

Review

What defines a gate? The enigmas of K⁺ channel structural mechanics

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Potassium (K⁺) channels vary in shape and size. Commonly, they form a functional pore through the membrane as an assembly of four subunits. For the predominant family of K⁺ channels at the plant plasma membrane, each subunit comprises a polypeptide of six transmembrane helices with a canonical voltage-sensor domain fused to a pair of pore-lining helices. These constructs give rise to a wide range of behaviours that are nonetheless ‘hard-wired’ within the channel protein structure. With cryo-EM data now available for four *Arabidopsis thaliana* K⁺ channels, it nonetheless remains an enigma how the structural mechanics give rise to channels that open—or gate—for K⁺ flux across widely differing voltages and how ligands, especially K⁺ itself, regulate this flux.

A necessity of K⁺ channels

Plants utilise the potassium ion (K⁺) almost universally as the major osmotic solute active in the cell. Potassium serves to maintain cellular turgor, it powers irreversible cell expansion during growth, and its transport enables the reversible changes in turgor that drive many cell and tissue movements, including those of stomata [1–4]. Potassium is a major factor in plant resilience to drought and salinity, and its transport contributes to pathogen resistance [2,5,6]. Potassium is equally important for cellular metabolism and thus also sustains the nutritional value of crops [7,8].

Potassium transport throughout the plant requires integral membrane proteins that enable the movement of the charged K⁺ ion across the hydrophobic barrier of membrane lipids. Not surprisingly, plants possess a large number of genes encoding K⁺ transporters, including both high-affinity transporters and K⁺ channels [1,9]. These transporters operate under different conditions and in different tissues [1,5,9,10], supporting plant growth even when the soil K⁺ concentration varies over three to four orders of magnitude [5,11]. The best estimates suggest that K⁺ channels contribute to more than 50% of the nutritional K⁺ uptake under most field conditions [5], and they account for roughly 70–80% of all K⁺ flux across the cell membranes of stomatal guard cells [4,12].

A feature of ion channels is their ability to facilitate flux at rates close to those of ion diffusion in free solution. Left unregulated, such a flux through a plasma membrane K⁺ channel would rapidly equilibrate a plant cell with its surroundings, in most common circumstances depleting the cell contents and collapsing the cell turgor. Thus, plant K⁺ channels, like the vast majority of ion channels in eukaryotic and prokaryotic cells, are regulated through a process known as gating—a rapid opening and closing of the channel pore—that limits ion movement through the channel.

Gating introduces a level of fine control that typically operates over time scales of milliseconds, subject to cellular signals or other environmental stimuli. For the K⁺ channels found at the plasma

Highlights

Voltage-sensitive potassium (K⁺) channels of the plant plasma membrane conform to a common set of structures, yet they exhibit strikingly different gating behaviours.

The cryo-EM structures for four of these K⁺ channels are now resolved and offer a wealth of detail on the mechanics of gating. Gating appears to arise through distributed interactions with a focus on the base of the channel pore, indicating a modular, ‘latching’ mechanism of semi-independent transitions that precede channel opening.

Many more questions now arise, especially around this apparent modularity and how it may be co-opted in different gating behaviours.

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membrane of virtually all plant cells, membrane voltage plays a particularly important role in gating [4,5,12,13]. These channels assemble functional units as tetramers around a central pore [14–16] and all feature gating structures that respond to the voltage across the membrane. This channel family includes the first two channel genes to be cloned in plants, *AKT1* [17] and *KAT1* [18] of the model plant *Arabidopsis*, encoding K^+ channels that gate open at negative voltages to facilitate K^+ uptake (Box 1) when the membrane is energised by the H^+ -ATPase.

Two other K^+ channels of *Arabidopsis*, *SKOR* [19] and *GORK* [20,21], show an opposing dependence on voltage and enable K^+ loss from the cell. Gating of these channels is activated by depolarising voltages and is also sensitive to the K^+ concentration outside ($[K^+]_o$) [21–26]. As a result, the channels open at voltages positive of the K^+ equilibrium voltage, E_K , thereby ensuring the necessary electrochemical driving force for efflux even as $[K^+]_o$ varies between 0.1 and 100 mM, and thus E_K varies by almost 200 mV (Box 1). *SKOR* is localised primarily to root xylem parenchyma cells, where it supports K^+ and water transport to the shoot [19]. *GORK* is expressed primarily in guard cells of the leaf and enables K^+ loss during stomatal closing [20,21].

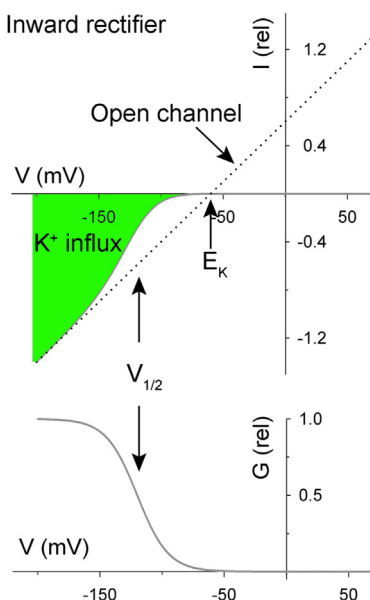
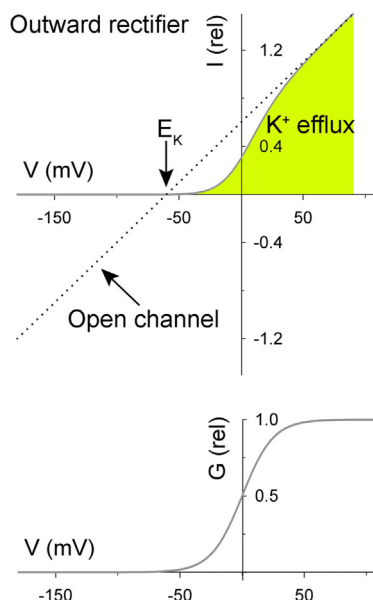
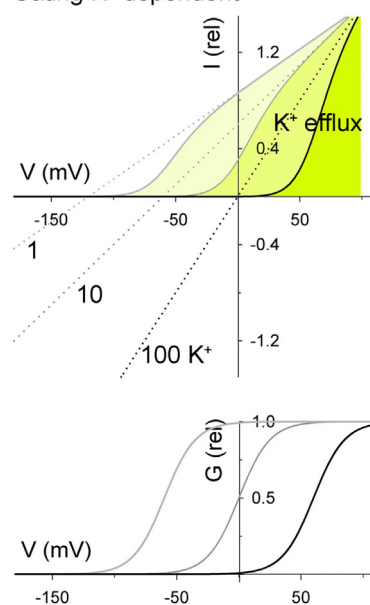
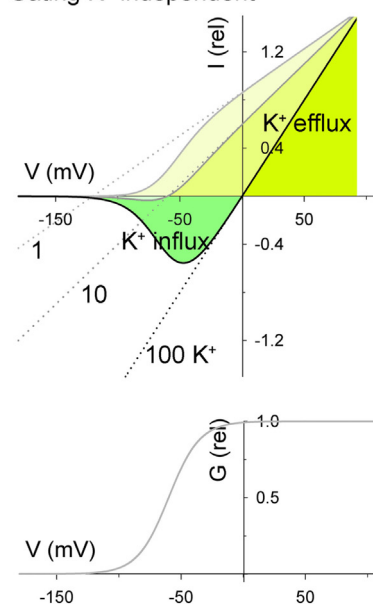
Remarkably, these four K^+ channels, the so-called inward-rectifying channels *AKT1* and *KAT1* and the outward-rectifying channels *SKOR* and *GORK*, belong to the same family of channel proteins and share the same basic structural features [27,28]. So, what then determines the gating of these K^+ channels? How can proteins with such very similar structures give rise to fundamentally differing sensitivities to membrane voltage and extracellular cation concentrations? The structures of all four channels are now resolved, adding significantly to our understanding of channel gating in plants. These structures also introduce fundamental puzzles that challenge our thinking about how channel structure determines function.

