The bare necessities of plant $K^+$ channel regulation

Cécile Lefoulon

1 Laboratory of Plant Physiology and Biophysics, Bower Building, University of Glasgow, Glasgow G12 8QQ, Scotland

*Author for communication: cecile.lefoulon@glasgow.ac.uk
†Senior author.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/general-instructions) is: Cécile Lefoulon (cecile.lefoulon@glasgow.ac.uk).

Abstract

Potassium ($K^+$) channels serve a wide range of functions in plants from mineral nutrition and osmotic balance to turgor generation for cell expansion and guard cell aperture control. Plant $K^+$ channels are members of the superfamily of voltage-dependent $K^+$ channels, or Kv channels, that include the Shaker channels first identified in fruit flies ($Drosophila melanogaster$). Kv channels have been studied in depth over the past half century and are the best-known of the voltage-dependent channels in plants. Like the Kv channels of animals, the plant Kv channels are regulated over timescales of milliseconds by conformational mechanisms that are commonly referred to as gating. Many aspects of gating are now well established, but these channels still hold some secrets, especially when it comes to the control of gating. How this control is achieved is especially important, as it holds substantial prospects for solutions to plant breeding with improved growth and water use efficiencies. Resolution of the structure for the KAT1 $K^+$ channel, the first channel from plants to be crystallized, shows that many previous assumptions about how the channels function need now to be revisited. Here, I strip the plant Kv channels bare to understand how they work, how they are gated by voltage and, in some cases, by $K^+$ itself, and how the gating of these channels can be regulated by the binding with other protein partners. Each of these features of plant Kv channels has important implications for plant physiology.

Introduction

Potassium ($K^+$) is the most abundant inorganic macroelement maintaining turgidity of the plant cell. As a positively charged species, it balances the negatively charged nucleic acids and proteins, and it serves as a cofactor for some enzymatic activities. The transport of $K^+$ is also a significant factor contributing to charge balance in the transport of other solutes across the cell membrane. Consequently, the transport of $K^+$ regulates cellular hydration and is a critical factor in cell expansion (Very and Sentenac, 2003; Wang and Wu, 2013).

Potassium transport through plasma membrane occurs through different types of transport systems, notably channels that can be highly selective for $K^+$, like the voltage-gated (Kv) channels that we will describe in this review, or less specific for cations like cyclic nucleotide-gated channel (CNGC), and transporters that can operate as uniporters or symporters with the proton as a driver ion, like high-affinity $K^+$ transporter (HKT) or high-affinity $K^+/K^+$ UPtake/K+ transporter (HAK/KUP/KT; Gambale and Uozumi, 2006; Waters et al., 2013; Santa-Maria et al., 2018; Dietrich et al., 2020). Channels are described as passive because they facilitate $K^+$ flux down the electrochemical gradient (Kochian et al., 1985) and generally operate with low transport affinity (Epstein et al., 1963) even if the Kv channel AKT1 can take up $K^+$ in the range between 10 and 100 $\mu$M (Spalding et al., 1999). The most prominent feature of Kv channels, however, is that their opening and closing are controlled by voltage.
Kv channels are not the only voltage-dependent channels in plants. CNGC channels and the vacuole TPC1 channel also show voltage sensitivity. Unlike plant Kv channels, however, they are not specific for $K^+$ and may carry also $Ca^{2+}$ and $Na^+$ (Guo et al., 2016; Li et al., 2017).

In this review, I focus on plant Kv channels, their structures, and their functions. I begin with an explanation of how $K^+$ is conducted through the channel pore and how the channels achieve a high degree of selectivity. Thereafter, I introduce the structural features of plant Kv channels, compare these among the superfamily of Kv channels, and describe the different types of plant Kv channels. A previous review (Jegla et al., 2018) addressed the evolution of Kv channels. Here I will focus on how Kv channels are regulated by voltage and our present understanding of how the gating by voltage is controlled. Finally, in closing, the review will address other proteins that regulate or affect gating.

**Plant Kv channels are highly selective for $K^+$**

Hille, in his 1978 review, noted that channels are enzymes and their substrates are ions. Indeed, from a thermodynamic standpoint, all transporters are enzymes that facilitate the conversion of solutes from one energetic state to another across a membrane. Binding affinity and ion specificity for a channel define their transport properties. Long before the crystal structure of any ion channel was available, electrophysiological and radiotracer flux studies provided key insights into how channels work. Beyond the concepts of their regulation, which I address in the subsequent sections, our understanding of ion permeation was well established early on. Hodgkin and Keynes (1955) showed that ions in a channel must penetrate in single file through a water-filled pore, and work in the early 1970s led researchers to understand that the ions must move through a series of energetic barriers and binding sites that provided selectivity while still enabling ions to cross the pore at rates very close to their free diffusion in solution (Bezanilla and Armstrong, 1972; Hille, 1973).

The presence of this selectivity filter in the $K^+$ channel pore was confirmed by mutating crucial residues, leading to the recognition of a highly conserved motif for $K^+$ selectivity (Heginbotham et al., 1992, 1994). Among others, these studies showed that swapping the selectivity motif of a highly selective $K^+$ channel with the motif from a poorly selective $K^-$ channel that is permeable to $Na^+$ and $K^+$ also swapped permeability. Furthermore, single mutations in the pore motif created channels that transport a range of different alkali ions with little or no selectivity for $K^+$.

We now know that Kv channels are formed by the assembly of four subunits, often referred to as $\alpha$-subunits. In general, voltage-gated ion channels are comprised of four homologous domains although, unlike Kv channels, these domains are contained within a single polypeptide in mammalian $Na^+$ and $Ca^{2+}$ channels. At the center, the tetramer assembly forms a hydrophilic pore (Figure 1A) with each subunit contributing a domain that lines the pore. This pore domain is determined by two transmembrane $\alpha$-helices, also referred to as Segments S5 and S6, linked together by a helical segment, the P-loop, that dips back toward the membrane (Figure 1). The P-loop contains the highly conserved TxGYG motif (TVGYG in Arabidopsis thaliana Kv channels but TTGYG in KAT1 and KAT2). This motif forms the $K^+$ selectivity filter that is found in all Kv $K^+$ channels, whether from plants, animals, or bacteria.

On entering the pore, the $K^+$ ion engages with the pore and is stripped of its hydration shell to become coordinated by the carbonyl oxygen atoms of the channel TxGYG motif (Figure 1, B and C). The model proposed by Hodgkin and Keynes (1955) suggests that the $K^+$ ions line up in a row and repulse one another so that each ion entering at one end of the pore “knocks” another ion out of the pore at the other end. Like newton balls, this “hard knock-on” model implies that the pore must be fully occupied by 4 $K^+$ atoms before a $K^+$ ion passes out of the pore at the far end. The theory is supported by molecular dynamic simulations (Köpfer et al., 2014), but crystal structures and more recent
simulations suggest that one out of every two occupancy sites on the motif is filled by water and only two K+ ions need to occupy the pore at a time before an ion passes out at the far end (Berneche and Roux, 2001; Morais-Cabral et al., 2001; Kratochvil et al., 2016). This model is often cited as the “soft knock-on” process (represented in Figure 1C). Potassium ions have to line up to enter the pore as it is tight enough to let only one K+ atom at the time. Because the pore size closely matches the hydration space for a K+ ion, larger ions like Ca2+ cannot permeate the pore, and smaller ions like Na+ cannot enter because carbonyl oxygens of the filter are too far from each other to compensate for the dehydration of a Na+ ion. Consequently, Kv channels are around 20–50 times less permeant to Na+ than K+ (Thiel and Blatt, 1991; Schachtman et al., 1991; Müller-Röber et al., 1995; Amtmann et al., 1997). Studies of the bacterial KcsA Kv channel show that the pore size and structure are maintained and strengthened by the interaction between amino acids located on the TxyGYG motif and on the tilted pore helix on the P-loop (Doyle et al., 1998). This conjunction between the pore loop and the adjacent helices most likely explains why mutations in plant Kv channel TxyGYG motif and in the pore helix will affect the ion selectivity of a channel. Mutated channels in these regions can become permeable to Rb+, Na+, and NH4+ (Figure 1B; Uozumi et al., 1995, Lacombe and Thibaud, 1998), or inhibition by Cs+ can be affected (Becker et al., 1996).

Plant Kv channel diversity and structure

Plant Kv channels are not Shaker but members of the CNBD channel subfamily

Although historically identified as Shaker channels, because Kv channels were first cloned from the Shaker mutant of

---

**Figure 1** Potassium conduction through plant Kv channel. A, Plant Kv channel α-subunit domains (left) comprise the domains of the Voltage Sensing Domain (VSD, blue), pore bearing the selective TT/VGYG filter (grey), C-linker (black), CNBD (red), ankyrin domains (turquoise), and hydrophobic and acidic residues (dark blue). Four α-subunits assemble to form a functional K+ channel with the pore at the center (right). B, Schematic of the KAT1 pore domain and key amino acids, notably the TxyGYG K+ permeability motif (grey), residues contributing to selectivity (cyan), and to Cs+ blockage (red). Sensitivity to K+ in gating (orange) is also associated with H+ sensitivity in the AKT2 channel (Geiger et al., 2002), whereas H+ sensitivity of KAT1 is separate (yellow ovals). Amino acids are found in AKT2 (orange ovals) and in SKOR and GORK (orange diamonds). C, Details of the K+ filter. The soft knock-on model is represented here. Motifs from two different subunits are shown, each bearing the TT(V)GYG sequence. Two additional motifs (not shown) are positioned in front and behind the plane of the image, and contribute to the binding sites labeled here as S0 to S4. To permeate the selectivity filter, a K+ ion must be stripped of its aqueous shell, which is replaced by coordination with oxygen atoms of the backbone carbonyls groups of the TTGYG residues. In this model, each K+ ion is separated by a water molecule. The weak interactions between the oxygen atoms and K+ ions are indicated by the dotted lines.
fruit flies, plant Kv channels show the greatest similarity to the Kv subfamily of cyclic nucleotide-binding domain (CNBD) channels, and more specifically the KCNH channels. These channels include the structurally similar ether-à-go-go (EAG), EAG-related gene (ERG), and EAG-like channels (ELK), that are found in nematodes (Caenorhabditis elegans), fruit flies, rodents, humans, and other mammals (Pilot et al., 2003). The structural evolution of the K⁺ voltage-dependent channels has been addressed recently (Jegla et al., 2018). In particular, Jegla et al. (2018) explain how the structure of plant Kv channels makes them unique compared to the Kv channels of other organisms. The authors conclude that they have to be classified on their own. I come to the same conclusion, but from an entirely different position of the operational perspective. This point notwithstanding, a knowledge on plant Kv channels benefits from comparing structures and the associated functional properties.

