6.10 Structure–Function Correlates in Plant Ion Channels

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Abbreviations

E_k	Nernst-equilibrium potential for potassium
ER	endoplasmic reticulum
K_{in}	inwardly rectifying potassium (channel)
K_{out}	outwardly rectifying potassium (channel)
K_{silent}	silent potassium (channel)
K_{weak}	weakly rectifying potassium (channel)
MPM	membrane–pore–membrane

Glossary

Heteromerization Different K^+ channel α subunits combine to form the ion conducting pore. This is different from homomerization, in which four identical channel α subunits assemble.

Membrane–pore–membrane motif The structure of the ion conducting pore in ion channels that is sandwiched by two transmembrane helices.

N-linked glycosylation Amino groups of arginine residues undergo glycosylation in the endoplasmic reticulum lumen.

Shaker A voltage-gated potassium channel that was first isolated from a mutant of the fruit fly *Drosophila melanogaster*. The Shaker mutant flies shake with their legs when narcotized.

Voltage-sensing domain The transmembrane domain in ion channels that senses the transmembrane voltage and induces conformational changes.

6.10.1 Introduction

In plants, membrane ion transport systems play an important role in maintaining cellular homeostasis and adaptation to environmental changes. This chapter focuses on K^+ transport systems because the most knowledge on structure–function correlates of plant ion transporters is available for these proteins. Potassium (K^+) is the most abundant cation in plant cells. It is present in concentrations that may reach up to 10% of the plant dry weight. K^+ has to be absorbed by the roots from the soil, where it is present only in low concentrations, and transported into the aerial part of the plant body. In particular, in leaves, K^+ is important for controlling the turgor pressure and the electrochemical membrane potential. The K^+ flux across the plasma membrane is highly associated with ion homeostasis and adaptation of the plant to environmental changes. For example, guard cells forming the stomata possess several active K^+ transport systems, which control swelling and shrinkage of the cells. This in turn controls CO_2 absorption from the air as well as the transpiration stream needed to pump up nutrients from roots to shoots. To satisfy the various needs for K^+ transport activities in plant cells, divergent types of K^+ channels and K^+ transporters have evolved in plants, and these were uncovered during research in

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the past two decades. The most knowledge was obtained on the model plant *Arabidopsis thaliana*. Comparison of these channels and transporters with others from different organisms increased the understanding of diverse K^+ transport systems and their regulation beyond the organism barrier.

6.10.2 Topology of K^+ Channels

6.10.2.1 Comparison of Plant Channels (KAT1 and AKT2) and Transporters (HKT) with Animal Channels

K^+ transport systems have evolved in eukaryotic as well as in prokaryotic organisms to adapt to specific conditions. Animal cells, for example, are characterized by a low internal Na^+ concentration compared to their extracellular space; therefore, they can use the Na^+ motive force to take up small molecules, such as amino acids and carbohydrates. Na^+ ions accumulating in the cytosol are transported to the extracellular space by the Na^+/K^+ exchanger (ATPase), which also supplies K^+ to keep the high content of K^+ in the intracellular space. In neuron cells and cardiac cells, K^+ efflux systems are employed to transmit the electrical signal across the membrane. In contrast, plants and bacteria have no Na^+/K^+ exchanger (ATPase) encoded in their genome. Instead of Na^+, they use the H^+ motive force to energize other transport processes. To maintain the high concentration of K^+ in the cytosol, these cells have other special K^+ uptake systems in their membrane.

At least five types of K^+ transport systems have been identified and classified in various organisms: K^+ channels, Trk/Ktr/HKT, Kup/KUP/HAK/KT, Kdp, and Kef/KEA. In the following sections, these transport systems in plant cells are presented mainly with respect to their membrane structure.

6.10.2.1.1 K^+ channels

The fundamental structure of K^+ channels in plant cells is very similar to that of K^+ channels in animals and bacteria. Plant K^+ channels can be subdivided into three families (**Figure 1**): (1) Shaker-type channels, with six hydrophobic transmembrane domains and a single pore domain; (2) Kir-like channels, with two transmembrane domains and a pore region; and (3) tandem pore K^+ channels (TPK family), with four transmembrane regions and two pore domains. At least nine genes encoding putative Shaker-type K^+ channels are

