selectivity filter.

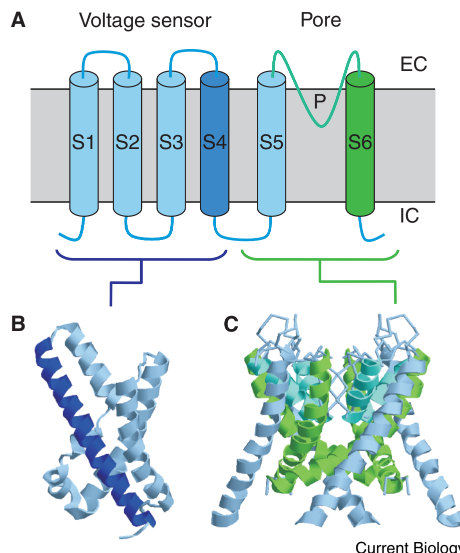

Figure 1.
(A) The transmembrane topology of a Kv channel subunit, showing the voltage sensor and pore domains. The intact channel is made up of four such subunits. The intracellular (IC) and extracellular (EC) faces of the membrane are labeled. (B) Structure of the voltage sensor domain of the bacterial voltage-gated channel KvAP (PDB code 1ORS), with the S4 helix in deep blue, and helices S1 to S3 in pale blue. (C) Structure of the pore domain from KvAP (PDB code 1ORQ), with the P helix and filter in cyan, and the S6 helix in green.

The filter region forms the extracellular end of the pore. The TVGYG motif is highly conserved amongst K^+ channels. The glycine residues of this motif enable the filter to adopt a conformation in which the mainchain carbonyl oxygen atoms point toward the center of the pore axis, generating five discrete binding sites for K^+ ions flowing through the pore. The filter region exhibits a degree of flexibility which may be responsible for 'fast gating' of K^+ channels [1]. The main activation gate, however, lies at the opposite end of the channel, at its cytoplasmic mouth.

Pore gating

The ion conduction pathway can switch between two main functional states, open and closed. The structural differences between these two states have been revealed by comparing the X-ray structures of KcsA, crystallised in a closed conformation, and of MthK, crystallised in an open conformation (Figure 2A). This comparison [2] suggested that bending or kinking of the inner pore-lining helices — M2 in KcsA and MthK, S6 in Kv channels — plays a key role in pore gating (Figure 2B). In KcsA, the M2 helices are undistorted, and converge to form a narrow hydrophobic constriction near the cytoplasmic entry to the pore. In contrast, in MthK or the bacterial voltage gated channel KvAP, the inner helices bend at a conserved glycine residue and so splay apart so as to open up the intracellular mouth.

It seems likely, therefore, that the conformational change responsible for gating involves a hinge-bending motion about the conserved glycine. As this glycine residue is highly conserved in K^+ channel sequences, it has been suggested that it forms the gating hinge in Kv channels. But in Kv channels from higher organisms — though not in KvAP — there is also a conserved PVP sequence motif carboxy-terminal to the conserved glycine, and this may act as a further molecular hinge. But it is not yet clear whether Kv pore opening is brought about by concerted or sequential motion at one or both hinge points. Molecular dynamics simulations of Kv channels suggest that the PVP motif provides a flexible element within the S6 helix (Figure 2C). Furthermore, mutations of this motif perturb channel gating [3].