The overall homologies among Kv channels aside, plant Kv channels lack the characteristic long cytosolic N-terminus of the Shaker channels that are known to bind to ancillary β-subunits (Hoshi et al., 1990; Schachtman et al., 1992; Long et al., 2005; Clark et al., 2020). These β-subunits are soluble proteins, essential for the function of the Shaker channels, and responsible for their so-called N-type inactivation (Figure 4D). N-type inactivation (autoinhibition) arises when the first 20–30 (largely positively charged) amino acids, which form a ball-like structure, physically enter the cytosolic end of the channel to plug and block ion entry to the channel pore. This process has been described as a “ball-and-chain” mechanism (Armstrong and Bezanilla, 1977; Zhou et al., 2001) and causes the closing of the channels some tens of milliseconds following channel activation with a change in membrane voltage. Plant Kv channels do not include such a domain and are not inactivated over time. The N-terminus is much shorter than typical of Shaker channels, but in some cases can interact with vesicle trafficking proteins, which introduces an additional level of regulation (see “SNARE proteins gate inward-rectifying Kv channels” section).

Plant Kv channels have an extended C-terminus, far longer than found among the Shaker subfamily (Figure 1A). This extended cytosolic domain generally comprises roughly half of the total sequence of residues and is recognized to be important for protein stability, tetramer assembly, and regulatory protein binding. The C-terminus contains, in order from the closest to pore domain to the furthest, the C-linker (see “Domain swapping and its implications for channel gating” section), the CNBD which is the typical domain carried by CNBD subfamily members, ankyrin domains, and an acid and hydrophobic residue domain (KHA; Very and Sentenac, 2003).

The CNBD domain is the typical domain found in cyclic nucleotide-gated (CNG) channels, hyperpolarization-activated cyclic nucleotide-gated channels (HCN), and ether-à-go-go type (KCNH) channels. CNG channels need this CNBD domain to bind to cyclic nucleotides (cGMP or cAMP depending on the channel) to be functional. The gating properties, maximal conductance, and kinetics of HCN channels are affected by the binding of cyclic nucleotides. Although KCNH channels do not depend on cyclic nucleotide binding, part of the protein mimics a binding pocket and incorporates a cyclic nucleotide. Plant Kv channels lack such a site mimic, but the CNBD is found in an “active” position by default, which precludes the need for cyclic nucleotide binding (Clark et al., 2020). In HCN channels, the CNBD domain rotates as part of channel gating leading to the open state (James and Zagotta, 2018). By contrast, the CNBD in plant Kv channels is critical for tetramerization (Daram et al., 1997; Dreyer et al., 2004). Some amino acids in the C-linker and CNBD of KAT1 (via the 39α-D-X-D motif) may be important for channel targeting to the plasma membrane, although tetramer stability has yet to be tested and could be compromised when changing these residues (Mikosch et al., 2006). Another region upstream of the KHA region seems to be important also for GORK tetramerization (Dreyer et al., 2004). The KHA region may contribute to channel clustering of the KST1 channel, the KAT1 homolog of potato, as clustering is lost when the KHA domain is removed (Ehrhardt et al., 1997).

Ankyrin domains are absent in KAT1 and KAT2 Kv channels. They consist of concave structure that is partially charged positive and another part is negative, at least in KAT1 channel (Sánchez-Barrena et al., 2020). These domains serve as binding sites for protein kinases, notably the CIPKs (Lee et al., 2007), and are described in “Calcium sensitive kinases and channels activation or inhibition” section.

Diversity in plant Kv channels

Although plant Kv channels share a common structure, their properties differ substantially from one to another. Broadly speaking, the channels have been subdivided among five groups (Pilot et al., 2003). This classification is appropriate for flowering plants (angiosperms), but the distinctions between groups, for example, Groups I and II, are not obvious in some species, including fern, bryophyte, and gymnosperm clades (Dreyer et al., 2020). Apart from phylogeny, Groups I and II channels properties are too similar to be separated: they are activated by membrane hyperpolarization and facilitate K⁺ influx into the cell (Figure 2A; inward-rectifying channels AKT1, KAT1 and KAT2 in Arabidopsis; Schachtman et al., 1992; Gaymard et al., 1996; Pilot et al., 2001; Xu et al., 2006). Members of Group III are weakly regulated by voltage and are represented by the AKT2 channel in Arabidopsis. AKT2 remains partially open at all voltages and therefore mediates a “leak” of K⁺ subject to the electrochemical driving force on the ion (Figure 2B; Lacombe et al., 2000). The channels of Group V are activated by membrane depolarization and mediate K⁺ efflux (Figure 2C; outward-rectifying channels GORK and SKOR; Gaymard et al., 1998; Ache et al., 2000). Finally, Group IV channels are also referred to as “silent” channel subunits. This group is
represented by KC1 in Arabidopsis. These channels are silent, because they do not form functional channel assemblies on their own, but function only in heterotetramers assembled with the other Kv channel subunits; in this context, they moderate the voltage sensitivity of channel gating (Duby et al., 2008).

\[
I = \frac{g_{\text{max}}(V - E_K)}{1 + \exp \left( \frac{V_1/2 - V}{F/R} \right)}
\]

(1)

\[
\Delta G = -F \delta V_{1/2}
\]

(2)

\[
I = g_{\text{max}}(V - E_K)
\]

(3)

The different characteristics of Kv channels reflect the different physiological processes in which they are involved. AKT1 channels participate in K⁺ uptake in roots; the SKOR channel allows the efflux of K⁺ from cells around xylem to translocate the ion from root to shoot; K⁺ distribution in vascular tissues is mediated by AKT2, for example; and KAT1, KAT2, and GORK are vital for the effective kinetics of stomatal movement. KAT1 and KAT2 facilitate stomata opening by mediating K⁺ influx into the guard cell; GORK contributes to K⁺ efflux for stomata closing (Very and Sentenac, 2003; Gambale and Uozumi, 2006; Lebaudy et al., 2007).

**Voltage gating in plant Kv channels**

**Up and down movement of the voltage-sensing domain**

For Kv channels, membrane voltage is the most important stimulus and its action changes the conformational state of the channel. These conformational changes, generally referred to as gating, define the open or closed states of the channel.

All Kv channels harbor so-called voltage sensor domains (VSDs; Figure 1A). In general, VSDs are structurally conserved and consist of the N-terminal end and four transmembrane α-helices, designated S1–S4. The fourth VSD segment contains a repeated sequence of positively charged amino acids that are positioned across the electrical field of the membrane. As a consequence, the position in the membrane of this domain—and much of the rest of the VSD through its assembly—is driven by changes in the membrane voltage. When the electric field across the plasma
The membrane becomes more negative (hyperpolarization), the VSD moves downward toward the cytoplasmic face of the membrane, and similarly, as the membrane becomes less negative inside (depolarization) the VSD shifts away from the cytosolic side of the membrane. These two end-point conformations are generally referred to as the “down” and “up” positions, respectively. These movements are coupled through the linkage to the pore-lining helices to open and close the channel pore (Figure 3A). For example, the “up” position for the KAT1 channel represents the channel in its closed state while the “down” position represents the channel in its open state.

Significantly, how this movement impacts on gating differs between inward- and outward-rectifying channels. KAT1 is an inward channel that opens at voltages more negative than −100 mV. To test the VSD movement in KAT1, basic residues in the S4 helix of the VSDs were substituted with cysteines (Latorre et al., 2003). Cysteine residues react with methylthiocyanate compounds, including the water-soluble MTSET. Thus, the reactivity of the residues provides information about the accessibility of substituted amino acids to the aqueous solution on one side or the other of the membrane in the open and closed states. For example, specific amino acids are accessible to MTSET added outside, but they become inaccessible when the channel is stimulated by voltage to drive it into the open conformation. The simplest explanation, therefore, is that these amino acids move in and out of range of MTSET on the cytosolic side when KAT1 is closed and open, respectively (Latorre et al., 2003).

By contrast, SKOR is an outward-rectifying channel that opens with positive-going voltages (depolarization) from the K⁺ equilibrium voltage (Eₖ; Figure 2C). SKOR is trapped in the open state by oxidizing agents, including H₂O₂ and MTSET, and this characteristic is observed when the channel is driven by voltage into “up” position (Garcia-Mata et al., 2010). Because hyperpolarization has opposite effects, closing outward-rectifying channels and opening inward-rectifying channels, we must conclude that VSD coupling to the pores differs between these two subsets of K⁺ channels.

It is of interest that some VSDs may act as channels in their own right. The Hv1/VSOP channel structure is very similar to the VSDs of Kv channels and incorporates a “proton wire” within the S1–S4 structure that conducts H⁺ across the membrane (Ramsey et al., 2006; Sasaki et al., 2006). Unlike Kv channels, Hv1 assembles functional channels as a dimer, the first subunit working as voltage sensor while the second subunit forms the pore. Functionality between voltage sensor and pore are interchangeable. The Hv1 VSD differs from the VSDs of the Kv channels, as it possesses an aqueous conduit in the center of the four transmembrane segments, allowing proton permeation (Ramsey et al., 2010). KAT1, by contrast, possesses a hydrophobic plug at the center of the VSD domain, formed by the union of the amino acids F102 and V70, which prevents H⁺ permeation (Lefoulon et al., 2014, Clark et al., 2020). Here the closed structure of the VSD is maintained by the formation of salt bridges between different positive and negative charged amino acids (Figure 3B).