A brief history

Much of our understanding, especially of K^+ channels, predates any structural data and has drawn on physico-mechanical analogies. Following the evidence for discrete, ion-specific conductances behind the action potential [29], Hodgkin and Keynes [30] noted that radiotracer K^+ flux did not behave as if ion movement was independent, as would be expected of a simple pore, but showed a flux ratio that was roughly 2.5 times the ratio of ion concentrations on the two sides of the membrane. They explained this behaviour using the concept of small, water-filled tubes through the membrane along which K^+ ions passed. Fluid mechanics and ion solvation in water implied that ion movement would bring with it water molecules, creating a wave of motion that could drag along other ions, a concept that would later be refined with the recognition of the multi-ion pore characteristics of K^+ channels [31,32], including those of plants [33,34].

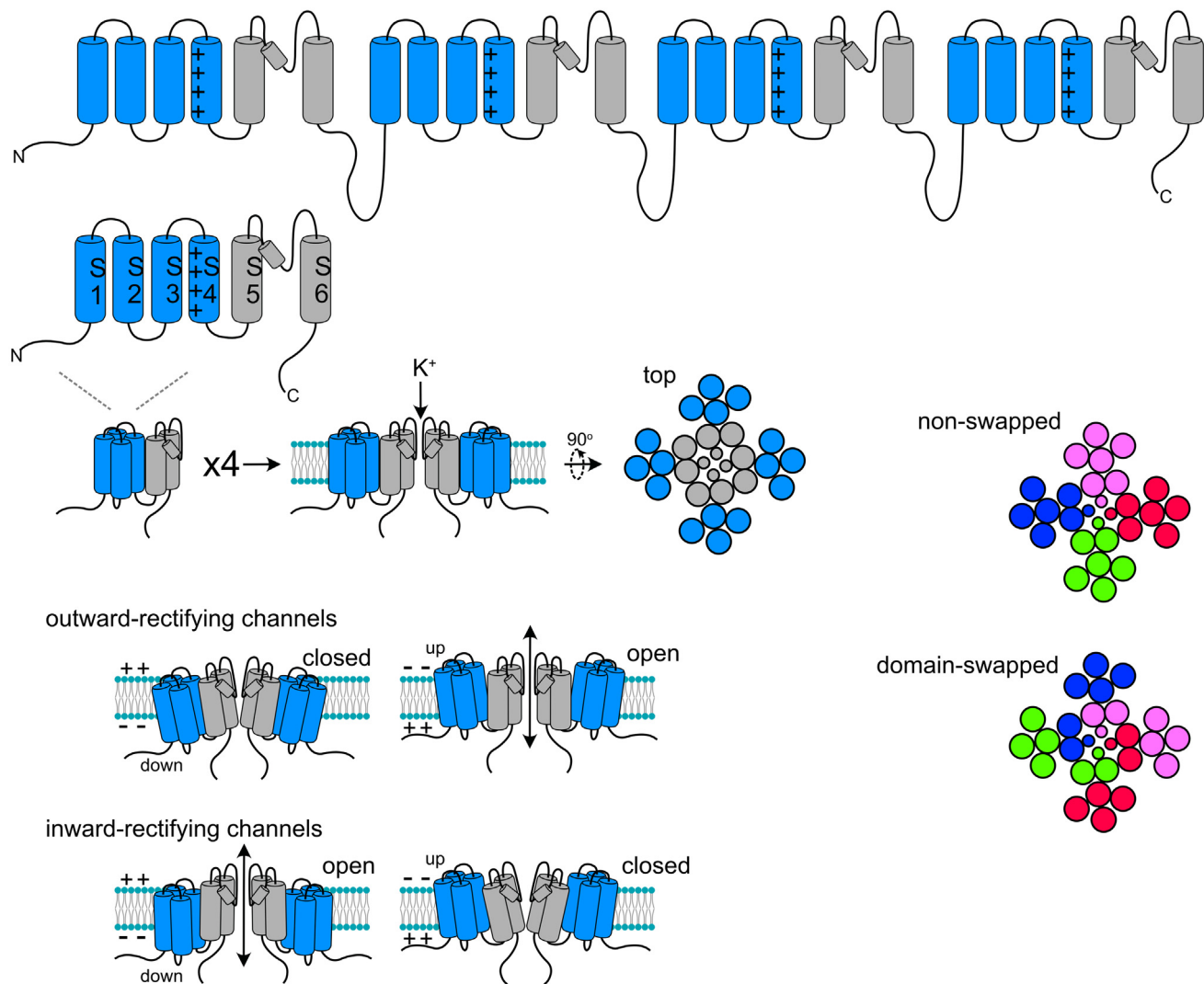
The findings led Mullins [35] to the idea of a pore that achieves selectivity based on the size of the ion and its interaction with the pore. In effect, the idea was that ion permeation occurred through a filter in which each ion was coordinated by the lining of the pore, thus replacing the hydrogen bonds normally formed with water in aqueous solution. That the oxygens of the channel protein might serve to coordinate K^+ ions in the pore, thereby introducing selectivity based on the size of the ion, was first proposed in the early 1970s and explained the relative permeabilities among alkali cations as well as other small molecules [36,37].