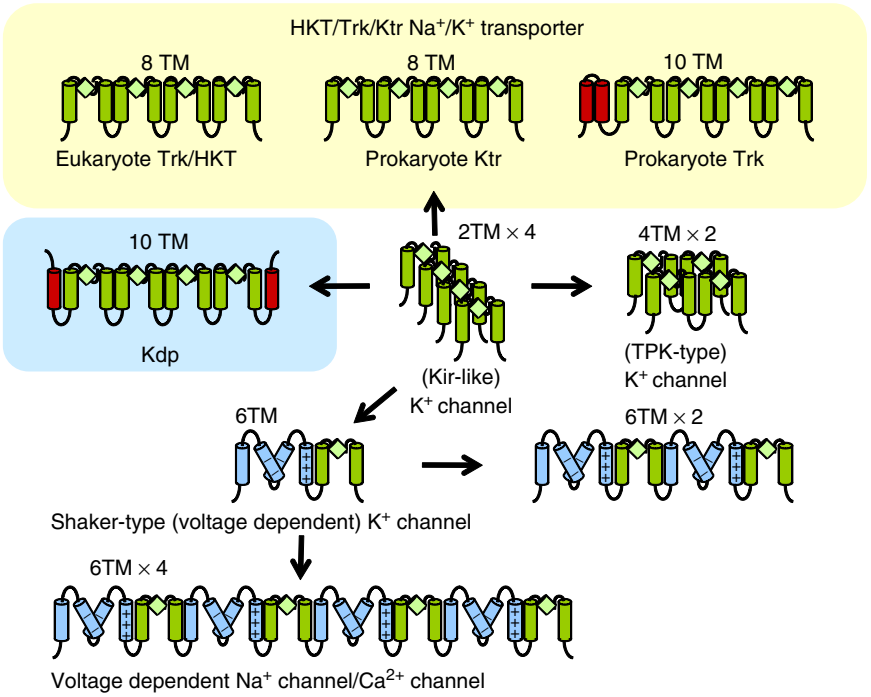

Figure 1 Membrane structure of cation channels and cation transporters. The membrane–pore–membrane (MPM) structure forms the ion conducting pore. MPM is highly conserved among cation channels and transporters (HKT/Trk/Ktr type) in prokaryotes and eukaryotes.[1,2,7–9,14,24] In voltage-gated channels, a voltage-sensing module can be found at the N terminus of MPM. Henn, D. K.; Baumann, A. Kaupp, U. B. Probing the transmembrane topology of cyclic nucleotide-gated ion channels with a gene fusion approach. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 7425–7429. 1999, Durell, S. R.; Hao, Y.; Nakamura, T.; Bakker, E. P.; Guy, H. R. Evolutionary relationship between K^+ channels and symporters. *Biophys. J.* **1999**, *77*, 775–788. Durell, S. R.; Guy, H. R. Structural models of the KtrB, TrkH, and Trk1,2 symporters based on the structure of the KcsA K^+ Channel. *Biophys. J.* **1999**, *77*, 789–807. Durell, S. R.; Bakker, E. P.; Guy, H. R. Does the KdpA subunit from the high affinity K^+-translocating P-type KDP-ATPase have a structure similar to that of K^+ channels? *Biophys. J.* **2000**, *78*, 188–99. Kato, Y.; Sakaguchi, M.; Mori, Y.; Saito, K.; Nakamura, T.; Bakker, E. P.; Sato, Y.; Goshima, S.; Uozumi, N. Evidence in support of a four transmembrane-pore-transmembrane topology model for the Arabidopsis thaliana N^{a+}/K^+ translocating AtHKT1 protein, a member of the superfamily of K+ transporters. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 6488–6493. M Ser, P.; Hosoo, Y.; Goshima, S.; Horie, T.; Eckelman, B.; Yamada, K.; Yoshida, K.; Bakker, E. P.; Shinmyo, A.; Oiki, S., Schroeder, J. I.; Uozumi, N. Glycine residues in potassium channel-like selectivity filters determine potassium selectivity in four-loop-per-subunit HKT transporters from plants. *Proc. Natl. Acad. Sci. USA*, **2002a**, *99*, 6428–6433.

present in the genome of the model plant *A. thaliana*. In addition, the genome encodes only one Kir-like (or KcsA-like) K^+ channel, *AtKCO3*, and five genes encoding the tandem form (*AtTPK1*, *AtTPK2*, *AtTPK3*, *AtTPK4*, and *AtTPK5*). In all plant K^+ channels, the pore segments spanning the K^+ permeation pathway are formed by the pore domains and the two flanking transmembrane regions as in the KcsA channel (membrane–pore–membrane motif (MPM)).[1] The membrane topology of one of the plant Shaker-type K^+ channels, KAT1, was determined experimentally, providing the basis for further structural assumptions.[2] Plant Shaker-type K^+ channels are voltage-gated channels. The deduced channel topology indicates that the voltage-sensing regions of the protein are located upstream of a single MPM motif.[3–5]

6.10.2.1.2 Trk/Ktr/HKT

As a first high-affinity K^+ transporter, the *HKT1* gene (TaHKT2;1) has been isolated from a wheat cDNA library.[6] This gene shares high homology with *Escherichia coli* Trk-type K^+ uptake transporters, other bacterial Ktr-type K^+ transporters, and *Saccharomyces cerevisiae* TRK-type K^+ transporters (**Figure 1**). This class of transporters has not been found in animal cells. Members of the group of Trk/Ktr/HKT transporters have eight transmembrane regions, $4 \times$ MPM, which has been determined by both experimental approach and bioinformatics predictions.[1,7,8] The MPM motif that is conserved in both K^+ channels and Trk/Ktr/HKT transporters strongly indicates the evolutionary-relevant relationship between the two protein types.[1,8] HKTs have two classes of transporters with respect to their K^+ selectivity.[9,10] Class 1 HKT proteins have a serine instead of glycine in the K^+-selective filter in the first pore region. Usually, these transporters mainly mediate only Na^+ but not K^+ transport into eukaryotic cells. However, a K^+ transport activity of AtHKT1 (class 1) has been found when expressed in *E. coli*.[11] Class 2 HKT proteins possess the conserved glycine in the four pore regions.

6.10.2.1.3 Kdp

Kdp is an ATPase isolated from bacteria. The Kdp gene is present in cyanobacteria but not in plant cells and animal cells. Kdp transporters from *E. coli* have been extensively characterized,[12] and their membrane structure was determined experimentally.[13] Kdp proteins show 10 membrane spanning domains. The atomic-scale structure model that was developed based on the crystal structure data for KcsA showed that the Kdp has four MPM motifs as shown in **Figure 1**.[14] The glycines of the four pore regions are also well conserved in Kdp transporters.

6.10.2.1.4 Kup/KUP/HAK/KT

KUP/HAK/KT genes have been deduced from plants by their homology to K^+ uptake permeases (KUP) from *E. coli*[15] and high-affinity K^+ transporters (HAK) from fungi.[16–20] The genome of *A. thaliana* comprises 13 genes of this type.[21] To date, the membrane structure has not been determined experimentally or by *in silico* predictions.

6.10.2.1.5 Kef/KEA

The *Arabidopsis* genome comprises six genes encoding the putative transporters KEA1–6. The function of KEAs has not been reported. Proteins of the KEA class show homologies to *E. coli* KefC and KefB.[22,23] The Kef transporters are regulated by glutathione and related components. The K^+ efflux mediated by Kef transport systems is involved in the modulation of the cytosolic pH.

6.10.2.2 Explanation of the Experimental Approaches

The determination of the membrane structure of transporter proteins provides important information for the analysis of their function and their interaction with other components. Hydropathy plots may help to allocate possible (trans)membrane structures. However, they cannot derive the real membrane structure, as illustrated by scientific discussions on several membrane proteins, such as glutamate receptors and the Na^+/H^+ antiporter. For voltage-dependent K^+ channels, initial studies identified the pore (P) region as the fifth hydrophobic transmembrane domain, H5, when counted from the N-terminal end. The first experimental data from studies with the aim to determine the structure of Shaker-type K^+ channels – in this case, cyclic nucleotide-gated channels from animal cells – were obtained by a gene fusion approach.[24]

It was a question whether the membrane structure of outwardly rectifying K^+ channels such as Shaker and HERG and inwardly rectifying K^+ channels such as KAT1 were identical or whether one type showed the reverse orientation with respect to the membrane. The current flow mediated by the two types shows an opposite orientation. The membrane topology of KAT1 was determined by a bacterial alkaline phosphatase fusion approach.[2] This approach can be applied only when the membrane proteins of interest are inserted in the proper conformation in the *E. coli* membrane. For the two plant K^+ channels KAT1 and AKT2, this conditions is fulfilled. Exogenous KAT1 and AKT2 complemented a K^+ uptake-deficient *E. coli* strain, indicating their proper integration into the membrane. Likewise, the *Arabidopsis* AtKUP1, AtKUP2, and AtHKT1 (AtHKT1;1) were proven functional in the *E. coli* membrane.[11,19,25] Probing of the membrane structure by the PhoA approach using *E. coli* as the expression system revealed that plant K^+ channels exhibit the same six-transmembrane structure as animal channels. During this study, it was found that the S3 of KAT1, which is part of the voltage-sensing module, was not integrated into the membrane by itself. The negatively charged residues in S3, which are highly conserved among all voltage-dependent K^+ channels, need to be masked by positively charged residues in S4 by electrostatic interaction during the insertion of S3 and S4.[2,24,26,27]

The membrane topology of AtHKT1 was also determined by the same bacterial approach.[7] The structure was confirmed by N-linked glycosylation mapping in dog pancreas endoplasmic reticulum (ER) membrane. This method is often used to determine the external side of the membrane protein because glycosylation only occurs in the ER lumen (external side) by oligosaccharyl transferase.[28] Moreover, the results obtained by the insertion of an epitope sequence between

different hydrophobic regions also support the membrane spanning structure.[29] The different approaches have provided consistent results on the AtHKT1 membrane structure, indicating that the membrane structure of AtHKT1 is similar to that of the pore-forming structure of K^+ channels.