**Domain swapping and its implications for channel gating**

How is the movement of Kv channel VSDs coupled to the opening and closing of the pore? And what determines whether the “down” position is coupled to channel opening or channel closing? The S4–S5 linkage between the VSD and pore domain appears to be critical. The mechanics of the mammalian Kv1-9 channels are best known from the crystal structures first resolved from Kv1.2 and Kv2.1. These channels have been described as “domain-swapped,” because the VSD domain of each subunit situates adjacent to the pore domain of the neighboring subunit (Figure 4A, right panel). In these channels, the gating mechanism gains leverage through this anchorage, enabling the VSD to pull on its pore domain through the linkage and dilating the channel pore, much like rotating the ring on an iris diaphragm to open a lens for light entry (Figure 4B, Long et al., 2005).

The structures of several CNBD channels—notably the human ERG, rat EAG1, human HCN1, the L. licerasiae LiiK, and C. elegans TAX-4 channels, all from the KCNH, HCN, and CNG channel families—were resolved in the past few years (Whicher and MacKinnon, 2016; James et al., 2017; Li et al., 2017; Lee and MacKinnon, 2017; Wang and MacKinnon, 2017). By contrast, these channels display a “non-domain-swapped” structure in which each pore domain abuts on the VSD domain of the same polypeptide (Figure 4A, left panel). We now know that KAT1 shows much the same characteristics (Clark et al., 2020). It is most likely that other plant Kv channels will follow suit. Certainly for each of these channels, the S4–S5 linkages are relatively short by contrast with the mammalian Kv1-9 channels, including the Shaker channels.

The short-length linker of nondomain-swapped channels adds a physical constraint that argues against their functioning like domain-swapped channels. The short linkers cannot extend across the same distance and thus preclude VSD leverage with the neighboring pore domain. Lörinz et al. (2015) and Tomczak et al. (2017) tested the relationship between VSD and pore domains by cutting the S4–S5 linkers of the rat EAG1 channel and coexpressing the split polypeptides in Xenopus oocytes. Their studies show that channel function is recovered when the linker is no longer intact. Even removing the S4–S5 linker entirely yielded channel currents that were similar to the currents of the intact EAG1 channel. These findings argue strongly against a mechanism relying on a direct physical coupling and “pull” through the VSD-pore domain linkage. Instead, the authors argue that the VSD may act as a plug that extends in down position below the inside of the pore to block K⁺ entry and efflux through the channel (Figure 4C).

The concept of a plug extension obviously cannot work with KAT1, because the “down” state of the VSD gives rise to an open KAT1 channel. Instead, the recent
crystallographic analysis of KAT1 (Clark et al., 2020) suggests a toggle-switch mechanism that incorporates part of the C-terminal cytosolic domain. This domain incorporates a so-called C-linker comprising two α-helices in a hairpin shape located in the cytoplasm just below the S6 transmembrane α-helix (Figure 4E). The analysis suggests that when KAT1 VSD goes "down" with the membrane hyperpolarization, the intracellular loop between the S4 and S5 α-helices presses against the C-linker of the neighboring subunit, thereby moving the S6 α-helix to open the pore (Clark et al., 2020).

Much the same mechanism has been proposed for gating of the HCN1 channel, which also opens when the VSD is in the "down" conformation and generates an inward current similar to KAT1 (Lee and MacKinnon, 2017). These findings give us a different perspective on the mechanics of KAT1 gating that may apply equally to other inward-rectifying K+ channels of plant. This point apart, they also show how little we know still about the mechanisms underlying gating of several Kv plant channels. Not least, although the GORK and SKOR channels are structurally similar to KAT1, the mechanism of their gating clearly cannot be understood as an opening by pressure on the C-linker with the VSD transting to the "up" position.

Differences between inward and outward gating
What defines the rectification of gating in the plant Kv channel subfamily? A strategy used by Gajdanowicz et al. (2009) and Riedelsberger et al. (2010) was to investigate the differences in sequence for the two channels by swapping domains one at a time to assess their importance for the gating control. Surprisingly, exchanging KAT1 and SKOR residues did not result in the exchange of channels gating properties but showed that gating could be modified, sometimes in unpredictable ways. Gajdanowicz et al. (2009) focused on differences between the S6 pore-lining α-helix. Using the voltage yielding half-maximal conductance, $V_{1/2}$ (explained in Figure 2 legend) as a measure of gating showed that this value is displaced to more negative voltages when key residues in the S6 α-helix are swapped with those of GORK. These mutations induced a bias suppressing KAT1 opening. By contrast, introducing KAT1 residues to the SKOR channel pore domain introduced a leak current.

Figure 3 VSD movements of KAT1 between the open and closed states. A, Movement of the VSDs within the membrane between the "up" and "down" states and the corresponding channel activity for K+ permeation. B, Interaction between amino acid residues across transmembrane α-helices within the VSD, as implicated from crystallographic and residue substitution analyses of KAT1 with molecular dynamic simulations. Residues $^{102}$F and $^{70}$V (yellow, hydrophobic) form the hydrophobic core of VSD. Basic residues (red) that provide positive charge to the S4 segment and form salt bridges (white lines) with acid amino acids (violet). Salt bridges incorporate knowledge of KAT1 and general Kv channel structure (black lines; Clark et al., 2020). Transition from the closed to open state realigns bridges and was identified through mutations F102W (F129W in KC1) that force the channel into the "up" state and D105E (D132E for KC1) that force the channel into the "down" state (Lefoulon et al., 2014).
with a partial loss of voltage control of the channel (Riedelsberger et al., 2010). This behavior could be explained if the mutated SKOR channel was unable to close completely. One potential interpretation from these studies is that the pore diameter of KAT1 is wider than that of SKOR, but molecular dynamic simulations suggest this is not the case and that the vestibule leading into the KAT1 pore is narrower than that of SKOR. Thus, for now, we must conclude simply that the two pores are structurally different in the two types of channels.

Chimeras constructed with the rEAG1 and HCN1 (Kasimova et al., 2019) do shed some light on the problem...
of what determines the rectification of a channel. Molecular
dynamic simulations and experimental analysis of these chi-
meras show that during the channel opening, negatively
carged amino acids from \( \alpha \)-helice S4 of HCN1 slide down
and a break in hydrogen bonding forms at a serine residue
in the middle of the S4 \( \alpha \)-helix. This amino acid is absent in
the EAG channels. Replacing this residue with a hydrophobic
residue in the chimeric HCN1 with the \( \alpha \)EAG1 pore domain
reversed the rectification of the channel. If we align this re-
region of the chimera with the plant Kv channels, it is clear
that the consensus sequence is slightly different. The resi-
dues RLLRL of HCN1 are replaced with RLWRL for all inward
that the consensus sequence is slightly different. The resi-
dues RLLRL of HCN1 are replaced with RLWRL for all inward
channels and with RLLxRV for GORK and SKOR.
Furthermore, an arginine is present at the HCN1 serine resi-
dues RLLRL of HCN1 are replaced with RLWRL for all inward
residues in the chimeric HCN1 with the \( \alpha \)EAG1 pore domain
reversed the rectification of the channel. If we align this re-
region of the chimera with the plant Kv channels, it is clear
that the consensus sequence is slightly different. The resi-
dues RLLRL of HCN1 are replaced with RLWRL for all inward

Kv channel gating by \( K^+ \)
One of the intriguing features of plant Kv channels is their
dependence on \( K^+ \) both as a permeant ion and as a gating
ligand. For the outward-rectifying channels GORK and
SKOR, but not for the inward-rectifying \( K^+ \) channels, exter-
nal \( K^+ \) regulates channel activity. These channels, and their
countertomorphs in other plant species (cf. Blatt, 1988; Blatt
and Gradmann, 1997; Jezek and Blatt, 2017) represent the only
known examples of enzymes for which \( K^+ \) binding alters ac-
tivity with a known mechanism. Increasing the \( K^+ \) con-
centrations outside has the effect of shifting \( V_{1/2} \) to more
positive voltages roughly in line with the shift in \( E_K \) (Figure
2C). In effect, increasing \( K^+ \) outside the cell favors the
closed channel conformation so that the membrane voltage
must be driven further to positive voltages in order to acti-
vate the channel. In guard cells, the interplay of \( K^+ \) with
voltage-dependent gating is crucial to ensure the capacity
for stomatal closure across a wide range of external \( K^+ \)
centrations (Blatt, 1988; Jezek and Blatt, 2017).

The mechanism for \( K^+ \)-dependent gating relies on \( K^+ \) en-
try and occupation of the channel pore. Single site muta-
tions of the SKOR channel in the TxGYG selectivity motif
and residues abutting on the inside of the S6 \( \alpha \)-helix lead to
a reduced \( K^+ \) sensitivity or a complete loss of \( K^+ \) regulation
in gating (Figure 1B; Johansson et al., 2006). Johansson et al.
(2006) also showed that this sensitivity alters with depletion
of \( K^+ \) from within the pore when extracellular \( K^+ \) is re-
duced. One interpretation is that \( K^+ \) occupation at the
pore entrance helps to stabilize the closed pore and increases
the activation energy for movement of the VSD to the “up” position. This interpretation accords with much ev-
dence that shows an, often tight coupling between the pore
and the VSD in Kv channels (Palovcak et al., 2014). In short,
constructions of the pore region and pressure through the
S6 \( \alpha \)-helix mediate \( K^+ \) sensing in these channels.

Kv channel gating by protons
By contrast with GORK and SKOR, the gating of KAT1 and
several other inward-rectifying \( K^+ \) channels is affected
substantially by \( H^+ \) with increasing \( H^+ \) concentrations out-
side facilitating KAT1 opening (Blatt, 1992). In the KAT1 ho-
logom of potato, KST1, the \( ^{265}H \) residue was suggested to
be responsible for pH sensing (Hoth and Hedrich, 1999).
However, subsequent work showed that an electron cloud of
\[ ^{268}E, ^{266}F, \text{and} ^{267}H \] is responsible for the pH sensitivity
of KAT1 (González et al., 2012). There is also evidence for
KAT1 that the first extracellular loop between the S1 and S2
\( \alpha \)-helices of the VSD and the cytosolic loop between the S2
and S3 \( \alpha \)-helices are involved in external and internal \( H^+ \)
sensing, respectively (Tang et al., 2000; Wang et al., 2016).
Thus, for example, exchange of the S1–S2 loop with that of
the maize ZmK2.1 homolog, which is insensitive to external
pH eliminates this \( pH \) sensitivity. In these instances, \( H^+ \)
Affects not only the \( V_{1/2} \) for gating, but also activation and
deactivation kinetics.