Cloning of the K^+ channel behind the Shaker mutant of *Drosophila* [38], and subsequently K^+ channels of vertebrates [39–41] and plants [17–21], enabled functional mapping to protein sequences. In general, K^+ channels were quickly recognised to assemble from four subunits

Box 1. Steady-state current–voltage (IV) relations describe the conductance and gating behaviours of K^+ channels**(A) Steady-state K^+ current****Inward rectifier****Outward rectifier****(B) Outward rectifier****Gating K^+ -dependent****Gating K^+ -independent**

(A) Relative conductances (G , below) describe channel gating, hence its rectification, as a function of membrane voltage. Multiplied by the open channel currents (dotted lines, above), its product defines the steady-state currents (solid lines, above) for the inward- (left) and outward-rectifying (right) K^+ channels. Here, the equilibrium voltage for K^+ (E_K) of -60 mV is typical of a plant cell with 10 mM K^+ outside, and the open-channel current is approximated as a linear (ohmic) relation. At any given voltage, negative current indicates K^+ influx (green shading) and positive current indicates K^+ efflux (yellow shading). Gating is defined by the midpoint voltage ($V_{1/2}$) at which $G = 0.5 G_{\max}$ and the maximum slope of the G - V curve, equivalent to the apparent gating charge. Note that $V_{1/2}$ for the outward rectifier, as shown (right), occurs at 0 mV. (B) Plant outward-rectifying K^+ channels exhibit a K^+ dependence to gating that is separate from the effects of K^+ as the permeant ion. Increasing $[K^+]$ outside shifts the open-channel relations (dotted lines) and E_K , marked by the voltage at which the open channel current is zero, to the right along the voltage axis. It also shifts the relative conductance curve (below left) in parallel. As a result, the steady-state IV relations (above left) remain biased for K^+ efflux (yellow shading). Were this not the case and gating were independent of K^+ (right), then increasing $[K^+]$ outside would lead to situations in which gating of the channels could allow K^+ influx (green shading).

analogous to the tetrameric repeats of Na⁺ channel proteins [42,43]. Within each Na⁺ channel repeat and K⁺ channel subunit were six transmembrane helical domains (Figure 1), designated S1–S6, the first four connected to S5 via an extended, semi-helical linker. The K⁺ channel pore and its vestibules were identified by mutations and domain exchanges affecting the sensitivities to block by small molecules, including peptide toxins that bound with high affinities [46] and the tetraethylammonium cation that enters the pore [47–49].



Trends in Plant Science

Figure 1. General features of mammalian Na⁺ channels and mammalian, Shaker and plant plasma membrane K⁺ channels. Mammalian Na⁺ channels (above) comprise a single polypeptide of four repeated domains, each comprising six transmembrane helices with four helices forming a voltage-sensor domain (VSD, blue) and two forming the lining of the pore (grey). This pattern of six transmembrane helices (S1–S6)—one with a density of repeated positively charged residues and the last two linked by a short helical loop—is evident in the K⁺ channels. The functional K⁺ channels are assembled from four of these pseudo-domains or subunits (centre and bottom left). Viewed from the top (centre), each functional channel comprises an inner ring of pore-lining helices (grey) and an outer ring of VSDs (blue). The same movement of the VSDs across the membrane, up and down, drives opening and closing of the pore, depending on the charge on the membrane (–+) and channel rectification (bottom left). Coupling between the VSD S4 helix and the S5 helix of the pore determines the arrangement of domains between K⁺ channel subunits (bottom right). Here colour-coding by subunit shows the arrangement of helices in non-swapped (CNBHD-type) and domain-swapped (Shaker and mammalian Kv1-like) channels. An expanded gating sequence plausible for the outward-rectifying channels GORK and SKOR is included in Figure 5.

Like permeation, much knowledge of gating predates the resolution of any channel structure. Hodgkin and Huxley [50] had proposed movements of so-called gating charges to sense changes in membrane voltage. Such movements would incur gating currents as these charged groups on the protein moved across the electrical field within the membrane, but the gating currents would be transient and orders of magnitude smaller than any ionic currents. Their recording posed major technical challenges. The first measurements from muscle and nerve of Na⁺ channel gating currents came in the early 1970s [51–53]. A decade later, recordings from K⁺ channels indicated a gating current with a limiting voltage dependence equivalent to the movement of at least four charges across the electrical field of the membrane [54,55].

With the cloning of Na⁺ channels and later the Shaker K⁺ channels, the unexpected concentration of charged amino acids, regularly spaced along the S4 helix in each K⁺ channel monomer and subunit repeat of the Na⁺ channel, immediately pointed to the long sought-after voltage sensor (Figure 1) and suggested a sliding or screw-like motion of the helix in response to changing voltage across the membrane [56]. Mutations introducing Cys residues at positions along this helix and elsewhere showed a substantial (>10 Å) movement across the membrane that exposed residues to chemical modification on one side or the other, depending on membrane voltage [57]. The idea that the S4 helix moved outward (or 'up') with depolarising voltages was also supported by measurements under voltage clamp with fluorometric residue labelling [58,59].

Testing the contribution of the repeated Arg and Lys residues in the S4 by mutations that neutralised or reversed charges showed corresponding reductions in the voltage dependencies of both Na⁺ and K⁺ channels [60,61]. Careful analysis indicated that between 12 and 14 positive charges crossed the membrane electric field during gating [62,63], but the effects were not functionally equivalent across all sites within the S4 helices and indicated that the voltage dependence was not solely a matter of electrostatics; the charge movements were subject to a cooperativity between K⁺ channel subunits [64,65].

The puzzles of plant K⁺ channels

The K⁺ channels of nerve and muscle, along with the Shaker proteins, are all channels that open on membrane depolarisation to enable K⁺ efflux from the cell. So-called 'anomalous' or inward-rectifying K⁺ channels are also present, notably in cardiac tissues but, prior to the cloning of the cardiac HCN channels, their rectification was widely associated with block from inside by Mg²⁺ [66,67]. So evidence for inward rectification in plant K⁺ channels, and the identification of AKT1 and KAT1 [17,18], challenged the established dogmas of voltage dependence in K⁺ channel activation. With the cloning of SKOR [19], this challenge was compounded. Here were both inward- and outward-rectifying K⁺ channels (Box 1) in plants, the gating of which was intrinsic to the channel proteins. The plant channels incorporated the same fundamental structure of six transmembrane helices, the same TxGYGD sequence in the pore, and similar voltage-sensor domains (VSDs) comprising the first four helices with a regular repeat of Arg and Lys residues in the S4 helix (Figure 1). These comparisons begged the overarching questions: How are the K⁺ channels of the plant plasma membrane related to their counterparts in mammals and flies? And what determines their voltage dependencies?