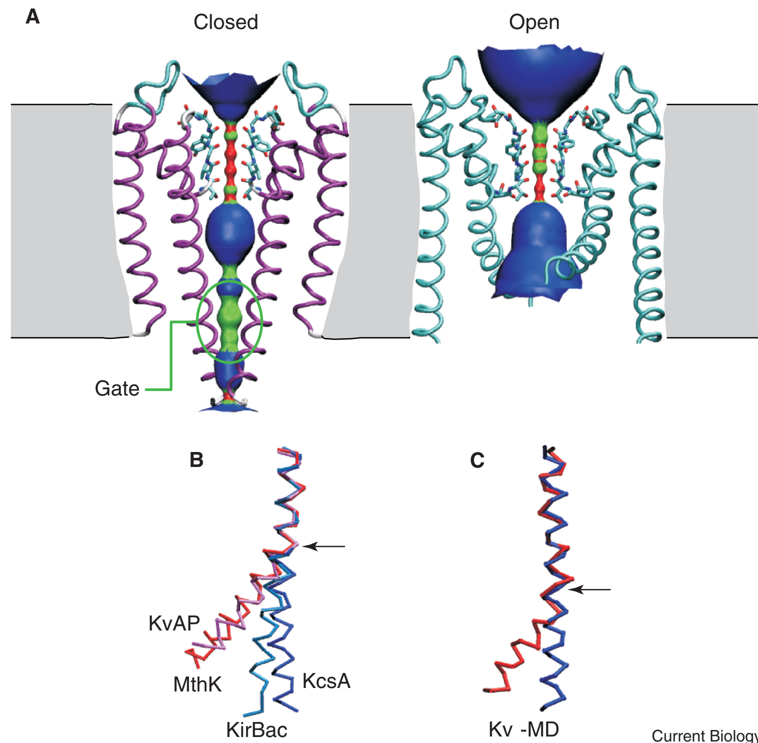

Figure 2. (A) Models of the *Shaker* Kv pore domain, the closed conformation model being based on the structure of KcsA, and the open conformation based on KvAP. In both cases, for clarity only two subunits of the core pore-forming domain (S5–P–S6) are shown. The surface lining the pore is shown, and the region of the hydrophobic cytoplasmic gate is indicated for the closed state model. (B) Comparison of the pore-lining helices from the X-ray structures of KcsA (blue, M2 helix, pore closed), KirBac1.1 (cyan, M2 helix, pore closed), MthK (red , M2 helix, pore open), and KvAP (purple, S6 helix, pore open). (C) Comparison of structures of the S6 helix taken from the start (blue) and end (red) of a molecular dynamics simulation of the *Shaker* Kv channel [11]. In both B and C, the amino-terminal halves — before the molecular hinge — of the helices are used for their superimposition, and the approximate locations of the molecular hinges are indicated by arrows.

The structure of KvAP

The X-ray structure of a bacterial Kv channel homolog, KvAP [4], revealed the structure of the voltage sensor and the pore, but did not resolve the relationship between them. The pore domain of KvAP (S5–P–S6) has the same architecture as that of other K^+ channels. It appears to be in an open state, as the S6 helices are kinked and the intracellular mouth of the channel is not occluded. The conformation and orientation of the S1–S4 domain in the X-ray structure of the intact channel are somewhat puzzling, however, and it has been suggested that this region of the protein may have somehow become distorted prior to or during crystallization. The X-ray structure of the isolated voltage sensor domain (Figure 3) agrees rather better with other experimental data, and so is presumed to be in an undistorted form.

Recent research has thus focused on how to reconstruct the manner in which the voltage sensor and pore domains of Kv channels are packed together, and on determining the changes in conformation and/or orientation of the voltage-sensor in response to membrane depolarisation.

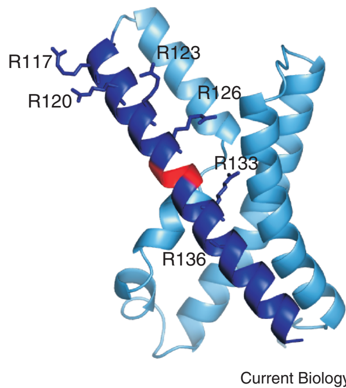

Figure 3. Crystal structure of the KvAP voltage sensor (PDB code 1ORS). Helices S1 to S3 are shown in pale blue, and helix S4 is shown in deep blue, with the putative hinge region [7] in red. The sidechains of the positively charged arginine residues of S4 that sense the change in voltage are shown in dark blue stick format.

Voltage sensing

Kv channels are activated as a result of the transmembrane movement of charge carriers located in the voltage sensing domain. This 'gating current' corresponds to the translocation of the equivalent of ~13 elementary charges per channel across the membrane. The S4 helices, bearing a regular array of positively charged amino acids, are the principal structural elements responsible for voltage-sensing. It is generally accepted that the first four arginines of S4 — R117 to R126 in S6 of KvAP (Figure 3) — account for the gating current, moving toward the extracellular solvent upon channel activation in response to membrane depolarization.

This much is known. But it has proved difficult to integrate the crystal structures of the intact KvAP channel and of the isolated KvAP voltage sensor [4] with physiological and biophysical data. This has resulted in formulation of a number of competing models for the orientation of the voltage-sensor domain relative to the pore domain in Kv channels and for how the conformation and orientation of the voltage sensor domain changes upon membrane depolarisation (Figure 4).

The canonical model

The canonical, or sliding-helix, model of Kv channel gating is derived from studies of S4 residue accessibility using cysteine scanning mutagenesis and thiol-reactive compounds, and from changes in fluorescence of probes attached to S4 upon channel activation [5]. Upon membrane depolarization, each S4 helix is proposed to slide or screw outwards toward the extracellular surface through a narrow proteinaceous vestibule, or 'canaliculus'. The canaliculi are proposed to provide an aqueous environment for most of the length of the S4 helices, with hydrophobic 'seal' midway along.

It is across this 'seal' that the transbilayer electric field is proposed to be focused. Upon depolarization, the S4 motion results in the arginine sidechain gating charges being relocated from an internally facing water pocket to an externally facing pocket. The remainder of the voltage-sensing domain is suggested to remain largely unperturbed. Thus, the motion of the S4 helix relative to the remainder of the protein is the fundamental voltage-sensing event, triggering subsequent conformational changes that result in channel opening of the pore domain.

The transporter model

The transporter model [6] is similar to the canonical model in that the S4 helix is also proposed to be buried within a canaliculus between the pore domain and the S1–S3 helices. In this case, however, the movement of S4 upon depolarisation is suggested to be quite subtle, so that, although the S4 helices do not undergo any substantial movement, they change the exposure of the gating charges (arginines) from the intracellular aqueous solution to the extracellular solution. Thus, the gating charges move across the entire transmembrane electric field, even though no large scale motion of S4 occurs.