AKT2 \( K^+ \) leakage and gating
The AKT2 channel incorporates elements of gating of the
inward-rectifying \( K^+ \) channels and those of a leak-like con-
ductance. As expected, the leak conductance responds near-
instantaneously to changes in voltage, whereas the rectifying
component of the AKT2 current exhibits slow activation ki-
netics and reaches a steady-state only after many tens of
milliseconds. These two current components are separable
based on their temporal characteristics so that subtracting
the instantaneous component yields current–voltage curves
with a modest voltage dependence (Figure 2B). The dual na-
ture of AKT2 means that it can mediate both influx and eff-
ux of \( K^+ \), depending on the membrane voltage and \( K^+ \)
centrations on the two sides of the membrane.

Three amino acids, distributed between the S4 and S5
loop and the C-linker, appear to determine the fractional
balance in AKT2 current between the leak and voltage-depen-
dent components; the latter is favored when these sites
are mutated to alanine or asparagine (Michael et al.,
2005; Figure 4E). Based on homology mapping to the struc-
ture of KAT1, these residues are closely juxtaposed on the
cytosolic face of the membrane (Gajdanowicz et al.,
2011; Clark et al., 2020). In KAT1, these residues are associ-
ated with the channel opening mechanics, and if mutated to ala-
nine the channel becomes biased to the closed state (Clark
et al., 2020). Here again, comparing these findings with our
knowledge of EAG1 noted above, it is clear that the S4–S5
loop is important for voltage gating control.

The leak conductance of AKT2 plays an important role in
the physiology of the plant. Mutating AKT2 channels to
eliminate the voltage-dependent component yields a chan-
nel that is nonetheless sufficient to complement the \( akt2 \)
utant phenotypes of slowed development and excitability
in leaves vascular tissue. By contrast, the \( akt2 \) phenotypes
are not rescued by channels mutated to give only the
voltage-dependent current component (Gajdanowicz et al.,
2011, Cuin et al., 2018). Such differences are not surprising,
as the voltage-dependent component, by nature, is special-
ized for activity over a limited range of physiological
voltages.
Of interest, however, AKT2 is normally expressed in the phloem and the delayed development evident in the akt2 mutant could be a consequence of altered transport, over longer distances, of metabolites such as sucrose (Deeken et al., 2002). Computational modeling suggests that switching between AKT2 gating "modes" of the leak and voltage-dependent conductances is sufficient to facilitate sucrose transport. How this facilitation is effected is more difficult to say. In general, all transport across a membrane is entangled through a common dependence on voltage as a driving force and as a consequence of ion transport. Kv channels are no exception and will influence other transport activities by altering the electrical balance across the membrane. It is likely that AKT2 is important for charge balance in conjunction with H⁺-coupled transport of sucrose. Regardless of the explanation, these two AKT2 mutations, and the knowledge of these domains in Kv channel gating, are clearly potential tools for manipulation and control plant growth.

**Regulation of voltage gating by heterotetramerization**

One aspect of Kv channels is their assembly as tetramers to form functional channels and, as a consequence, the potential for mixed gating characteristics through the assembly of heterotetramers (Pilot et al., 2001; Xicluna et al., 2007; Lebaudy et al., 2010). Heterotetramers are widely found among the inward-rectifying channels and yield voltage dependencies that can vary depending on the subunit identity. Obviously, such variations can occur only over the longer timescales of channel gene transcription, translation, channel assembly, and export from the endoplasmic reticulum. A good example of differences with tetramer assemblies is KAT1 and KAT2. Channels assembled of KAT2 alone activate at very negative voltages, typically with V₁/₂ values near and more negative than −180 mV, whereas gating of KAT1 homotetramers yields currents with V₁/₂ values ~40–50 mV more positive than KAT2 V₁/₂ values, and the gating of the heterotetramers falls between these two limits (Lebaudy et al , 2010). Analogously, heterotetramers of AKT2 and KAT2 show a reduced K⁺ leak conductance compared to the AKT2 homotetramer assembly, as well as an inverted sensitivity to pH and insensitivity to Ca²⁺ (Xicluna et al., 2007).

One other key example is that of KC1. Although this channel subunit fails to assemble functional channels on its own, KC1 heteromers with AKT1 are activated at substantially more negative voltages than is observed when AKT1 is expressed on its own. KC1 has a similar “inhibiting” effect on the other Kv channel subunits (Duby et al., 2008, Jeanguenin et al., 2011). The KC1 subunit is essential for K⁺ channel function nonetheless: kc1 mutant plants wholly lack the inward-rectifying K⁺ current normally observed in Arabidopsis roots, suggesting roles in channel stability and, as noted below, in association with other regulatory proteins (Honsbein et al., 2009). Finally, there is some evidence that developmental regulation of KC1 expression is important for adjusting the gating characteristics of root tissues to conditions of low external K⁺ concentration (Figure 2A; Geiger et al., 2009).

**Regulation of voltage gating by clustering**

One last mechanism of gating control, yet to be fully resolved, depends on Kv channel clustering. There has long been evidence that plant Kv channels congregate locally in clusters within the surface of the plasma membrane (Hurst et al., 2004; Meckel et al., 2004; Sutter et al., 2006; Sutter et al., 2007). For KAT1, such clustering is associated with channel traffic and is affected by signals such as abscisic acid (Hurst et al., 2004; Sutter et al., 2006; Sutter et al., 2007). Cell biological and electrophysiological analyses support the occurrence of these channels in assemblies of tens of channel tetramers that are studded across the cell surface.

In at least one instance, that of the GORK K⁺ channel, clustering is associated with gating. As with KAT1, the GORK channel is found in clusters of ~600 nm diameter across the cell surface but, unlike KAT1, these clusters are stable at plasma membrane in the face of hormone exposure (Eisenach et al., 2014). However, the studies of Eisenach et al. (2014) show that GORK clustering is highly sensitive to the external K⁺ concentration, with clusters dispersing rapidly as the K⁺ concentration increases. Eisenach et al. also report that these changes in GORK clustering are blocked by external Ba²⁺, just as is gating, suggesting a link between gating and clustering. Indeed, we can imagine that interactions between the VSDs of GORK, when clustered, could be important for regulating channel activity by fostering or enhancing channel activity even at modest membrane voltages when external K⁺ is sufficiently reduced that the electrochemical driving force prevents its entry into the cell. Whether such behavior extends to other channels remains to be seen. The KST1 K⁺ channel was described to form clusters through its KHA domain. However, the KST1 current remains unchanged when KHA region is deleted (Ehrhardt et al., 1997).

**Kv channel gating regulation by ancillary proteins**

**SNARE proteins gate inward-rectifying Kv channels**

The plasma membrane is an interface of exchange, not only of ions and solutes via trans-membrane transport, but also of vesicular cargos that deliver lipids, proteins, and other material to the membrane and secrete cargoes at cell surface into the apoplast. This traffic of membrane vesicles is driven by a complex machinery of proteins that maintain exchanges between subcellular compartments. Among these proteins, the Soluble NSF-Attachment protein REceptor proteins (SNAREs) drive the final steps in fusion of vesicles with their target membranes (Chen and Scheller, 2001). SNAREs assemble functional complexes of four discrete coiled-coil
proteins defined by the core coiled-coil residues: the Qa-, Qb-, and Qc-SNAREs (or Qbc-SNARE) that normally localize to the target membrane, and a vesicular R-SNARE (Bassham and Blatt, 2008).

Conventional wisdom holds that membrane vesicle traffic affects ion transport over periods of many minutes to hours by modifying the population of channels and pumps at the cell surface. For example, abscisic acid induces KAT1 endocytosis with halftime of roughly 10 min (Sutter et al., 2007), and blocking exocytosis of the channel back to the plasma membrane slows stomatal opening over many tens of minutes (Eisenach et al., 2012). However, work over the past two decades has shown that SNARE proteins also regulate ion channel activities directly through physical interactions that take place with channels that are already present at the membrane.

The KAT1 channel is a prime example. Early studies of the Qa-SNARE SYP121 (=SYR1/PEN1) showed that direct injection of the SYP121AC protein fragment (=Sp2 fragment), including the coiled-coil domain but lacking the membrane anchor, was sufficient to block abscisic acid regulation of the major K+ and Cl− currents in tobacco (Nicotiana tabacum) guard cells, although the currents were still evident under voltage clamp (Leyman et al., 1999). Subsequent work (Honsbein et al., 2009; Grefen et al., 2010) showed that the inward-rectifying K+ channels KAT1 and KC1 bound selectively with SYP121, the binding altering the voltage-dependent characteristics of channel gating. Such changes in gating could not be explained as a consequence of vesicle traffic. Indeed, Grefen et al. (2015) showed that the interaction had a mutual effect with the SNARE altering gating and the channel enhancing SYP121-dependent vesicle traffic (see also Karnik et al., 2017).

We now know that, along with SYP121, the Qbc-SNARE SNAP33 and the R-SNARE VAMP721 bind to the channel VSD domain through a conserved RYxxWE motif located at the N-terminal cytosolic end of the channel and close to the S1 α-helix (Figure 5; Grefen et al., 2015; Zhang et al., 2015; Waghmare et al., 2019). Additionally, the channel binds with the regulatory protein SEC11, a member of the SEC1/MUNC18 protein family that regulates SNARE assembly and secretory traffic (Karnik et al., 2013, 2015; Waghmare et al., 2019). Of interest, the interaction of each of these SNAREs and SEC11 have different effects on channel gating, while the combination of the SNAREs in complex leads to a channel current that is little altered relative to the channels when expressed on their own (Waghmare et al., 2019). These findings, along with supporting isothermal calorimetry and Co-IP assays, lead to the conclusion that channel binding occurs in sequence with the several SNAREs during the vesicle fusion process.