Initially, the K⁺ channels in plants were grouped with Shaker channels as the only other known K⁺ channels similarly comprising six transmembrane helices with VSDs assembled from the first four helices. With hindsight, a more detailed look uncovers substantial differences, as have been reviewed both from evolutionary and functional perspectives [27,28,68]. Shaker and mammalian Kv1-like channels incorporate VSDs coupled to the S5 helix via a long cytoplasmic linker that enables a 'domain-swapped' arrangement (Figure 1) with each VSD positioned against the

pore-lining helices of the next subunit [27,69]. The plant counterparts all exhibit much shorter linkers that position each VSD against the pore-lining helices of the same subunit. Equally, the plant K^+ channels incorporate an extended C-terminal cytosolic tail (Figure 2)—for AKT1, SKOR, and GORK, over half the total primary sequences—that includes a cyclic nucleotide-binding homology domain (CNBHD), a sequence homologous to the cyclic nucleotide-binding domain (CNBD) of cyclic nucleotide-gated channels (CNGCs) of animals [70,71]. This domain is also found in plant CNGCs, but it is not present in metazoan Shaker and Kv1-like channels. With the exceptions of KAT1, KAT2, and KAT3 (=KC1), the plant K^+ channels also harbour an extended C-terminal domain (Figures 2 and 3) of ankyrin (ANK) repeats that are not found in the metazoan channels. So while both lineages have a common connection to Archean voltage-gated K^+ channels, the Shaker-like channels were probably lost during the evolution of plants [27].

With the cryo-EM structures for KAT1 [72,73], AKT1 [74,75], SKOR [76], and now GORK [44,77,78], we have a wealth of detail. Like other K^+ channels, each of these channels incorporates voltage

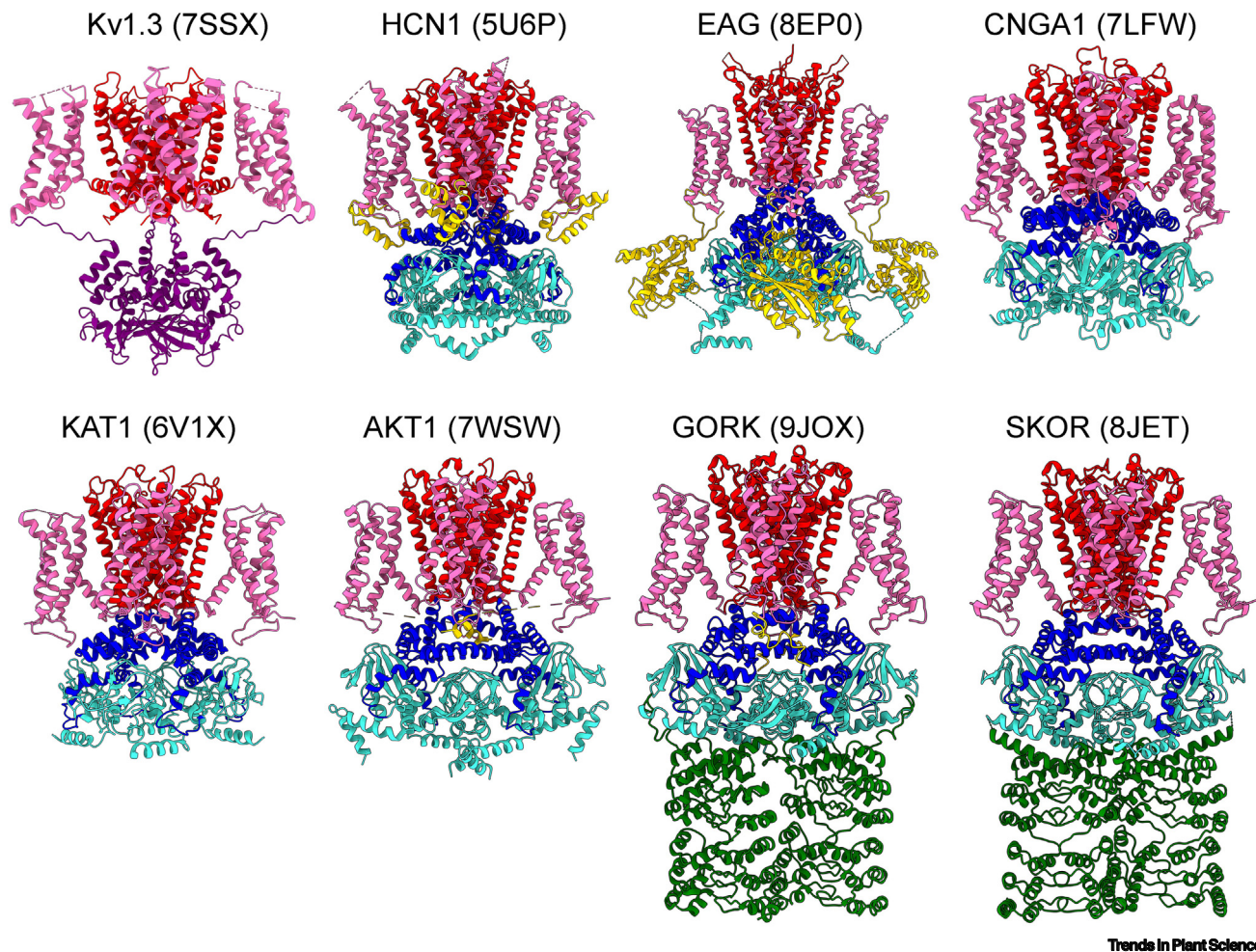
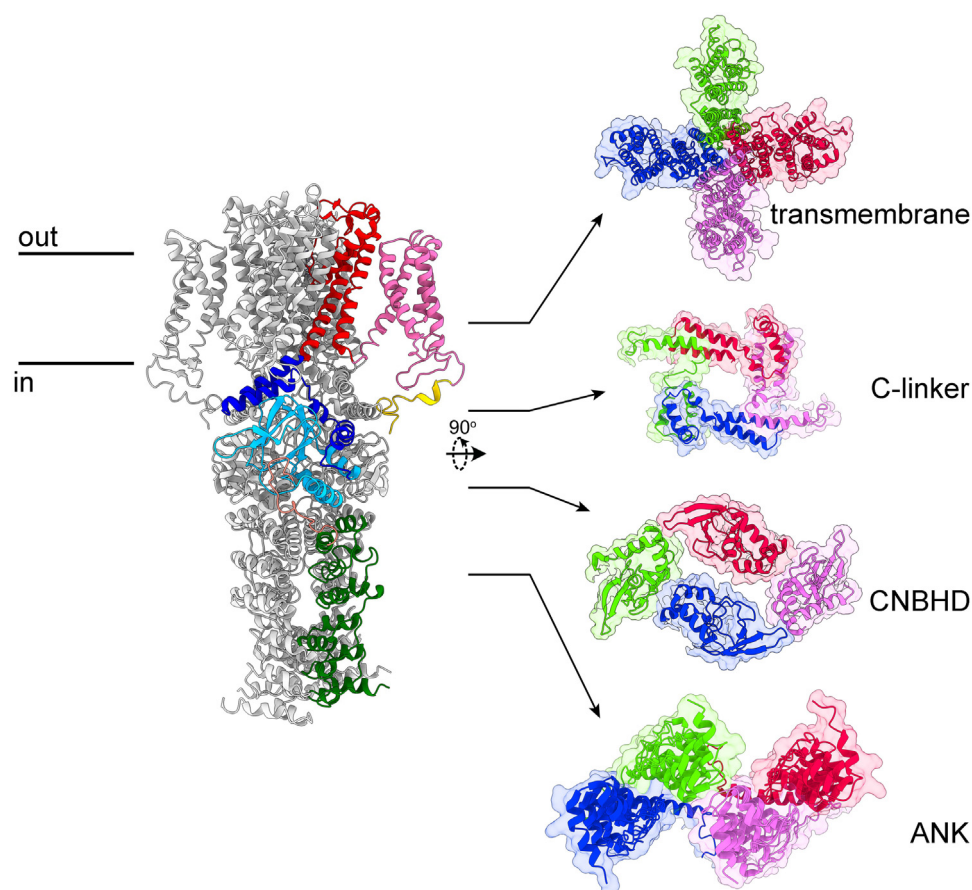