6.10.3 Selectivity Filter of K^+ Channels and the HKT K^+ Transporter

A typical K^+ channel shows the characteristic signature sequence of Gly-Tyr-Gly in its K^+ selectivity filter.[30] This unique and highly conserved motif has not yet been found in other types of channels and transporters, including Na^+ channels and Ca^{2+} channels. As noted previously, it was concluded – based on experimental data and atomic-scale homology modeling – that Trk/Ktr/HKT and Kdp transporters may share a selectivity filter sequence similar to that of K^+ channels.

The transporter TaHKT2;1 shows K^+ and Na^+ transport in yeast and *Xenopus* oocytes,[6,31] whereas AtHKT1;1 mediates mainly Na^+ transport in the same systems.[11] In an attempt to identify the residues responsible for K^+ transport, a glycine residue in the first pore region of the four times repeated MPM structure of TaHKT2;1 was identified to determine the K^+ transport activity (**Figure 2**).[9] In the case of AtHKT1;1, this Gly is converted to Ser. However, glycine residues at the same position in the second, third, and fourth pore regions are preserved. Experimental data on the KtrB transporter from *Vibrio alginolyticus* revealed that all four conserved Gly are part of a K^+-selective filter.[32,33] This result was further supported by *in silico* modeling.[1,8] From this, it was predicted that the conserved glycine residues in the transporters correspond to the first Gly in the signature sequence of K^+ channels. A similar structure was predicted for the selective filter of Kdp.[14] Also, the *E. coli* Kdp transporter exhibits the glycine residues in the second, third, and fourth pore regions. The intriguing conservation of the glycine residue in the pore regions may indicate an important evolutionary step in developing the selectivity filter of K^+ channels and Trk/Ktr/HKT and Kdp transporters.

6.10.4 Channel or Transporter: What Determines Protein Function?

Based on their structures and functions, membrane transport proteins can be divided into three groups: channels, transporters, and pumps. In general, ion channels allow ions to diffuse passively down their electrochemical gradients, but transporters and pumps use energy to transport ions actively against these gradients. The transport rates of ion and water channels are much greater than those of transporters. Typically, a channel molecule can transport more than 10^6 ions per second, but the rates of ion transporters are usually in the range of 10^2–10^4 ions per second. What is the cause of the difference between channels and transporters? It could be assumed that structural differences between transporters and channels likely determine their different activities. Therefore, comparisons of the amino acid sequences may provide good information to address the questions. Fortunately, a structural model of the MPM motifs of Ktr/Trk/HKT-type transporters has been proposed based on the structure of the bacterial KcsA channel.[8] This model provides landmarks of structurally similar regions, such as the pore regions, allowing a more precise comparison of K^+ channels and Trk/Ktr/HKT-type transporters in other regions. Comparison of their amino acid sequences shows that positively charged residues are well conserved in the $M2_D$ helices of Ktr/Trk/HKT-type transporters, unlike the M2 segments of most K^+ channels (**Figure 3**). Moreover, the atomic-scale model developed by Durell and Guy[8] indicates that an arginine residue is placed nearby the ion conducting pore. It was predicted that this highly conserved positive residue in the middle of $M2_D$, which is not present in K^+ channels, may be one of the determinants for the special properties of transporters. This hypothesis was tested experimentally, and the results

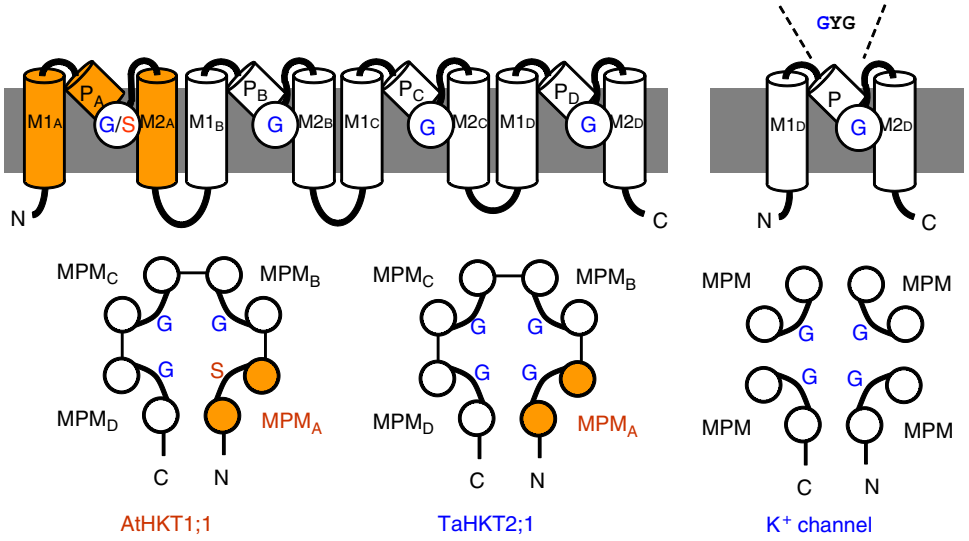

Figure 2 Membrane structure and ion selective filter of HKT-type transporters and K^+ channels. Four Gly in the individual pore regions allow the transport of K^+, whereas three Gly and one Ser do not. Transporters with these properties prefer the transport of Na^+.

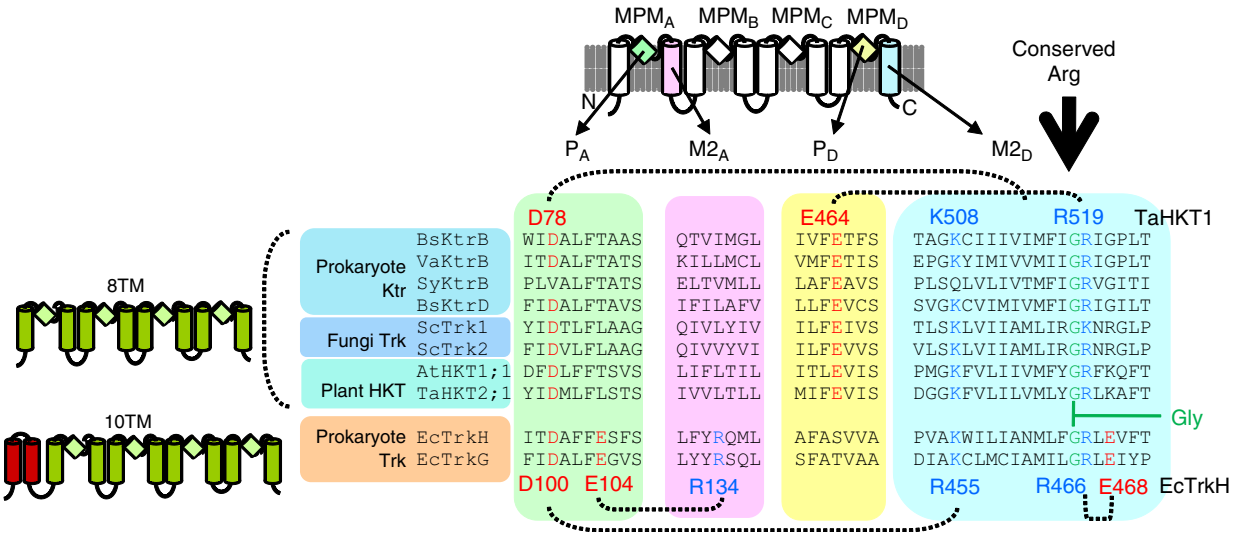

Figure 3 Conserved charged residues in Trk/Ktr/HKT. Possible electrostatic interactions are indicated. Reproduced from Kato, N.; Akai, M.; Zulkifli, L.; Matsuda, N.; Kato, Y.; Goshima, S.; Hazama, A.; Yamagami, M.; Guy, H. R.; Uozumi, N. Role of positively charged amino acids in the M2$_D$ transmembrane helix of Ktr/Trk/HKT type cation transporters. *Channels (Austin)* **2007**, *1*, 161–171. Copyright by Landes Bioscience.