How might we understand SNARE binding and its influence on channel gating? Experimental evidence shows that SYP121 binding with KC1 (in complex with AKT1) and with KAT1 favors channel activation, displacing V_{1/2} to more positive voltages in each case (Honsbein et al., 2009; Grefen et al., 2010, 2015). For KAT1, SYP121 binding can be shown to favor the channel open lifetime (Lefoulon et al., 2018), effectively reducing the energy barrier for transition from the closed to the open state (Figure 6A). Of interest in this context, the KAT1 crystal structure (Clark et al., 2020) shows that RYxxWE motif interacts with the highly positive charged S4, especially through G3E (last residue of the motif) that forms hydrogen bonds with 184R of the S4 α-helix in the closed ("up") position. A simple explanation, then, is that SNARE binding interferes with hydrogen bonding through G3E that otherwise would stabilize the channel in the closed state, thus promoting channel opening (Figure 3B).

The interactions between these Kv channels and the SNAREs make physiological sense in the context of cell expansion. SNARE-channel interactions confer a mutual control on both solute uptake and exocytosis so that the increase in cell surface area and volume is coordinated with the increase in osmotic content. The importance of this coordination is evident from work with dominant-negative protein fragments that retain binding but are disrupted in their normal functions. Thus, the early studies with the SYP121AC protein fragment showed that its expression allowed uncontrolled accumulation of osmotic solute, mainly K+ salts, and a highly significant increase in turgor (Sokolovski et al., 2008; Grefen et al., 2015). The complementary effect was observed on expressing the KC1 VSD alone (Grefen et al., 2015); in this case, the VSD promoted secretory traffic without enhanced K+ uptake, leading to a decrease in K+ and total osmotic content.

Analysis of several mutants yielded analogous findings. In roots, SYP121 and VAMP721 bind with KC1-AKT1 heterotrimers (Honsbein et al., 2009; Zhang et al., 2015). AKT1 dependent root growth was completely inhibited in the syp121 mutant along with the K+ current, thus phenocopying akt1 and kc1 null mutant plants. By contrast, plants overexpressing VAMP721, which inhibits KC1-AKT1 activity, showed roots that grew only very slowly in low K+, much as is observed in the kc1 mutant plants (Zhang et al., 2015). Finally, in stomata, SYP121AC expression suppressed stomatal closure (Sokolovski et al., 2008) and the syp121 mutation slowed stomatal reopening leading to plants that grew poorly, notably at low humidity and under high light. In each case, these characteristics are readily traced back to the coordination of the K+ currents with secretory vesicle traffic.

14-3-3 proteins modify Kv channel gating to prevent channel rundown

14-3-3 proteins are chaperones that bind specifically to phosphoproteins and regulate their function. They are involved in different signaling pathways, making the connection between environmental stimuli and protein regulation to provoke a physiological change (Denison et al., 2011). 14-3-3 proteins are known to regulate receptor kinases and other enzymes, to affect transcription factors including ABI5, and also to control the activities of H+-transporting ATPases and ion channels like TPK1, HvKCO1, and the Kv
channels KAT1 and GORK (Baunsgaard et al., 1998; Sinnige et al., 2005; Van den Wijngaard et al., 2005; Latz et al., 2007). Several 14-3-3 proteins bind to the C-terminus of GORK in vitro when phosphorylated (Van Kleeff et al., 2018), and the outward-rectifying K⁺ current in root protoplasts is inhibited when exposed to barley 14-3-3 proteins (Van den Wijngaard et al., 2005). These effects appear limited to overall activity and do not affect the voltage dependencies of the channels. The effect of 14-3-3 proteins on inward-rectifying K⁺ channels contrasts with these observations: in this case, the 14-3-3 proteins appear to enhance the conductance and displace V₁/₂ to less negative voltages (Van den Wijngaard et al., 2005).

In general, the specificity of channels for 14-3-3 protein binding is weak. For example, KAT1 will also interact with endogenous 14-3-3 proteins of Xenopus when expressed in oocytes, and these interactions lead to the same enhancement and shifts in V₁/₂ as seen with the plant 14-3-3 proteins (Sottocornola et al., 2006, 2008). 14-3-3 proteins interact with the penultimate serine of KAT1 (Figure 5) to sustain KAT1 activity and prevent the loss of channel activity in isolation, what is often referred to as channel rundown (Hoshi, 1995). Channel activity can be sustained also if ATP is added to the bathing solution and through phosphomimetic mutation of the penultimate serine (S676D) of KAT1 or with the addition of fusicoccin which binds and stabilizes 14-3-3 interactions (Saponaro et al., 2017). From a physiological context, we can imagine that 14-3-3 proteins enhance and stabilize KAT1 activity in coordination with H⁺-ATPases thereby greatly enhancing K⁺ uptake. Indeed, it is known that H⁺-ATPases are activated by light, through phosphorylation and 14-3-3 protein binding (Kinoshita and Shimazaki, 2002; Inoue and

![Figure 5](https://academic.oup.com/plphys/article-lookup/1874/20262968/2)
Proton fluxes promote the hyperpolarization that activates KAT1 and the channel activity is also supported by the binding of 14-3-3 proteins.

Channel phosphorylation does not necessarily affect channel gating

While there is a large body of data on Kv channel regulation by elevated cytosolic-free Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]), we still have little idea in many cases about how this regulation is affected. For example, electrophysiological studies of guard cells have long shown that the inward-rectifying K\textsuperscript{+} channels are strongly inhibited by elevating [Ca\textsuperscript{2+}], to 400–500 nM and above (Lemtiri-Chlieh and MacRobbie, 1994; Grabov and Blatt, 1997; Grabov and Blatt, 1999; Sokolovski et al., 2008). Thus, we don’t know if [Ca\textsuperscript{2+}] acts directly on channels or is mediated secondarily through another Ca\textsuperscript{2+}-binding protein. The actions of protein (de-)phosphorylation can affect the amplitude of [Ca\textsuperscript{2+}] transients. As an example, we know that [Ca\textsuperscript{2+}] spikes observed after challenging guard cells with nitric oxide are reduced by adding kinase antagonists to the medium (Sokolovski et al., 2005).

One certain fact is that [Ca\textsuperscript{2+}], acts as a regulator of Kv channels through phosphorylation and may serve both upstream and downstream kinases. However, for Kv channels such actions are commonly associated with changes in the pool of activatable channels, in other words with an apparent change in the amplitude of channel conductance rather than with any changes in the voltage dependence or kinetics of channel activation (Figure 6B). The best-known example is in the activation of Ca\textsuperscript{2+}-dependent protein kinases by [Ca\textsuperscript{2+}]. Two predominant families of kinases appear that respond to [Ca\textsuperscript{2+}], and regulate Kv channels: the calcium-dependent protein kinases (CPKs) and CBL-interacting protein kinases (CIPKs). The latter kinases require the presence of calcineurin B-like (CBL)-binding proteins. CBLs chelate Ca\textsuperscript{2+} and then anchor and target CIPK to activate their target proteins for phosphorylation (Kudla et al., 1999; Shi et al., 1999; Halfter et al., 2000). One well-known example is the AKT1 channel, which requires the CIPK23/CBL1 or CIPK23/CBL9 pairs in vivo and when expressed in oocytes for activity (Table 1; Li et al., 2006). These kinases modify the channel maximum conductance, following a pattern of an “all or nothing” effect (Xu et al., 2006). However, AKT1 displays K\textsuperscript{+} currents when overexpressed in tobacco.
protoplasts, where the native CIPK/CBL kinase pair is likely to be active (Hosy et al., 2005).

The activity of the GORK channel, similarly, is subject to regulation by phosphorylation and, again, the effect is separate from gating per se. GORK is functional in Xenopus oocytes and phosphorylation by CPK21 on residue 649S modulates GORK conductance (Curran et al., 2011; Van Kleeff et al., 2018; Figure 5, Table 1). The phosphomimetic S649E mutation increases GORK activity when expressed in Xenopus oocytes, but it does so without changing GORK gating properties (Lefoulon et al., 2016). Indeed, these studies showed that the density of channel protein with the wild-type GORK and GORK S649E mutant at the membrane was identical, despite the difference in activities. One simple conclusion, thus, is that phosphorylation acts as a switch to engage a population of GORK channels that would otherwise be inactive. However, to date, there remains a question about how elevating [Ca2+]i acts to modify gating.

Phosphorylation can also affect channel traffic indirectly. The action of CIPK6/CBL4 is a good example, as the kinase pair interacts with AKT2 but does not phosphorylate the channel (Held et al., 2011). Instead, the CIPK6/CBL4 pair promotes AKT2 traffic, resulting in increase in channel population and conductance of AKT2 at the membrane. Here, again, the effect of kinases depends on Ca2+, but although its mode of action differs from that of CPK21 on GORK the consequence in ensemble channel activity is virtually the same.

Conclusion

Mechanistic studies of the Kv channel family in plants have already added substantially to an understanding of their differing roles in plants, from nutrition to the regulation of stomata. These studies provide detail of the transport mechanics and its regulation and are now poised to help guide research toward engineering plants with improved efficiencies in nutritional control and water conservation. For example, the use of a chimera between the K+ channel Kcv, from Chlorella PBCV-1 virus, linked to a photosensor module from the Avena sativa phototropin has enabled enhanced stomata opening and closing kinetics, thus improving plant growth under fluctuating light conditions (Papanatsiou et al., 2019). Kv channels are not only controlled by voltage, but also contribute to membrane voltage through the transport of K+. There are many examples of how Kv channels contribute to voltage, among these we know that the phenotypes of the double mutant of the H+-ATPases aha1aha2 are largely rescued by elevating the K+ concentration in the growth media (Haruta and Sussman, 2012). Voltage deregulation of Kv channels has multiple physiological effect, for example, on phloem in affecting sugar transport and plant cell growth. Similarly, both experimental and model simulations of overexpression with channels and H-ATPases show that, modifying gating rather than Kv channel populations is the most effective way to manipulate cellular physiology (Wang et al., 2014a, 2014b).

In short, understanding the biophysical and regulatory features of the Kv channels is opening opportunities for bioengineering (see “Outstanding questions” section). The next steps will be to develop networks of interacting transport proteins in order to access the complete sets of pathways essential to Kv channel regulation.

Acknowledgments

The author thanks Michael R. Blatt (Glasgow) for support during the preparation of this review and for suggestions on its preparation.
Funding

This work was supported by grants from the UK Biotechnology and Biological Sciences Research Council BB/L001276/1, BB/M01133X/1, BB/N006909/1, and BB/T006153/1 to Prof. Blatt. These grants are part of the lab where the author works.