Figure 2. Comparison of the known structures of Shaker and Kv1-type with CNBD channels from mammals (above) and CNBHD channels from plants (below). Protein data bank (PDB) codes are indicated in parentheses. Pore-lining helices are shown in red, voltage-sensor domains (VSDs) in pink, C-linkers in blue and CNB (H)Ds in cyan. N-terminal domains, where resolved, are shown in yellow and ankyrin (ANK) domains in green. Unresolved linkages are indicated with dashed lines. Note that the ANK domain of AKT1 remains unresolved.



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Figure 3. Segmental projections of the GORK K^+ channel highlight the transition from fourfold to twofold symmetry. Side view (left) with one subunit colour-coded to show the pore-lining helices in red, voltage-sensor domain (VSD) in pink, C-linker in blue, cyclic nucleotide-binding domain (CNBHD) in cyan, ankyrin (ANK) domain in green, and the N terminus in yellow. Segmental projections (right) are colour-coded by subunit and show a fourfold symmetry in the transmembrane region that progressively collapses through the C-linker and CNBHD to the twofold symmetry of the ANK domain. Note the 'elbow-on-shoulder' arrangement of C-linkers beneath the membrane. Analysis after Zhang *et al.* [44].

sensors, the VSDs, that move within the membrane in response to voltage. They also include a selectivity filter within a short helical pore loop of carbonyl oxygens from the TxGYGD signature sequence that coordinates the K^+ ion and mimics the shell of water molecules that otherwise surround a K^+ ion in free solution. Cation entry to the filter is favoured by negatively charged amino acids that line the much larger cavities on either side of the filter, thereby directing the bulk of the membrane voltage across the narrow, 12-Å distance of the filter over which the ions diffuse. Within the filter, K^+ ions introduce a mutual repulsion along the chain of occupied coordination sites with the 'knock-on' effect of pushing the neighbouring ions through the filter [44,73]. Despite these shared features, questions remain, notably around the voltage dependencies of gating and their molecular mechanics.

The C-linker gating hub

The plant K^+ channel structures resolved to date highlight a number of other features that align them with the pacemaker or animal hyperpolarisation-activated cyclic nucleotide-gated (HCN) channel subfamily and plant CNGC channels. Notably, the plant K^+ channels, like their HCN

and CNGC counterparts, include the C-linker that plays a major role in the conformational changes of channel gating [79]. In each of the plant K⁺ channels (Figure 3), the C-linker follows immediately after a sharp bend in the S6 helix and forms a helix-turn-helix hairpin structure of four short helical stretches (A', B', C', and D'). Together the four C-linkers form a ring around and immediately below the pore, each C-linker abutting its neighbour in an 'elbow-on-shoulder' arrangement. In KAT1, the C-linkers are arranged in true fourfold symmetry around the pore [73]. In GORK, SKOR, and AKT1, by contrast, the C-linkers alternate between a 'flat' and 'kinked' arrangement [44,74,76]. The result is a pseudo fourfold symmetry around the pore that introduces a similar displacement of the underlying CNBDs.

With the non-domain-swapped architecture of the VSDs, the short S4–S5 linker positions the base of each VSD abutting the cytosolic end of the S6 helix from the same subunit. Single-site mutations along the C-linker of GORK identified two key acidic residues located on the C-linker A' helix that associate with the VSD and S6 helix, profoundly affecting the voltage dependence of gating [44]. These data confirm the coupling between the C-linker ring and the base of the pore. Similarly, mutations of KAT1 that localise around the VSD–pore interface suggest that direct interaction between the VSD and the C-linker hairpin is a primary determinant of gating polarity [73]. In short, the C-linker of the plant K⁺ channels emerges as a critical hub for gating, coupling movements between the pore and VSD and, as we note below, the CNBHD [80].

When does a cyclic nucleotide binding domain bind cyclic nucleotides?

Viewed from the outside, the four CNBHDs situate below the ring of C-linkers (Figure 3). Each CNBHD incorporates a set of leading helices, a seven-stranded β -roll, and terminates with three further helices, similar to mammalian HCN channels [71,81] and the CNGCs in bacteria and fungi [27]. Cyclic nucleotide binding of the HCN channels depends on the β -roll structure that forms a binding pocket with residues contributing electrostatic and hydrogen-bonding interactions, notably a highly conserved Arg residue (R⁵⁴⁹ in human HCN1) that coordinates the negatively charged cyclic phosphate moiety [82,83]. Studies of Mlotik1, a homologue of mammalian CNGCs from the bacterium *Mesorhizobium loti* [84–86], have shown that the CNBD is displaced towards the membrane surface on cAMP binding, consolidating the tetrameric structure of the channel.