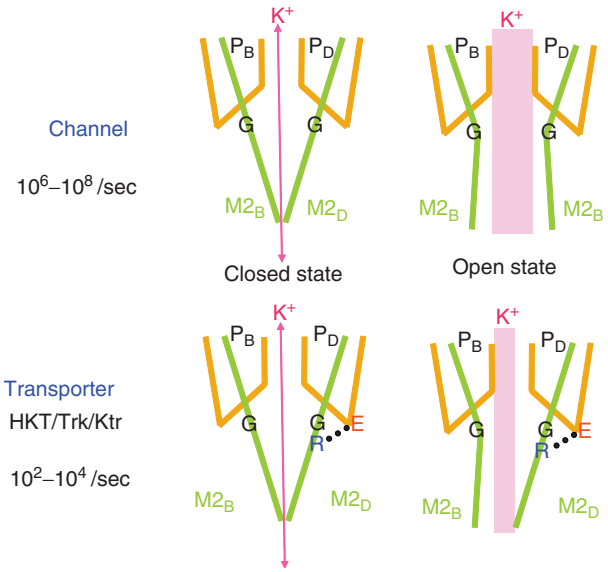

Figure 4 Possible model for the functional difference between channels and transporters. The region involving the salt bridge in Ktr/Trk/HKT-type transporters may undergo conformational changes during the transport cycle. Among Ktr/Trk/HKT family members, a Gly–Arg motif in the center of M2$_D$ is highly conserved. In K$^+$ channels, such a Gly residue serves as a hinge region where M2 (or S6 in 6TM K$^+$ channels) bends when the channel opens. If the C-terminal region of M2$_D$ moved away from the central axis of the ion conducting pore during the open state, the salt bridge between the R (R519 in TaHKT2;1) and the E (E464 in TaHKT2;1) in Ktr/Trk/HKT may provide a barrier preventing the free diffusion of ions down their electrochemical gradient; that is, it may prevent transporters from being channels Reproduced from Kato, N.; Akai, M.; Zulkifli, L.; Matsuda, N.; Kato, Y.; Goshima, S.; Hazama, A.; Yamagami, M.; Guy, H. R.; Uozumi, N. Role of positively charged amino acids in the M2$_D$ transmembrane helix of Ktr/Trk/HKT type cation transporters. *Channels (Austin)* **2007**, *1*, 161–171. Copyright by Landes Bioscience.

indicated that the conserved Arg near the middle of the M2$_D$ segment was essential for the K$^+$ transport activity of bacterial KtrB and plant HKTs. Single replacement of the positively charged residues in the M2$_D$ helices with Gln abolished the K$^+$ uptake activity of *Synechocystis* KtrB and decreased the cation uptake activity of wheat TaHKT2;1 and AtHKT1;1.[29] The activity was not affected when Arg was converted to Lys. The positive charge of the Arg, R519 in TaHKT2;1, was predicted in the model to be neutralized electrostatically to avoid blocking of cation passage through the pore. Based on the structural model, E464 in the fourth pore region, P$_D$, in Ktr/HKT, but not present in Trk is likely to be in the vicinity of R519 (**Figure 3**). The possible electrostatic interaction of R519–E464 in TaHKT1 was proposed to reduce the electrostatic barrier. It was assumed that the local distribution of positively charged amino acids in M2$_D$ helices would be associated with the characteristics of this transporter family because the presence of an unpaired positively charged residue within a cation transporter would be an electrostatic barrier to the passage of cations.

Among Ktr/Trk/HKT family members, a Gly residue immediately preceding the Arg in M2$_D$ is also fully conserved (**Figure 3**). The analogous residue in K$^+$ channels such as KcsA is also usually a Gly (**Figure 2**), which serves as a hinge region where M2 (or S6 in 6TM K$^+$ channels) bends when the channel opens.[34] If the C-terminal region of M2$_D$ moves away from the central axis of the ion conducting pore during the open state, the salt bridge of R519 with E464 in TaHKT1 may provide a barrier preventing the free diffusion of ions down their electrochemical gradient; that is, it may prevent transporters from being channels (**Figure 4**).[29] In bacterial Trk, the Arg may interact with the adjacent negatively charged residue in M2$_D$ (**Figure 3**). In other words, the electrostatic interactions of positively charged residues in the middle of M2$_D$ with negative residues (e.g., close to the selectivity filter) confer special functional transporter properties on Ktr/Trk/

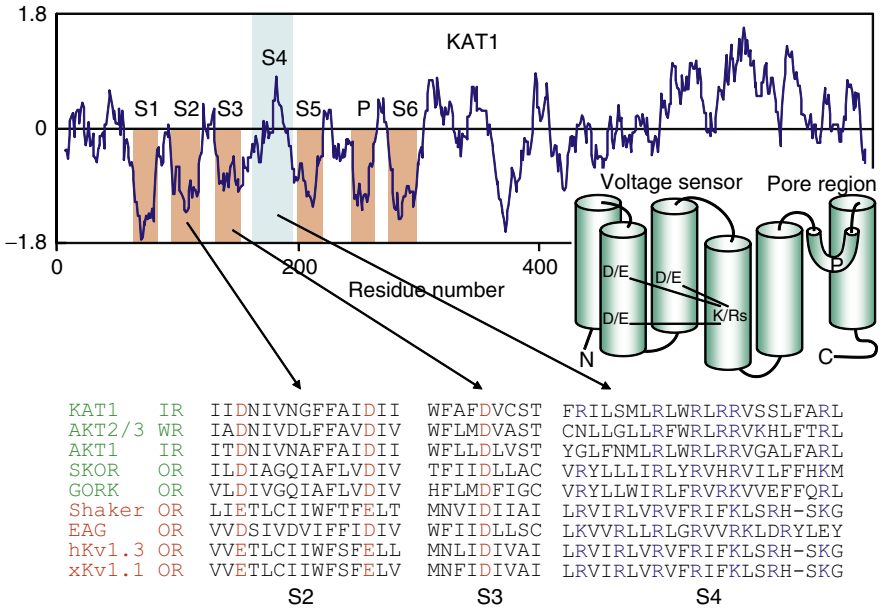

Figure 5 Voltage-dependent K^+ channels. The conserved positive and negative residues in the hydrophobic segments S2, S3, and S4 are present in animal and plant Shaker-type K^+ channels. FIg. 1 in Sato et al. JBC 2003, Vol. 278, No. 15, Issue of April 11, pp. 13227–13234, 2003, Molecular Dissection of the Contribution of Negatively and Positively Charged Residues in S2, S3, and S4 to the Final Membrane Topology of the Voltage Sensor in the K Channel, KAT1*. Copyright by American Society for Biochemistry and Molecular Biology.