Conflict of interest statement. None declared.

References

Ache P, Becker D, Ivashikina N, Dietrich P, Roelfsema MRG, Blatt MR (1997) Inactivation of the sodium channel Nav1.9 in Aplysia californica. Nature 386: 697–700.

Becker D, Dreyer I, Hoth S, Reid JD, Busch H, Lehnen M, Palme Baunsgaard L, Fuglsang AT, Jahn T, Korthout HA, de Boer AH, Bassham DC, Blatt MR (2008) SNAREs: cogs and coordinators in signaling and development. Plant Physiol 147: 1504–1515.

Baunsgaard L, Fuglsang AT, Jahn T, Korthout HA, de Boer AH, Palmgren MG (1998) The 14-3-3 proteins associate with the plant plasma membrane H (+)-ATPase to generate a fusicoccin binding complex and a fusicoccin responsive system. Plant J 13: 661–671.

Becker D, Dreyer I, Hoth S, Reid JD, Busch H, Lehnen M, Palme, K, Hedrich R (1996) Changes in voltage activation, Cs + sensitivity, and ion permeability in H5 mutants of the plant K + channel KAT1. Proc Natl Acad Sci USA 93: 8123–8128.

Berneche S, Roux B (2001) Energetics of ion conduction through the K + channel. Nature 414: 73–77.

Bezanilla F, Armstrong CM (1992) K + channels of stomatal guard cells. Characteristics and function. Annu Rev Physiol 54: 511–536.

Blatt MR (1988) Potassium-dependent, bipolar gating of K + channels in guard cells. J Membr Biol 102: 235–246.

Blatt MR (1992) K + channels of stomatal guard cells. Characteristics of the inward rectifier and its control by pH. J Gen Physiol 99: 615–644.

Blatt, MR, Gradmann D (1997) K + -sensitive gating of the K + outward rectifier in Vicia guard cells. J Membr Biol 158: 241–256.

Chen YA, Scheller RH (2001) SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol 2: 98–106.

Chérél I, Michaud E, Platet N, Mouline K, Alcon C, Sentenac H, Thibaud JB (2002) Physical and functional interaction of the Arabidopsis K + channel AKT2 and phosphatase AtPP2CA. Plant Cell 14: 1133–1146.

Clark MD, Contreras GF, Shen R, Perozo E (2020) Electromechanical coupling in the hyperpolarization-activated K + channel KAT1. Nature 583: 145–149.

Corratgé-Failie C, Ronzier E, Sanchez F, Prado K, Kim JH, Lanciau N, Leonhardt N, Lacombe B, Xiong TC (2017) The Arabidopsis guard cell outward potassium channel GORK is regulated by CPK3. FEBS Lett 591: 1982–1992.

Cuin TA, Dreyer I, Michaud E (2018) The role of potassium channels in Arabidopsis thaliana long distance electrical signalling: AKT2 modulates tissue excitability while GORK shapes action potentials. Int J Mol Sci 19: 926.

Curran A, Chang IF, Chang CL, Garg S, Miguel RM, Barron YD, Li Y, Romanosky S, Cushman JC, Gribskov M, et al. (2011) Calcium-dependent protein kinases from Arabidopsis show substrate specificity differences in an analysis of 103 substrates. Front Plant Sci 2: 36.

Daram P, Urbach S, Gaynard F, Sentenac H, Chérél I (1997) Tetramerization of the AKT1 plant potassium channel involves its C-terminal cytoplasmic domain. EMBO J 16: 3455–3463.

Deeken R, Geiger D, Fromm J, Koroleva O, Ache P, Langefeld-Heyser R, Sauer N, May ST, Hedrich R (2002) Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of Arabidopsis. Planta 216: 334–344.

Denison FC, AL Paul, Zupsanska AK, Ferl RJ (2011) 14-3-3 proteins in plant physiology. Semin Cell Dev Biol 22: 720–727.

Dietrich P, Moeder W, Yoshioka K (2020) Plant cyclic nucleotide-gated channels: New insights on their functions and regulation. Plant Physiol 184: 27–38.

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K + conduction and selectivity. Science 280: 69–77.

Dreyer I, Porée F, Schneider A, Mittelstädt J, Bertl A, Sentenac H, Thibaud JB, Mueller-Roeber B (2004) Assembly of plant Shaker-like Kout channels requires two distinct sites of the channel α-subunit. Biophys J 87: 858–872.

Dreyer I, Sussmilch FC, Fukushima K, Riadi G, Becker D, Schultz J, Hedrich R (2020) How to grow a tree: plant voltage-dependent cation channels in the spotlight of evolution. Trends Plant Sci 26: 41–52.

Duby G, Hossy E, Fitzames C, Alcon C, Costa A, Sentenac H, Thibaud JB (2008) AtKC1, a conditionally targeted Shaker-type subunit, regulates the activity of plant K + channels. Plant J 53: 115–123.

Eisenach C, Chen ZH, Greifen C, Blatt MR (2012) The trafficking protein SYP121 of Arabidopsis connects programmed stomatal closure and K + channel activity with vegetative growth. Plant J 69: 241–251.

Eisenach C, Papanatsiou M, Hillert EK, Blatt MR (2014) Clustering of the K + channel GORK of A. thaliana parallels its gating by extracellular K +. Plant J 78: 203–214.

Epstein E, Raines DW, Elzam OE (1963) Resolution of dual mechanisms of potassium absorption by barley roots. Proc Natl Acad Sci USA 49: 684–692.

Ehrhardt T, Zimmermann S, Müller-Röber B (1997) Association of plant K + in channels is mediated by conserved C-termini and does not affect subunit assembly. FEBS Lett 409: 166–170.

Förster S, Schmidt LK, Kopic E, Anschütz U, Huang S, Schlücking K, Köster P, Waadt R, Larrieu A, Batistic O, et al. (2019) Wounding-induced stomatal closure requires jasmonate-mediated activation of GORK K + channels by a Ca2 + sensor-kinase CBL1-CIPK5 complex. Dev Cell 48: 87–99.

Gajdanowicz P, García-Mata C, Gonzalez W, Morales-Navarro SE, Sharma T, González-Nilo FD, Dreyer I (2009) Distinct roles of the last transmembrane domain in controlling Arabidopsis K + channel activity. New Phytol 182: 380–391.

Gajdanowicz P, Michaud E, Sandmann M, Rocha M, Corrêa LGG, Ramírez-Aguilar SJ, Gomez-Porras JL, Gonzalez W, Thibaud JB, von Dongen JT, et al. (2011) Potassium (K +) gradients serve as a mobile energy source in plant vascular tissues. Proc Natl Acad Sci USA 108: 864–869.

Gambale F, Uozumi N (2006) Properties of shaker-type potassium channels in higher plants. J Membr Biol 210: 1–19.

García-Mata C, Wang J, Gajdanowicz P, Gonzalez W, Hills A, Donald N, Riedelsberger J, Ammann A, Dreyer I, Blatt MR (2010) A minimal cysteine motif required to activate the SKOR K + channel: molecular basis of K + conduction and selectivity. Science 328: 69–77.

Gambale F, Cerutti M, Loreau C, Lemaillart G, Urbach S, Revalle M, Devauchelle G, Sentenac H, Thibaud JB (1996) The baculovirus/insect cell system as an alternative to Xenopus oocytes: First characterization of the AKT1 K + channel from Arabidopsis thaliana. J Biol Chem 271: 22863–22870.
Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferrièe N, Thibaud JB, Sentenac H (1998) Identification and disruption of a plant shaker-like outward channel involved in K+ release into the xylem sap. Cell 94: 647–655.

Geiger D, Becker D, Lacombe B, Hedrich R (2002) Outer pore residues control the H+ and K+ sensitivity of the Arabidopsis potassium channel AKT3. Plant Cell 14: 1859–1868.

Geiger D, Becker D, Vosloh D, Gambarle F, Palme K, Rehers M, Amschuetz U, Dreyer I, Kudla J, Hedrich R (2009) Heteromeric AtKC1-AKT1 channels in Arabidopsis roots facilitate growth under K+ limiting conditions. J Biol Chem 284: 21288–21295.

Hachiya T, Nishida Y, Takenami J, Hirose M, Fujita S, Sato J, Gateck T, Satoh Y, Hagiwara S, Kohata K, Okumura K, Shimazaki Y, Kasahara T, Takahashi S, Seki M (2015) The pH sensitivity of the plant K+ uptake channel KAT1 is built from a sensory cloud rather than from single key amino acids. Biochem J 442: 57–63.

Grabov A, Blatt MR (1997) Parallel control of the inward-rectifier K+ channel by cytosolic free Ca2+ and pH in Vicia guard cells. Planta 201: 84–95.

Grabov A, Blatt MR (1999) A steep dependence of inward-rectifying potassium channels on cytosolic free calcium concentration increase evoked by hyperpolarization in guard cells. Plant Physiol 119: 277–288.

Grefen C, Chen Z, Honsbein A, Donald N, Hills A, Blatt MR (2010) A Novel Motif Essential for SNARE Interaction with the K+ Channel KC1 and Channel Gating in Arabidopsis. Plant Cell. 22: 3076–3092.

Grefen C, Karnik R, Larson E, Lefoulon C, Yang W, Waghmare S, Zhang B, Hills A, Blatt MR (2015) A vesicle-trafficking protein commandeer Kv channel voltage sensors for voltage-dependent secretion. Nat Plants 1: 1–12.

Guo J, Zeng W, Chen Q, Chen L, Yang Y, Cang C, Ren D, Jiang Y (2016) Structure of the voltage-gated two-pore channel TPC1 from Arabidopsis thaliana. Nature 531: 196–201.

Halfter U, Ishitani M, Zhu JK (2000) The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci USA 97: 3735–3740.

Haruta M, Sussman MR (2012) The effect of a genetically reduced plasma membrane protonotive force on vegetative growth of Arabidopsis. Plant Physiol 158: 1158–1171.

Heginbotham L, Abramson T, MacKinnon R (1994) Potassium channels in myelinated nerve: selective permeability to small cations. J Gen Physiol 61: 669–686.