While animal CNBD channels are activated or regulated by cyclic nucleotides [87,88], it appears that CNBHDs of the voltage-dependent channels of plants have lost their capacity for cyclic nucleotide binding [27]. The CNBHDs of the plant K⁺ channels lack residues of the conserved β -rolls needed to interact with bound cyclic nucleotides and, like the fly ether-à-go-go (EAG) K⁺ channel [89], the plant K⁺ channels do not bind cyclic nucleotides. Additions of non-hydrolysable 8-bromo forms of cAMP and cGMP similarly have no impact on activity [44,73,90].

Instead, the binding pocket in GORK is occupied by Arg⁵¹² located in a short sequence of residues—the so-called A-linker—between the CNBHD and the ANK domain that acts like an intrinsic ligand [44]. Not surprisingly, mutations of Arg⁵¹² greatly reduce GORK activity without affecting its voltage dependence [44]. For now, similar intrinsic ligands in SKOR, AKT1, and KAT1 have yet to be examined, although SKOR shows a very similar structure in the A-linker region. It is worth noting, too, that the CNBHDs of the plant CNGC1 and CNGC5 channels incorporate intrinsic ligands within their structure and do not respond to cAMP or cGMP [91], despite earlier reports suggesting a sensitivity to cyclic nucleotides [92,93]. Given the structural plasticity of CNBHDs, their capacity to bind other ligands, metabolites, or signalling molecules remains an open question.

While the findings suggest that intrinsic ligand-like interactions are a conserved feature of plant CNBHD channels, including the voltage-gated K^+ channels, they also raise the question why the CNBHD should be retained as part of the channel structure. A comparison with HCN1 and HCN4 may be informative here. Cyclic nucleotide binding to the HCN channels does not lead to channel opening in itself but has the effect of displacing channel voltage dependence to more positive voltages and accelerating opening on depolarisation [94,95]. Recent high-resolution cryo-EM studies of HCN1 and HCN4 [71,96] have shown that binding leads to conformational changes that reposition residues from the channel N-terminus between the base of the VSD and the C-linker, so augmenting the mechanical continuity of these domains [96–98].

Our own studies show that the N-termini of GORK interact with residues of the CNBHDs and intercalate with the C-linkers, and these interactions displace channel gating to more positive voltages, effectively reducing channel activity in a voltage-dependent manner (Box 1 and Figure 4). GORK forms clusters at low-millimolar and submillimolar K^+ concentrations outside that depend on interchannel interactions between the N-termini [24,26], thus connecting channel clustering and K^+ -dependent gating. Deletions of the GORK N-terminus lead to substantial negative-going shifts in gating, promoting channel activity, as well as a loss of clustering [24,44]. Within the GORK structure, the effect of N-terminal deletions is to displace the CNBHDs upwards, consolidating the tetrameric structure and partially dilating the channel pore [44]. The N-terminus may also form contacts with the C-linker in AKT1 [74,75]. These observations leave no doubt that the N-termini of these channels play an important 'latching' role in gating, whether by voltage or by K^+ , and their interactions below the channel pore may explain why the bulky CNBHD domains are retained as a necessary part of the gating mechanics in these channels.

K^+ channel symmetry—a tail that wags the dog?

A striking feature of the plant K^+ channels that include ANK domains is a transition from fourfold to twofold symmetry [44,76–78]. Cryo-EM analyses of KAT1 show a fourfold symmetry in the non-domain-swapped structure that is consistent with EAG (KCNH) and other CNB(H)D and Shaker-type channels [73]. By contrast, the C-terminal cytosolic domains of GORK and SKOR adopt transitional configurations that collapse into a twofold symmetrical arrangement in the ANK domain (Figures 2 and 3). Much the same arrangement may be deduced for AKT1; although the full channel structures resolved to date do not include the ANK domain [74,75], the AKT1 ANK domain itself follows the canonical ribbon-like arrangement of helical repeats and appears important for binding the CBL1–CIPK23 kinase complex [99]. The symmetry reduction in these channels is shaped by the C- and A-linkers connecting the VSD, CNBHD, and ANK domains, and it is likely to correlate with domain composition.

What role might symmetry play in channel gating? Symmetry transitions do present a gating mechanism that is conserved in some multimeric ion channels. For example, animal and plant glutamate receptors (iGluRs/GLRs) undergo asymmetric rearrangements with ligand binding that appear to propagate to the pore [100–102]. Even so, the unique symmetry and arrangement within the GLR3.4 structure indicate that plant GLR channels differ from the animal iGluR family [100] and suggest that plants may exploit such transitions in different ways.

How important, then, are the symmetry transition and the ANK domain to the plant K^+ channels? The answer is not so clear-cut. True, an analysis of two dominant coupling sites in GORK suggests that the pre-open channel associates with a change in symmetry transition. Notably, key mutations located in the C-linkers, VSDs, and S6 helices of GORK promote a fourfold symmetry within this structural ring of the pre-open channel [44]. These mutations also enhance channel activity by shifting its gating negative by 40–50 mV and stabilise the open channel, giving