HKT proteins. In addition, from the amino acid sequence, other possible electrostatic interactions in Ktr/Trk/HKT could be deduced that might be involved in stabilizing the structure.

6.10.5 Integration of K^+ Channels into the Membrane

Plant cells express K^+ channels, which are homologous to the Shaker channel isolated from *Drosophila melanogaster*.[35] However, these channels show a much larger functional variability. Because plant cells have an ionic condition different from that of animal cells, they express not only depolarization-activated K^+ channels but also hyperpolarization-activated ones.[4] KAT1, the first cloned K^+ channel from a plant,[36] is considered one of the models for hyperpolarization-activated (inward-rectifying) K^+ channels. It shares the common structural feature of six transmembrane segments (S1–S6) and the pore (P) with other voltage-dependent K^+ channels. The cytosolic C-terminal region has been proposed to involve regulation through phosporylation by specific kinases – calcium-dependent kinases or the ABA-mediated kinase, SnRK2.6.[37,38] The fourth transmembrane segment, S4, which is only weakly hydrophobic and contains several positively charged residues, is part of the voltage sensor. The positively charged residues in S4 were shown to interact with negatively charged residues in S2 (**Figure 5**). Interestingly, the interacting charged residues are conserved in voltage-dependent K^+ channels from prokaryotes and eukaryotes.[39] The voltage-gating mechanism was well illustrated on the basis of a large amount of experimental data.[40] On the other hand, the hydropathy profile of KAT1 shows a relatively low hydrophobicity for S4 and a highly hydrophobic property for the pore region, which does not explain the final topology of the channel. Although the final topology of Shaker-type K^+ channels has been determined, mainly on the basis of crystal structure data,[41,42] there is little topogenesis evidence concerning the formation of the channel structure.

6.10.5.1 Integration of the Voltage-Sensing Domain into the Membrane

A series of hydrophobic domains in polytopic membrane proteins are sequentially integrated by means of the translocation into the ER membrane. Voltage-dependent K^+ channels show a hydrophobicity similar to that illustrated for KAT1 in **Figure 5**, including domains (S2–S4) with highly conserved charged residues. Together with the S1 segment, these regions form the voltage-sensing module. In mutagenesis experiments, it has been shown that electrostatic interactions within this voltage-sensing module are crucial for its function in voltage gating.[39,43] This conclusion was further substantiated by evaluating the interaction between charged residues in S2–S4 in *in vitro* translation and translocation experiments.[26,27,44] The analysis of the topogenic function of the different parts of KAT1 and Shaker B showed that S1 inserts into the membrane and retains in it as a signal anchor. The following S2 is integrated as a stop-transfer sequence.[44] The sequential insertion of S1 and S2 occurred in KAT1 as in Shaker B. The integration of S3, however, revealed some differences. The S3 region of KAT1 shows a lower hydrophobicity than that of Shaker. Consequently, the S3 of Shaker still shows some ability to integrate into the membrane alone, whereas the S3 of KAT1 does not. Especially, a negative residue located in the middle of S3

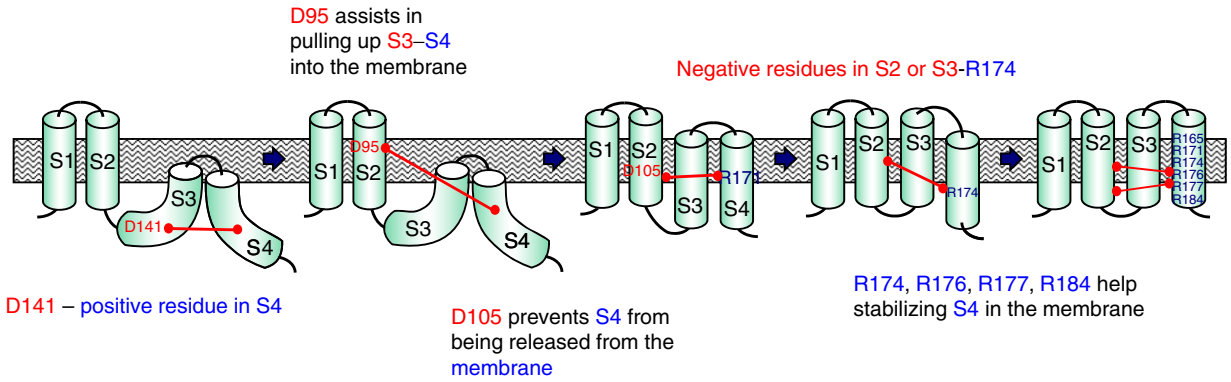

Figure 6 Membrane integration of the voltage sensor (S3 and S4) of KAT1. Almost all integral membrane proteins on the membrane organelles of the exocytic and endocytic pathway are synthesized on membrane-bound ribosomes and are cotranslationally integrated into the membrane. There, they acquire their final topology. A series of hydrophobic domains are sequentially integrated via the translocon into the endoplasmic reticulum membrane.[26,27] Modified from Sato, Y.; Sakaguchi, M.; Goshima, S.; Nakamura, T.; UOZUMI, N. Molecular dissection of the contribution of negatively and positively charged residues in S2, S3, and S4 to the final membrane topology of the voltage sensor in the K^+ channel, KAT1. *J. Biol. Chem.* **2003**. Copyright by American Society for Biochemistry and Molecular Biology.

prevents the insertion of S3 into the membrane by itself.[24,26,27,44] Nevertheless, the S3 segments of both channels require the presence of the S4 segment for fully efficient membrane integration. Salt bridges between S3 and S4 support the insertion of the combined element. On the other hand, the membrane-unstable S4 needs the preceding S1–S3 transmembrane segments for proper membrane insertion. In KAT1, for example, two negative residues in S2 (E283 and E293 in Shaker) stabilize S4 in the membrane. During the insertion of S4, the negative residues in S2 of Shaker B and KAT1 interact with each other. This S2–S4 interaction affects not only topogenesis but also channel functionality. For the Shaker channel, it was observed that reversal mutations between E283 in S2 and R368 in S4 stabilize the closed confirmation.[45] Durell et al.[40] predicted in their structural model a tight S2–S4 interaction only in the open conformation, indicating that electrostatic interactions might be mostly transient phenomena. Based on systematic mutations and *in vitro* translocation experiments, several possible membrane integration steps of the voltage-sensing module of KAT1 were proposed (**Figure 6**).[27] However, available data indicate that the cooperative insertion of the voltage-sensing module is a property not only of KAT1 but also common to voltage-dependent K^+ channels, and that a balance between electrostatic and hydrophobic forces determines the degree of cooperativity between the different regions.