Hillin B (1973) Potassium channels in myelinated nerve: selective permeability to small cations. J Gen Physiol 61: 669–686.

Hillin B (1978) Ionic channels in excitable membranes. Current problems and biophysical approaches. Biophys J 22: 283–294.

Hodgkin AL, Keynes RD (1955) The potassium permeability of a giant nerve fibre. J Physiol 128: 61–88.

Honsbein A, Sokolovski S, Grefen C, Campanoni P, Pratelli R, Paneque M, Chen Z, Johnson I, Blatt MR (2009) A tripartite SNARE-K+ channel complex mediates in channel-dependent K+ nutrition in Arabidopsis. Plant Cell 21: 2859–2877.

Hoshi T, Zagotta WN, Aldrich RW (1990) Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science 250: 533–538.

Hoshi T (1995) Regulation of voltage dependence of the KAT1 channel by intracellular factors. J Gen Physiol 105: 309–328.

Hosy E, Duby G, Véry AA, Costa A, Sentenac H, Thibaud JB (2005) A procedure for localisation and electrophysiological characterisation of ion channels heterologously expressed in a plant context. Plant Methods 1: 1–14.

Hoth S, Hedrich R (1999) Distinct molecular bases for pH sensitivity of the guard cell K+ channels KST1 and KAT1. J Biol Chem 274: 11599–11603.

Hurst AC, Meckel T, Tayefeh S, Thiel G, Homann U (2004) Trafficking of the plant potassium inward rectifier KAT1 in guard cell protoplasts of Vicia faba. Plant J 37: 391–397.

Inoue SI, Kinoshita T (2017) Blue light regulation of stomatal opening and the plasma membrane H+ -ATPase. Plant Physiol 174: 531–538.

James ZM, Borst AJ, Hailin Y, Frenz B, Dimaio F, Zagotta WN, Veesler D (2017) CyoEM structure of a prokaryotic cyclic nucleotide-gated ion channel. Proc Natl Acad Sci USA 114: 4430–4435.

James ZM, Zagotta WN (2018) Structural insights into the mechanisms of CNBD channel function, J Gen Physiol 150: 225–244.

Jegla T, Busey G, Assmann SM (2018) Evolution and structural characteristics of plant voltage-gated K+ channels. Plant Cell 30: 2898–2909.

Jezek M, Blatt MR (2017) The membrane transport system of the guard cell and its integration for stomatal dynamics. Plant Physiol 174: 487–519.

Johannson I, Wulfetange K, Porec F, Michard E, Gajdanowicz P, Lacombe B, Sentenac H, Thibaud JB, Mueller-Roeber B, Blatt MR, et al. (2006) External K+ modulates the activity of the Arabidopsis potassium channel SKOR via an unusual mechanism. Plant J 67: 570–582.

Jegla T, Busey G, Assmann SM (2018) Evolution and structural characteristics of plant voltage-gated K+ channels. Plant Cell 30: 2898–2909.

Karnik R, Zhang B, Waghmare S, Aderhold C, Grefen C, Blatt MR. (2015) Binding of SEC11 Indicates Its Role in SNARE Vesicle Fusion and Identifies Two Pathways for Vesicular Traffic to the Plasma Membrane. Plant Cell 27: 675–694.

Karnik R, Grefen C, Bayne R, Honsbein A, Köhler T, Kiooumourtzoglou D, Williams M, Bryant NJ, Blatt Michael R (2013) Arabidopsis Sec11/Munc18 Protein SEC11 Is a Competitive and Dynamic Modulator of SNARE Binding and SYP121-Dependent Vesicle Traffic. Plant Cell. 25: 1368–1382.

Karnik R, Waghmare S, Zhang B, Larson E, Lefoulon C, Gonzalez W, Blatt MR (2017) Commandeering channel voltage sensors for secretion, cell turgor, and volume control. Trends Plant Sci 22: 81–95.

Kinoshita T, Shimazaki KI (2002) Biochemical evidence for the requirement of 14–3–3 protein binding in activation of the guard-cell plasma membrane H+ -ATPase by blue light. Plant Cell Physiol 43: 1359–1365.

Kochian LV, Xin-Zhi J, Lucas WL (1985) Potassium Transport in Corn Roots : IV. Characterization of the Linear Component. Plant Physiol 79: 771–776.

Kasimova MA, Tewari D, Cowgill JB, Ursuezac WC, Lin JL, Delemotte L, Chanda B (2019) Helix breaking transition in the S4 of HCN channel is critical for hyperpolarization-dependent gating. Elife 8: e53400.

Köpfer DA, Song C, Gruene T, Sheldrick GM, Zachariae U, de Groot BL (2014) Ion permeation in K+ channels occurs by direct Coulomb knock-on. Science 346: 352–355.

Kratochvil HT, Carr JK, Matulef K, Annen AW, Li H, Maj M, Ostmeyer J, Serrano AL, Raghuhrman H, Moran SD, et al. (2016) Instantaneous ion configurations in the K+ ion channel selectivity filter revealed by 2D IR spectroscopy. Science 353: 1040–1044.
Kudla J, Xu Q, Harter K, Gruissem W, Luan S (1999) Genes for calcineurin B-like proteins in Arabidopsis are differentially regulated by stress signals. Proc Natl Acad Sci USA 96: 4718–4723.

Lacombe B, Thibaud JB (1998) Evidence for a multi-ion pore behavior in the plant potassium channel KAT1. J Membr Biol 166: 91–100.

Lacombe B, Pilot G, Michaud E, Gaymard F, Sentenac H, Thibaud JB (2000) A shaker-like K+ channel with weak rectification is expressed in both source and sink phloem tissues of Arabidopsis. Plant Cell 12: 837–851.

Lan W, Lee S, Che Y, Jiang Y, Luan S (2011) Mechanistic Analysis of AKT1 Regulation by the CBL–CIPK–PP2CA Interactions. Mol Plant 4: 527–536.

Lorrec B, Olcese R, Basso C, Gonzalez C, Munoz F, Cosmelli D, Alvarez O (2003) Molecular coupling between voltage sensor and pore opening in the Arabidopsis inward rectifier K+ channel KAT1. J Gen Physiol 122: 459–469.

Latz A, Becker D, Hekman M, Müller T, Beyhl D, Marten I, Eing Latorre R, Olcese R, Basso C, Gonzalez C, Muller T, Beyhl D, Marten I, Eing L (2018) Gating control by AKT1-mediated K+ uptake by the KAT1 K+ channel leaveraged through phosphomimetic-activating mutations. J Biol Chem 291: 111–120.

Lebaudy A, Vern AA, Sentenac H (2007) K+ channel activity in plants: genes, regulations and functions. FEMS Lett 581: 2357–2366.

Lebaudy A, Pascaud F, Vern AA, Alcon C, Dreyer I, Thibaud JB, Lacombe B (2010) Preferential KAT1–KAT2 heteromerization determines inward K+ current properties in Arabidopsis guard cells. J Biol Chem 285: 6265–6274.

Lee SC, Lan WZ, Kim BG, Li L, Cheong YH, Pandey GK, Lu G, Buchanan BB, Luan S (2007) A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. Proc Natl Acad Sci 104: 15959–15964.

Lee CH, MacKinnon R (2017) Structures of the human HCN1 hyperpolarization-activated channel. Cell 168: 111–120.

Lehouc Q, Karkn R, Hansbein A, Gutova PV, Grefen C, Riedelsberger J, Poblete T, Dreyer I, Gonzalez W, Blatt MR (2014) Voltage-sensor transitions of the inward-rectifying K+ channel KAT1 indicate a latching mechanism biased by hydration within the voltage sensor. Plant Physiol 166: 960–975.

Lehouc Q, Bogenlin M, Moreau B, Vern AA, Szponarski W, Daunat M, Michaud E, Gaillard I, Chérèl I (2016) The Arabidopsis AtPP2CA protein phosphatase inhibits the GORK K+ efflux channel and exerts a dominant suppressive effect on phosphomimetic-activating mutations. J Biol Chem 291: 6521–6533.

Lehouc Q, Waghmare S, Karkn R, Blatt MR (2018) Gating control and K+ uptake by the KAT1 K+ channel leveraged through membrane anchoring of the trafficking protein SYP121. Plant Cell Environ 41: 2668–2677.

Lemtriti-Clieif, F., MacRobbie, EAC (1994) Role of calcium in the modulation of Vicia guard cell potassium channels by abscisic acid: a patch-clamp study. J Membr Biol 137: 99–107.

Leyman B, Geelen D, Quintero FJ, Blatt MR (1999) A tobacco syntaxin with a role in hormonal control of guard cell ion channels. Science 283: 537–540.

Li L, Kim BG, Cheong YH, Pandey GK, Luan S (2006) A Ca2+ signaling pathway regulates a K+ channel for low-K response in Arabidopsis. Proc Natl Acad Sci USA, 103: 12625–12630.

Li M, Zhou X, Wang S, Michailidis I, Gong Y, Su D, Li H, Li X, Yang J (2017) Structure of a eukaryotic cyclic-nucleotide-gated channel. Nature 542: 60–65.

Long SB, Campbell EB, MacKinnon R (2005) Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science 309: 897–903.

Lörrinczi É, Gómez-Posada JC, de La Peña P, Tomczak AP, Fernández-Trillo J, Leipscher U, Stühmer W, Barros F, Pardo LA (2015) Voltage-dependent gating of KCNH potassium channels lacking a covalent link between voltage-sensing and pore domains. Nature Commun 6: 1–14.

Meckel T, Hurst AC, Thiel G, Homann U (2004) Endocytosis against high turgor: intact guard cells of Vicia faba constitutively endocytose fluorescently labelled plasma membrane and GFP-tagged K + -channel KAT1. Plant J 39: 182–193.

Michaud E, Lacombe B, Porée F, Mueller-Roever B, Sentenac H, Thibaud JB, Dreyer I (2005) A unique voltage sensor sensitizes the potassium channel AKT2 to phosphoryregulation. J Gen Physiol 126: 605–617.

Mikosch M, Hurst AC, Hertel B, Homann U (2006) Diadic motif is required for efficient transport of the K+ channel KAT1 to the plasma membrane. Plant Physiol 142: 923–930.