This model is consistent with fluorescence label experiments, and also with measurements of a transmembrane proton flux observed when the outermost S4 arginine is replaced by a histidine.

The paddle model

The paddle model is derived from comparison of the crystal structures of the voltage sensor domain of the bacterial channel KvAP, and of the intact KvAP channel [4]. The critical component of the model is a paddle-like structure formed by S4 and the carboxy-terminal half of the S3 helix (S3b). The paddles are attached to the channel through flexible S3 loops and S4–S5 linkers and, with the exception of the critical S4 arginine residues, have an amino acid composition that is mainly hydrophobic.

In this model, the paddles are positioned loosely around the periphery of the channel and are exposed to the membrane environment. In the full-length crystal structure, the paddles are located close to the intracellular surface; biophysical studies, however, indicate that the paddles can also be exposed to the extracellular solution. From these findings it was inferred that the paddles translocate their cargo of gating charges across the entire bilayer *en masse* in response to changes of membrane potential.

A prediction of the paddle model is that the positively charged S4 helices are significantly exposed to the surrounding lipid environment during their translocation from one side of the membrane to the other. This might be expected to provide a high energy barrier to channel activation. It is conceivable, however, that local reorganisation of lipid packing around the paddle could lower such barriers, and so the model cannot be excluded on theoretical grounds.

The twisted S4 model

The results of recent site-directed spin labeling experiments of KvAP in a lipid bilayer environment [7] indicate that the S4 helices are at the protein–lipid interface, rather

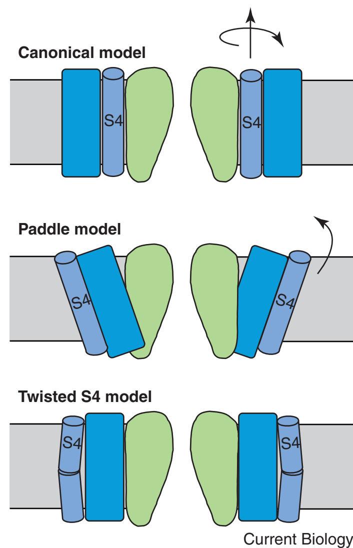

Figure 4. Three models of the organization of the voltage sensor domain (blue) relative to the pore domain (green) in Kv channels. The S4 helix is shown in deep blue, and for clarity only two of the four subunits are shown. In the canonical model (top), the S4 helix is sandwiched between the pore domain and the S1–S3 helices of the voltage sensor domain. Thus the S4 helix is not exposed to the surrounding lipid bilayer (gray). Upon activation of the channel the S4 helix is thought to twist and translate relative to the remainder of the protein, as indicated by the arrows. In the paddle model (middle), the S4 helix is on the exterior surface of the channel, fully exposed to the lipid bilayer. The arrow shows the presumed direction of movement of the voltage sensor domain upon channel activation. In the twisted S4 model (bottom), the S4 helix is on the surface of the protein. However, the hinge region in the middle of S4 allows the two helical segments to be twisted relative to one another so that the arginine sidechains are not exposed to the surrounding lipid bilayer.

than buried within a canaliculus. This is in apparent agreement with the paddle model, but more detailed examination of spin label accessibility data indicates that most of the arginine sidechains are shielded from the lipid environment. These data can be explained by a modification to the structure of the voltage-sensor, whereby there is flexible linker or hinge in the middle of S4 (Figure 3), enabling one half of S4 to rotate relative to the other.

This model would allow the S4 helix to be at the protein lipid interface, but with the positive charges pointing inwards toward the remainder of the protein (Figure 4). It is speculated that the flexible linker may permit differential rearrangements of the two halves of the S4 helix in response to voltage changes.

The way forward?

Further experimental and computational studies are required before we reach a complete structural understanding of the mechanisms of voltage-sensing and voltage-gating of Kv channels. Progress is likely to be made using a range of indirect techniques to complement the structural data that have come from X-ray crystallography. In particular, spectroscopic [7] and chemical modification [8] studies of Kv channels *in situ* in lipid bilayers will help to resolve the structure of the resting (closed state) channel. These methods will then have to be deployed in combination with a transmembrane voltage difference to reveal the change in sensor conformation and orientation during the transition from the closed to the open state.

It is likely that computational methods will be used to integrate data from these diverse sources in a molecular mechanism. Given the proposed changes in conformation of the voltage sensor upon activation of Kv channels, it is important to characterize the intrinsic flexibility of this domain. Molecular dynamics simulations offer one possibility for exploring the conformational dynamics of the sensor domain (our unpublished data).

A number of other techniques may also yield information on the location of the voltage sensor relative to the membrane. For example, toxins that interact with the sensor, for example Vstx1 [9], provide valuable probes. Their location — and hence by extension the location of the voltage-sensor — relative to a lipid bilayer may be established via molecular simulations. Similarly, electron microscopy [10] may reveal the overall shape of Kv molecules trapped in different conformational states. Thus, by combining information from these disparate sources, a complete mechanism of Kv voltage-sensing and gating may emerge.

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