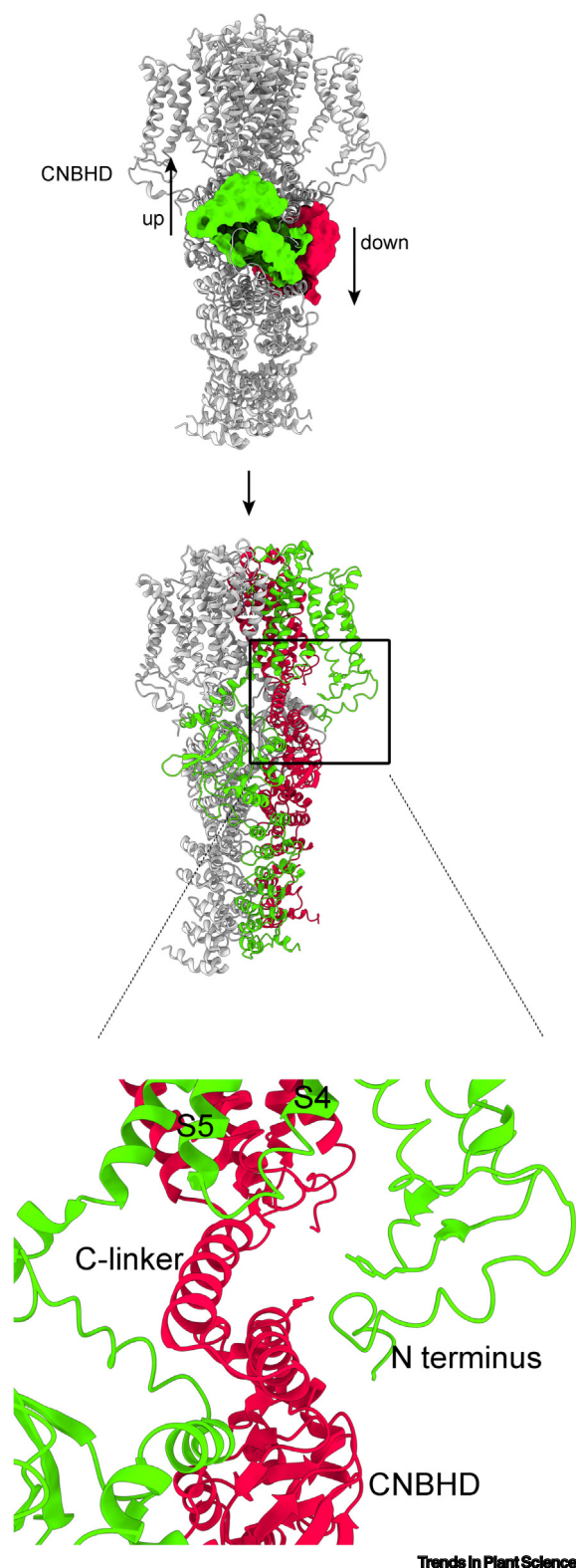


Figure 4. Modular gating of GORK engages an upward movement of the cyclic nucleotide-binding homology domains (CNBHDs) and altered interaction of the N-termini with the C-linkers. Structure of the wild-type GORK channel (top) shows CNBHDs (space-filled, with two subunits colour-coded green and red) alternately positioned down and displaced 10 Å up towards the inner membrane surface. N-terminal deletions that favour the open channel also lead to positioning of all four CNBHDs up to the membrane. Positioning (centre and below) with two subunits colour-coded show the association and intercalation of the N-terminus from one subunit with the C-linker of the neighbouring subunit that constrains conformations to the open channel. Analysis after Zhang *et al.* [44].

rise to extraordinarily slow current deactivation kinetics once activated. In AKT1, the twofold symmetric organisation appears to hinder C-linker reorientation, and phosphorylating the C-linkers that promotes the channel current results in a fourfold symmetric conformation, suggesting a symmetry switch behind channel activation [74].

One difficulty with this interpretation is simply that none of the structures resolved to date show a plant K^+ channel in the open conformation. Crystal and cryo-EM structures are necessarily resolved from protein isolated in the absence of an electrical field (0 mV). So it is no surprise that the structures of KAT1 and AKT1 reveal a closed pore. These channels normally open only with a substantial voltage, negative on the inside. For GORK and SKOR, by contrast, the lack of an open structure is more surprising, as these channels normally are open in the absence of an electric field, especially when the K^+ concentration outside is below 30 mM (Box 1) [21,23,24]. Yet the GORK structures resolved in 10 mM K^+ , and with N-terminal deletion mutants that give full channel activity at 0 mV, yielded only marginal dilations in pore diameter [44]. These structures showed the VSD in the 'up' (outward-shifted) conformation that is consistent with the open channel (Box 1 and Figure 5). In other words, the open (conductive) pore in these channels does not appear linked directly either to the VSD or a transition in symmetry. That inward rectifiers such as KAT1 [73] maintain a fourfold symmetry with a closed pore underlines this point and raises a question whether transitions from twofold to fourfold symmetry are a consequence rather than a driver for channel opening in GORK, SKOR, and AKT1.

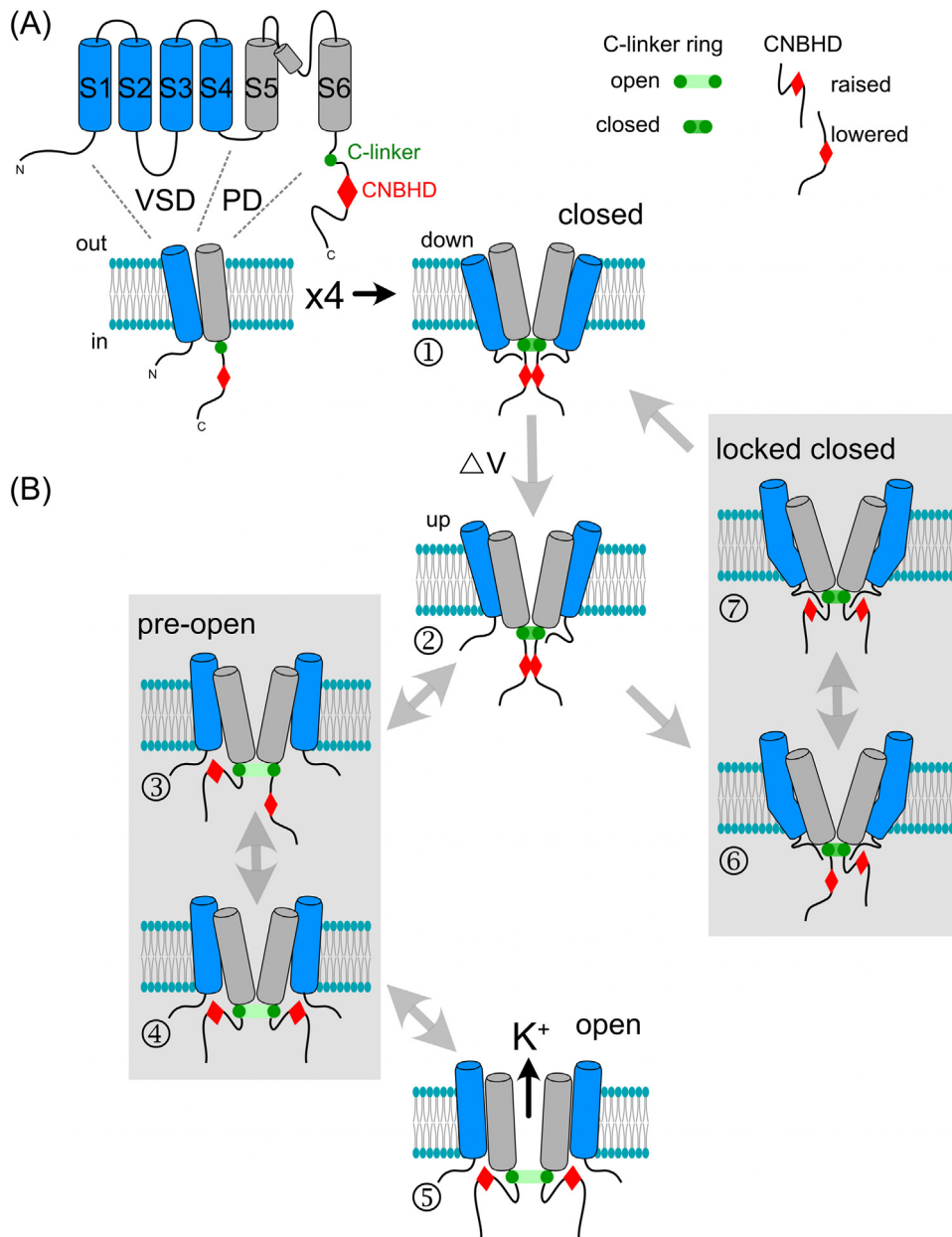
A second concern relates to the ANK domain itself. Although the last ANK repeat was proposed to contribute to tetramer assembly [103], deletions of the ANK domain nevertheless have yielded functional channels. Deleting either the final ANK repeat or the entire ANK domain of GORK had no effect on the voltage dependence of channel gating or on the ensemble channel conductance [44]. The A-linker incorporates the pseudo-ligand for CNBHD interactions and therefore may affect current amplitude [78], but any such action is clearly separable from that of the distal ANK domain. In short, any conformations adopted by this C-terminal domain appear unlikely to have a defining role in channel activity.