Mora-Cabrall JH, Zhou Y, MacKinnon R (2001) Energetic optimization of ion conduction rate by the K+ selectivity filter. Nature 416: 37–42.

Müller-Röber B, Ellenberg J, Provat N, Willmitzer L, Busch M, Becker D, Dietrich P, Hoth S, Hedrich R (1995) Cloning and electrophysiological analysis of KST1, an inward rectifying K+ channel expressed in potato guard cells. EMBO J 14: 2409–2416.

Palovcak E, Delemotte L, Klein ML, Carnevale V (2014) Evolutionary imprint of activation: the design principles of VSDs. J Gen Physiol 143: 145–156.

Papanatsiou M, Petersen J, Henderson L, Wang Y, Christie JM, Blatt MR (2019) Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. Science 363: 1456–1459.

Pilot G, Lacombe B, Gaymard F, Chérèl I, Bouchereau J, Thibaud JB, Sentenac H (2001) Guard Cell Inward K+ Channel Activity in Arabidopsis Involves Expression of the Twin Channel Subunits KAT1 and KAT2. J Biol Chem 276: 3215–3221.

Pilot G, Pratelli R, Gaymard F, Meyer Y, Sentenac H (2003) Five-group distribution of the Shaker-like K+ channel family in higher plants. J Mol Evol 56: 418–434.

Ramsey IS, Moran MM, Chong JA, Clapham DE (2006) A voltage-gated proton-selective channel lacking the pore domain. Nature 440: 1213–1216.

Ramsey IS, Mokrab Y, Carvacho I, Sands ZA, Sansom MS, Clapham DE (2010) An aqueous H+ permeation pathway in the voltage-gated proton channel Hv1. Nat Struct Mol Biol 17: 869.

Riedelsberger J, Sharma T, Gonzalez W, Gajdanowicz P, Morales-Navarro SE, Garcia-Mata C, Mueller-Roever B, Gonzalez-Nilo FD, Blatt MR, Dreyer I (2010) Distributed structures underlie gating differences between the kin channel KAT1 and the Kout channel. Mol Plant 3: 236–245.

Rosnier E, Corrátge-Faille C, Sanchez F, Prado K, Brière C, Leonhardt N, Thibaud JB, Xiong TC (2014) CPK13, a noncanonical Ca2+-dependent protein kinase, specifically inhibits KAT2 and KAT1 shaker K+ channels and reduces stomatal opening. Plant Physiol 166: 314–326.

Schachtman DP, Schroeder JJ, Lucas WJ, Anderson JA, Gaber RF (1992) Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. Science 258: 1654–1658.

Sánchez-Barrena MJ, Chaves-Sanjuan A, Raddatz N, Mendoza I, Cortés Á, Gago F, González-Rubio JM, Benavente JL, Quintero FJ, Pardo JM et al. (2020) Recognition and activation of the plant AKT1 potassium channel by the kinase CPK23. Plant Physiol 182: 2143–2153.

Santa-Maria GE, Oliferuk S, Moriconi JJ (2018) KT-HAK-KUP transporters in major terrestrial photosynthetic organisms: A twenty years tale. J Plant Physiol 226: 77–90.

Saponaro A, Porro A, Chaves-Sanjuan A, Nardini M, Rauh O, Thiel G, Moroni A (2017) Fusococcin activates KAT1 channels by stabilizing their interaction with 14-3-3 proteins. Plant Cell 29: 2570–2580.

Sasaki M, Takagi M, Okamura Y (2006) A voltage sensor-domain protein is a voltage-gated proton channel. Science 312: 589–592.

Schachtman DP, Tyeeman SD, Terry BR (1991) The K+ /Na+ selectivity of a cation channel in the plasma membrane of root cells.
does not differ in salt-tolerant and salt-sensitive wheat species. Plant Physiol 97: 598–605.
Shi J, Kim KN, Ritz O, Albrecht V, Gupta R, Harter K, Luan S, Kudla J (1999) Novel protein kinases associated with calcineurin B-like calcium sensors in Arabidopsis. Plant Cell 11: 2393–2405.
Sinnige MP, ten Hoopen P, van den Wijngaard PW, Roobeek I, Schoonheim PJ, Mol JN, de Boer AH (2005) The barley two-pore K+ channel HvKCO1 interacts with 14-3-3 proteins in an isoform specific manner. Plant Sci 169: 612–619.
Sokolovski S, Hills A, Gay RA, Blatt MR (2008) Functional Interaction of the SNARE Protein NtSyp121 in Ca2+ Channel Gating. Ca2+ Transients and ABA Signalling of Stomatal Guard Cells. Mol Plant 1: 347–358.
Sokolovski S, Hills A, Gay R, Garcia-Mata C, Lamattina L, Blatt MR (2005) Protein phosphorylation is a prerequisite for intracellular Ca2+ release and ion channel control by nitric oxide and abscisic acid in guard cells. Plant J 43: 520–529.
Sottocornola B, Visconti S, Orsi S, Gazzarrini S, Giacometti S, Olivari C, Camoni L, Aducci P, Marra M, Abenavoli A, et al. (2006) The potassium channel KAT1 is activated by plant and animal 14-3-3 proteins. J Biol Chem 281: 35735–35741.
Sottocornola B, Gazzarrini S, Olivari C, Romani G, Valuzzi P, Thiel G, Moroni A (2008) 14-3-3 proteins regulate the potassium channel KAT1 by dual modes. Plant Biol 10: 231–236.
Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lewis BD (1999) Potassium phosphorylation is a prerequisite for intracellular Ca2+ release and ion channel control by nitric oxide and abscisic acid in guard cells. Plant J 43: 520–529.
Sutter JU, Campanoni P, Tyrrell M, Blatt MR (2006) Selective mobility and sensitivity to SNAREs is exhibited by the Arabidopsis KAT1 K+ channel at the plasma membrane. Plant Cell 18: 935–954.
Sutter JU, Sieben C, Hartel A, Eisenach C, Thiel G, Blatt MR (2007) Abscisic acid triggers the endocytosis of the Arabidopsis KAT1 K+ channel and its recycling to the plasma membrane. Curr Biol 17: 1396–1402.
Tang XD, Marten I, Dietrich P, Iwashikina N, Hedrich R, Hoshi T (2000) Histidine118 in the S2–S3 linker specifically controls activation of the KAT1 channel expressed in Xenopus oocytes. Biophys J 78: 1255–1269.
Thiel G, Blatt MR (1991) The mechanism of ion permeation through K+ channels of stomatal guard cells: voltage-dependent block by Na+. J Plant Physiol 138: 326–334.
Tomczak AP, Fernández-Trillo J, Bharill S, Papp F, Panyi G, Stühmer W, Isacoff EY, Pardo LA (2017) A new mechanism of voltage-dependent gating exposed by KV10. 1 channels interrupted between voltage sensor and pore. J Gen Physiol 149: 577–593.
Uozumi N, Gassmann W, Cao Y, Schroeder JI (1995) Identification of strong modifications in cation selectivity in an Arabidopsis inward rectifying potassium channel by mutant selection in yeast. J Biol Chem 270: 24276–24281.
Van Kleeft PJM, Gao J, Mol S, Zwart N, Zhang H, Li KW, De Boer AH (2018) The Arabidopsis GORK K+ channel is phosphorylated by calcium-dependent protein kinase 21 (CPK21), which in turn is activated by 14-3-3 proteins. Plant Physiol Biochem 125: 219–231.
Very AA, Sentenac H (2003) Molecular mechanisms and regulation of K+ transport in higher plants. Ann Rev Plant Biol 54: 575–603.
Waghmare S, Lefoulon C, Zhang B, Liliekyte E, Donald N, Blatt MR (2019) K+ channel-SEC11 binding exchange regulates SNARE assembly for secretory traffic. Plant Physiol 181: 1096–1113.
Wang Y, Hills A, Blatt MR (2014a) Systems analysis of guard cell membrane transport for enhanced stomatal dynamics and water use efficiency. Plant Physiol 164: 1593–1599.
Wang W, MacKinnon R (2017) Cryo-EM structure of the open human ether-à-go-go-related K+ channel hERG. Cell 169: 422–430.
Wang Y, Noguchi K, Ono N, Inoue SI, Terashima I, Kinoshita, T. (2014b) Overexpression of plasma membrane H + -ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. Proc Natl Acad Sci USA 111: 533–538.
Wang L, Yang SY, Guo MY, Huang YN, Sentenac H, Véry AA, Su YH (2016) The S1–S2 linker determines the distinct pH sensitivity between ZmK2.1 and KAT1. Plant J 85: 675–685.
Wang Y, Wu WH (2013) Potassium transport and signaling in higher plants. Ann Rev Plant Biol 64: 451–476.
Waters S, Gilliham M, Hrmova M (2013) Plant high-affinity potassium (HKT) transporters involved in salinity tolerance: structural insights to probe differences in ion selectivity. Int J Mol Sci 14: 7660–7680.
Whicher JR, MacKinnon R (2016) Structure of the voltage-gated K+ channel Eag1 reveals an alternative voltage sensing mechanism. Science 353: 664–669.
Van den Wijngaard PW, Sinnige MP, Roobeek I, Reumer A, Schoonheim PJ, Mol JN, Wang M, De Boer AH (2005) Abscisic acid and 14-3-3 proteins control K+ channel activity in barley embryonic root. Plant J 41: 43–55.
Xicluna J, Lacombe B, Dreyer I, Alcon C, Jeanguenin L, Sentenac H, Thibaud JB, Chérel I (2007) Increased functional diversity of plant K+ channels by preferential heteromerization of the shaker-like subunits AKT2 and KAT2. J Biol Chem 282: 486–494.
Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, Wu WH (2006) A potassium kinase, interacting with two calcineurin B-like proteins, regulates K+ transporter AKT1 in Arabidopsis. Cell 125: 1347–1360.
Zhang B, Karnik R, Wang Y, Wallmeroth N, Blatt MR, Grefen C (2015) The Arabidopsis R-SNARE VAMP721 interacts with KAT1 and KC1 K+ channels to moderate K+ current at the plasma membrane. Plant Cell 27: 1697–1717.
Zhou M, Morais-Cabral JH, Mann S, MacKinnon R (2001) Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. Nature 411: 657–661.