6.10.5.2 Topogenesis of the Pore-Forming Structure

Although the pore region P is highly hydrophobic, it does not substantially exhibit any topogenic function. All tested P regions of Kir 2.1, KcsA, and KAT1 do not have the ability to form a membrane-spanning domain alone. Instead, the P region combines with the neighboring domains, and together they form a functional module. Topogenic examinations of the segments M1 (S5), P, and M2 (S6) in Kir 2.1 (IRK1), KcsA, and KAT1 revealed that M1 (S5) and M2 (S6) possessed the topogenic function that can enter the membrane (**Figure 7**). The crystallography data show a loop structure for the P region, which is possibly formed after insertion within the membrane as indicated by other experiments. To monitor the location of the P region, the Kir 2.1 channel was engineered by a small change in a side chain (Gln140 replaced by Asn) to contain a novel N-glycosylation site in the P region. This site is located at the bottom of the loop and far from the ER lumen. The attachment of carbohydrates to the P region shows that the P region is exposed to the luminal space before the channel takes on its final topology (**Figure 7**). The X-ray structure of KcsA[46] indicates that the turn between the pore helix and the stretched GYG motive (TATT in KcsA and TQTT in Kir 2.1) is probably responsible for the bend in the middle of the P region. However, the removal of the turn by replacement of TQTT in Kir 2.1 with LQLL, because Leu tends to form a helix, failed to make the P region transmembrane. This suggests that despite its hydrophobicity and its prediction score for being transmembrane, the P region shows little propensity to take up a transmembrane configuration.[47] Notably, the nontopogenic function of the hydrophobic pore segment is one of the determinants contributing to the final membrane structure of K^+ channels (**Figure 7**).

6.10.6 Gating of Plant K^+ Channels

6.10.6.1 Same Structure, Different Function

As outlined previously, voltage-dependent K^+ channels show a common structural framework. This also holds true for the family of voltage-dependent plant K^+ channels. Nevertheless, at the functional level, this family exhibits an astonishing diversity and can be subdivided empirically into four different subgroups[5]: inwardly rectifying (K_{in}), silent (K_{silent}), weakly rectifying (K_{weak}), and outwardly rectifying (K_{out}) channel subunits. This separation parallels a similar subdivision based on primary sequence alignment.

Channels built by subunits from the first subgroup (K_{in} channels) normally facilitate K^+ uptake into the cell. These channels activate (open) at membrane voltages of -80 to

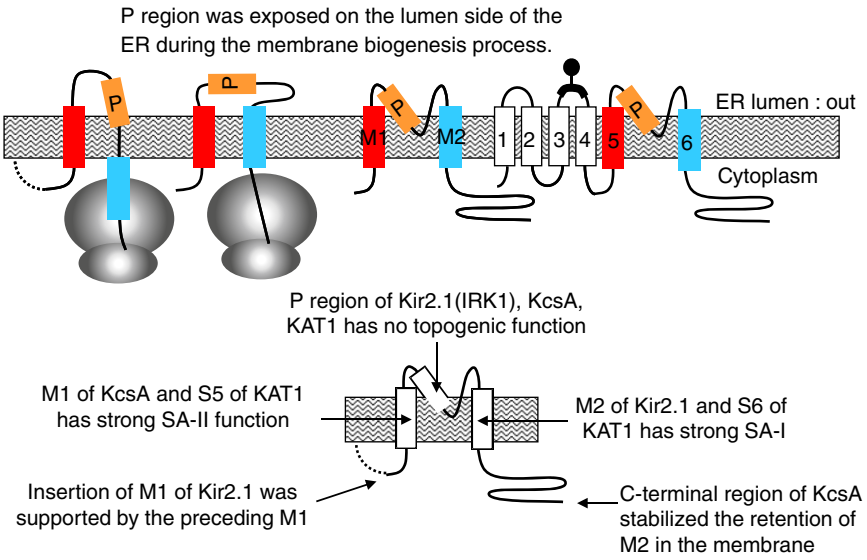

Figure 7 Membrane topogenesis of pore-forming region in Kv1.2(IRK1), KcsA, and KAT1(S5-P-S6).[27] Modified from Umigai, N.; Sato, Y.; Mizutani, A.; Utsumi, T.; Sakaguchi, M.; Uozumi, N. Topogenesis of two transmembrane type K^+ channels, Kir 2.1 and KcsA. *J Biol Chem*, *278*, **2003** 40373–40384. Copyright by American Society for Biochemistry and Molecular Biology.

-100 mV, a threshold that is insensitive to the external K^+ concentration (**Figure 8(a)**). The model plant *A. thaliana* comprises five genes coding for K_{in} channel subunits: *AKT1*, *KAT1*, *KAT2*, *SPIK*, and *AKT6* (not yet characterized).

A second subgroup comprises silent (K_{silent}) channel subunits. Like their mammalian counterparts, they do not form functional channels when expressed on their own. However, they have a strong affinity for heteromerization with K_{in} subunits. The sole gene in this category in *Arabidopsis* is *AtKC1*. The AtKC1 subunit is expressed in the root epidermis, where it preferentially assembles with the K_{in} subunit AKT1. This assembly affects K_{in} channel behavior in two ways. First, it affects the voltage dependence of gating by displacing channel activation to more negative values (**Figure 8(b)**, dashed gray line).[48] Second, it introduces a hypersensitivity of the pore structure toward the external K^+ concentration (**Figure 8(b)**, gray line).[49]

The third group of weakly rectifying (K_{weak}) channels is represented in *Arabidopsis* by the gene *AKT2/3*. Subunits of this group are functional as homotetramers but also assemble with K_{in} channel subunits and confer their rectification properties to the heteromeric channels. Following expression in heterologous expression systems, such as *Xenopus* oocytes or COS cells, K_{weak} channels exhibit two current components. These result from two channel subpopulations characterized by different gating modes (**Figure 8(c)**). One subpopulation shows gating analogous to that of K_{in} channels (**Figure 8(c)**, mode 1) activating (open) at membrane voltages of -80 to -100 mV. In the second subpopulation, this activation threshold is shifted by >250 mV to more positive voltages. As a consequence, these channels show little sensitivity to voltage. They are open over the entire physiological voltage range typical of a leaklike, albeit K^+-selective, current (**Figure 8(c)**, mode 2). Channels can be switched between the two gating modes by post-translational modifications. Essential for this regulation is phosphorylation of two highly conserved serine residues specific to the K_{weak} channels. One is localized in the S4–S5 linker and the other in the linker between the S6 transmembrane helix and the cytosolic C terminus. Phosphorylation of both residues favors the conversion to the leaklike mode 2. However, phosphorylation of both sides appears not to be sufficient for inducing the huge shift of the activation threshold. Another important entity is a lysine residue in the voltage-sensor S4 that is highly conserved among K_{weak} subunits but not present in other plant voltage-gated K^+ channel subunits.[50] Channels formed by the three subunit types K_{in}, K_{weak}, and K_{silent} are commonly associated with channel opening upon hyperpolarization. In normal conditions, this permits the influx of K^+ into plant cells. In general, K_{weak} and K_{silent} subunits can thus be considered as special cases within the broader grouping of inward-rectifying channels. This global classification is further supported by the ability of K_{in}, K_{weak}, and K_{silent} subunits to assemble heteromeric channels with one another.