Capturing the open state

To date, no plant K^+ channel has been resolved in a bona fide open state. For the inward-rectifying K^+ channels that open on hyperpolarisation, including KAT1 and AKT1, capturing the open-state conformation is particularly challenging, as we have noted above. For depolarisation-activated channels such as GORK and SKOR, the lack of voltage during purification is not a constraint, and the structures resolved to date show the VSDs in the 'up' conformation that is expected of the open channel.

Most likely, the structures of GORK captured by Zhang *et al.* [44] include a long-lived closed state expected at high K^+ concentrations [22,25] and one or more pre-open states associated with a partially dilated pore captured at lower K^+ concentrations (Figure 5). These findings, along with the kinetic studies by Johansson *et al.* [25], hint at a mechanism in GORK and SKOR analogous to so-called 'filter gating', as is known for mammalian K2P channels [45]. Such gating relies on permeant ion occupation of the pore to determine channel opening and closing and is therefore subject to ion concentrations on both sides of the membrane. However, unlike K2P channels, both GORK and SKOR show little evidence of gating by internal K^+ [24–26,104]. So the question of whether a mechanism like filter gating operates in the plant channels remains an open one.

That the GORK and SKOR channel pores have been found closed with the VSD in the 'up' conformation does accord with the idea that the coupling between the VSDs and the open pore is indirect



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Figure 5. A plausible modular (or latched) gating sequence for GORK connecting the closed and open states of the channel. Schematic of the GORK K⁺ channel structure (A) indicating the voltage-sensor domain (VSD) and pore domain (PD), C-linker, and CNBHD. The sequence takes account of cryo-EM evidence for GORK of Zhang *et al.* [44] and the kinetic analysis for SKOR gating by Johansson *et al.* [25]. Transition from closed to open (B) engages depolarisation-driven movement of the VSD [(1)–(2)] from 'down' to the 'up' position followed by disengagement of the N-terminus [(2)–(3)] and conformational changes of the C-linker and CNBHD [(3)–(4)] before the pore opens [(5)]. In higher external K⁺ concentrations, the channel may also transit to one or more 'locked closed' conformations [(6)–(7)] before relaxing to the closed state [(1)], a dependence that shares similarities with so-called 'filter gating' channels [45].

among CNB(H)D channels. The situation contrasts with that of the Shaker-like channels and is supported by the resolution of intermediate conformational states. Intermediate steps have also been resolved for KAT1 [105], and allosteric coupling occurs in the mammalian HCN and human

ERG channels [106–108]. The idea of a modular or sequential ‘latching’ mechanism in gating is also supported by findings of HCN channels with the pore open and closed, in both configurations with VSDs in the ‘up’ configuration [70,81,96,109]. Ultimately, a clearer picture of these dynamics will come once structures are resolved for the plant K^+ channels with the pores open.

Concluding remarks: An outlook on gating

It remains a remarkable fact that, in *Arabidopsis* and all other plant species examined so far, the plasma membrane K^+ channels are very similar in sequence, yet they exhibit profound differences in gating. As a class, the inward-rectifying K^+ channels—KAT1, AKT1, and their homologues—give currents on membrane hyperpolarisation and operate a gating mechanism over a fixed voltage range, independent of the permeant ion. The outward-rectifying K^+ channels, by contrast, incorporate a gating mechanism that is subject both to voltage and, uniquely, to the extracellular K^+ concentration. Gating of these channels, including SKOR and GORK, is not fixed but instead activates over a voltage range that shifts in parallel with the prevailing equilibrium voltage for K^+ .

Our expanding knowledge of the structures of these K^+ channels is certain to inform on their differing functional mechanics and will be greatly accelerated once a channel is resolved with an open pore. Clearly, innovative approaches are needed to secure a metastable open conformation that can survive protein purification and preparation. Recent studies of vertebrate CNG and EAG channels using time-resolved cryo-EM have provided details of the conformational trajectories as the channels pass through intermediate states [110,111]. For plants, time-resolved cryo-EM and K^+ mimetics may provide solutions, especially as GORK gating has been reported to present a latching mechanism, semi-independent of the VSD [44]. Further support may come from *in silico* modelling, including AlphaFold [112], molecular dynamic simulations, and especially elastic network modelling that expands molecular dynamic simulations to timescales that are truly relevant to gating [113]. Such efforts will be important in bridging the gap between structural snapshots and functional kinetics in order to decode the dynamics of channel gating.

Once these challenges are overcome, we will have a much clearer picture of what determines the differences in voltage sensitivity between inward- and outward-rectifying channels in plants, and how a permeant ion such as K^+ also contributes to gating as an allosteric regulatory factor. What is already clear is that no single domain defines the gating of the plant K^+ channels. It seems likely, therefore, that the gating characteristics of these channels will emerge from the distributed conformations and interactions between domains within each channel protein (see also Outstanding questions).

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Declaration of Generative AI and AI-assisted technologies in the writing process

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Outstanding questions

What is needed to resolve the structures of an open potassium (K^+) channel?

With both inward- and outward-rectifying K^+ channels sharing the same non-swapped domain structure and assemblies with cytosolic C-linker, cyclic nucleotide-binding homology domain, and ankyrin domains, what determines the differences in current rectification?

How do the unstructured N termini of GORK, SKOR, and potentially AKT1 channels affect C-linker and cyclic nucleotide-binding homology domain conformational stability?

What is the role of the ankyrin domain structure in gating and in channel organisation at the membrane?

Can a latching process of gating be ascribed to discrete domain modules with a common order of events across the different channels? And could the order in which these events are co-opted explain major differences in gating between the various K^+ channels?

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