In contrast, outwardly rectifying channel subunits only assemble between themselves. K_{out} channels activate (open) with membrane depolarization (**Figure 8(d)**). The model plant *Arabidopsis* comprises two genes coding for K_{out} channel subunits: SKOR and GORK. Outward-rectifying channels facilitate K^+ efflux, usually in charge balance with Cl^- channels, and contribute to solute loading of the xylem as well as solute loss from guard cells during stomatal closure. Interestingly, unlike inward-rectifying channels, outward-rectifying channels exhibit a unique sensitivity of the gate to both the membrane voltage and the extracellular K^+ concentration.[51] The channels open only at voltages positive for Nernst-equilibrium potential for potassium (E_K), and thus ensure K^+ efflux regardless of the extracellular K^+ concentration. This ability to adapt channel gating to the prevailing K^+ concentration outside makes good physiological sense because it guarantees that the channels open only when the driving force for net K^+ flux is directed outward, even when extracellular K^+ varies over concentrations from 10 nM to 100 mM.

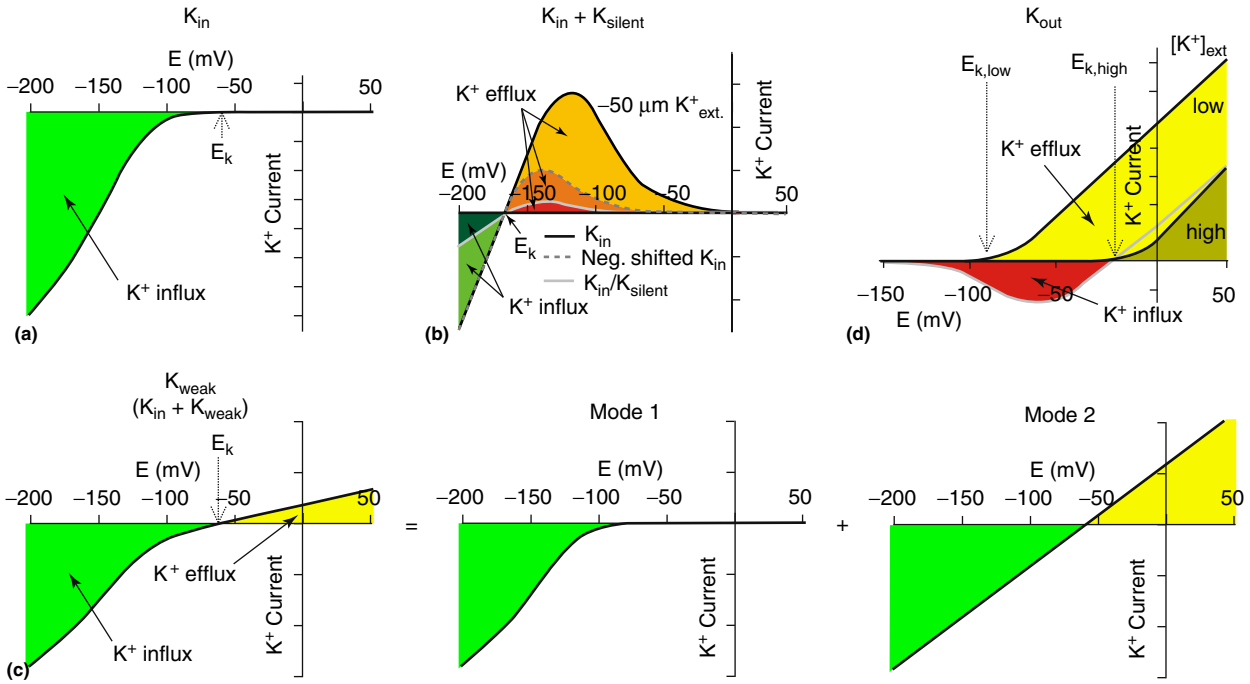

Figure 8 The family of voltage-gated plant K^+ channels shows a huge functional variability. Although the subunits share the same structure, they may form inward-rectifying channels (a), modulated inward-rectifying channels (b), weakly rectifying channels (c), or outward-rectifying channels (d). Modified from figure 3 in Dreyer, I.; Blatt, M. R. What makes a gate? The ins and outs of Kv-like K^+ channels in plants. *Trends Plant Sci*, **2009**, *14*, 383–390.

6.10.6.2 K^+ Sensing of Plant K_{out} Channels: A Unique Mechanism

The mechanism underlying the K^+-sensing phenomenon of plant K_{out} channels was identified by Johansson et al. (**Figure 9**).[51] The K^+ sensor is actually the selectivity filter of the channel that interacts with the gating machinery and adjusts its structure according to the external K^+ concentration. When the channel is open, K^+ ions permeate along their electrochemical gradient (**Figure 9(a)**). Upon closure, which occurs stochastically, the permeation pathway is occluded at its cytosolic end (**Figure 9(b)**). Now, the selectivity filter of the channel is accessible only from the extracellular side and not from the intracellular side. When the extracellular K^+ concentration is high (e.g., 30 or 100 mM), the probability that K^+ ions place one or more of the K^+ coordination sites within the selectivity filter is also relatively high. In this condition, the selectivity filter strongly interacts with the gating machinery and induces another conformational change. Consequently, the closed state of the channel is stabilized (**Figure 9(c)**). To reopen the channel, more energy is necessary, indicated by a more positive activation threshold. In contrast, when the extracellular K^+ concentration is low (e.g., 1 or 3 mM), the probability that K^+ ions place at least one of the K^+ coordination sites within the selectivity filter is low. In this condition, the selectivity filter collapses,[52] also altering its interaction with the gating machinery. Consequently, no further conformation change is induced that might stabilize the closed channel (**Figure 9(d)**). Reopening of the channel is achieved stochastically by reverting the initial closing step (**Figures 9(d)** and **9(e)**). However, the collapsed structure of the selectivity filter still does not allow the flux of K^+ through the channel. Only when the internal K^+ concentration is high enough the cavity of the channel is flushed by K^+ ions, which then enter the selectivity filter and again alter its structure (**Figure 9(e)**). The channel is now open and again allows K^+ permeation along the electrochemical gradient (**Figure 9(a)**). In the gating scheme displayed in **Figure 9**, the only K^+-dependent step is the transition from **Figure 9(d)** to **Figure 9(b)**; however, this explains all essential features of the K^+ sensor of plant K_{out} channels, such as the shift of the voltage-dependent activation curve of the channel toward more positive voltages with increasing extracellular K^+ (**Figure 8(d)**).

6.10.7 Assembly of Plant K^+ Channels: Diversity through Heteromerization

Like their animal homologs, functional plant voltage-gated K^+ channels are assembled by four α subunits. As shown in co-expression experiments in heterologous expression systems, such as *Xenopus* oocytes and yeast cells, or in plant expression systems, these are in principle endowed with the potential to form not only homomeric but also heteromeric channels. However, assembly is not completely indiscriminate, as might be concluded from initial results. For instance, heteromerization was demonstrated among different K_{in} channel α subunits (e.g., KAT1 and KAT2) and between different K_{out} channel α subunits (e.g., GORK and SKOR) but not between K_{out} and K_{in} channel α subunits (e.g., SKOR and KAT1). The molecular basis for this discrimination was shown to be located within

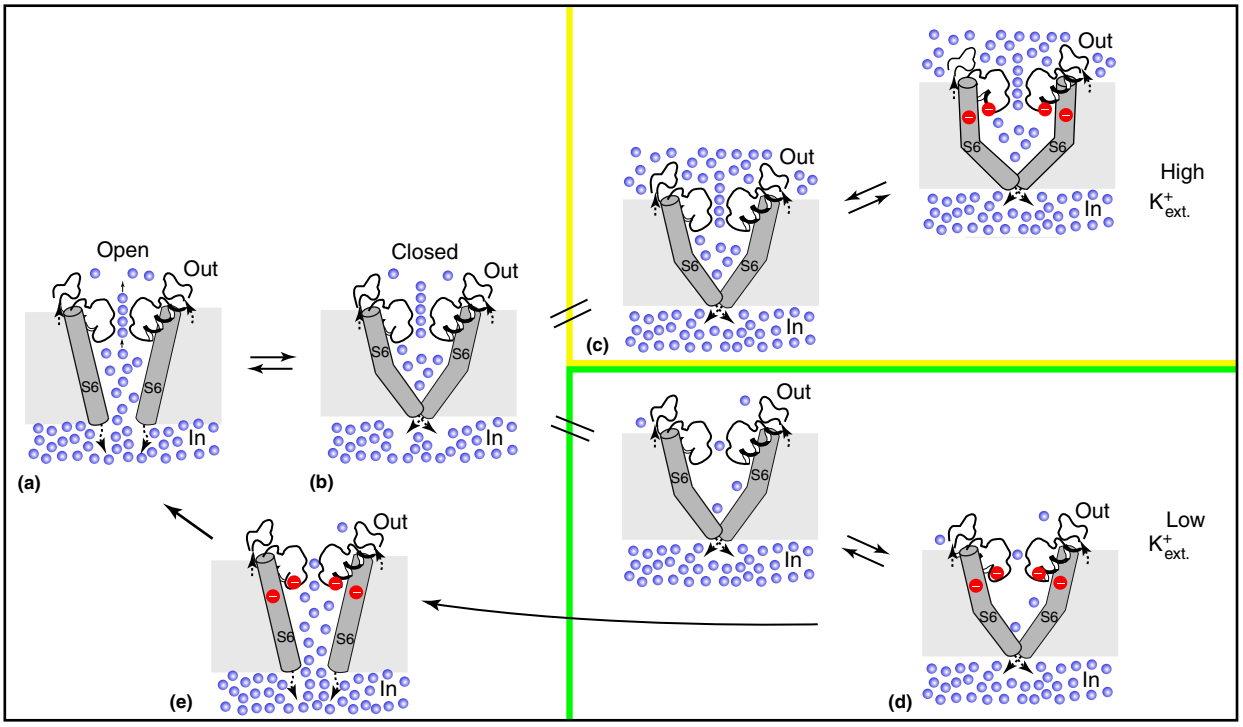

Figure 9 Schematic illustration of the mechanism of K^+ sensing in outward-rectifying plant K^+ channels. Details are explained in the text. Modified from Jeanguenin, L.; Lebaudy, A.; Xicluna, J.; Alcon, C.; Hosy, E.; Duby, G.; Michard, E.; Lacombe, B.; Dreyer, I.; Thibaud, J. B. Heteromerization of Arabidopsis Kv channel alpha-subunits: Data and prospects. *Plant Signal Behav*, **2008** *3*, 622–625. Copyright by Landes Bioscience.

the cytoplasmic C-terminal regions of the α subunits.[53] However, functional heteromeric channels were reported for combinations of different hyperpolarization-activated plant K^+ α subunits – that is, between K_{in} and K_{weak} α subunits and between K_{in} and K_{silent} α subunits.

Not only can heteromeric channels be formed of subunits originating from the same species but also subunits from different species have been shown to form functional heteromeric channels, indicating that channel heteromerization occurred earlier than species separation during evolution.[48]

The aggregation process of voltage-gated plant K^+ α subunits was proposed to be similar to that of their animal counterparts.[54–56] The subunits assemble first into dimers, and then two dimers form a functional tetrameric K^+ channel. In some cases, such as for the pairs KAT2/AKT2 and AtKC1/AKT1, it has additionally been shown that heteromerization is preferred to homomerization, emphasizing the potential role of heteromerization in plant cell physiology. Obviously, a prerequisite for subunits to assemble into heteromeric channels *in planta* is some overlap of both time and spatial expression patterns. For K_{out} channels, such as SKOR in GORK in *Arabidopsis*, this condition appears not to be fulfilled. By contrast, many cell types have been shown to express different hyperpolarization-activated K^+ channel α subunits. For instance, root epidermal/cortical cells in *Arabidopsis* express both AKT1 and AtKC1, and guard cells have been reported to express the subunits KAT1, KAT2, AKT2, AtKC1, and AKT1.[57]

Therefore, it not surprising that the ultimate proof of the existence of heteromeric channels in plants has been provided for inward-rectifying channels.[58] The assumption that plant K^+ channels would only be homomers was confuted by using a set of three specially designed transgenic plants. The first plant, *kat2-1*, was a null-allele mutant for the K_{in} channel α subunit gene *KAT2*. The second plant, *dnkat2*, was the corresponding wild-type plant comprising an additional dominant-negative version of the *KAT2* gene. In addition to the native KAT2 α subunit, this plant expresses a polypeptide in which the selectivity filter motif GYGD was replaced by RRGD. These dnKAT2 α subunits assemble as the native ones. However, when one of these is integrated into a tetrameric channel, the pore is occluded – that is, the channel is not functional. The third plant, named *kincless*, was a cross between the knockout *kat2-1* and the dominant-negative *dnkat2*. This plant does not express the native wild-type KAT2 α subunit anymore but only its dominant-negative version, dnKAT2. The phenotypes of the three plants and that of the wild-type were compared under different conditions. Assuming that plant K^+ channels exist only as homomers, it was expected that the phenotype of *kincless* should not differ from that of the other three plants if WT = *kat2-1* = *dnkat2*. Under such a condition, KAT2 would not be a locus that determines the trait of the plant. However, it was shown that *kincless* differed from the other three plants, confuting the assumption that plant K^+ channels were only homomers.[58] Interestingly, the question, "Do heteromeric K^+ channels exist *in planta*?", which is relative to the molecular scale, was answered after carrying out simple analyses of a macroscopic phenotype.

It is proposed that heteromerization increases the functional diversity of voltage-gated plant K^+ channels and, hence, allows the fine-tuning of the electrical features of the

plasma membrane of plant cells.[48] Although heteromeric channels might inherit most of their functional features from their constitutive α subunits, they may significantly differ in a few of them. The hyperpolarization-activated K^+ channel α subunits characterized so far display fairly similar high selectivity for K^+, block by Cs^+, and activation by hyperpolarization. However, they may significantly differ in their activation voltage threshold and in their apparent gating charge. They may also differ in other functional features, such as their sensitivity to Ca^{2+} and pH and susceptibility to post-translational modifications such as (de)phosphorylation.

The power of heteromeric assembly was illustrated on the K_{in}/K_{silent} pair AKT1/AtKC1.[49] Earlier studies showed that K_{silent} α subunits alone are not capable of forming functional channels. Only in combination with K_{in} subunits do K_{silent} subunits reveal their functional properties. It was shown that K_{silent} subunits alone remain within the ER and apparently require the coexpression of another α subunit to be targeted to the plasma membrane as part of heteromeric channels.[59–61] In *Arabidopsis* roots, the K_{silent} α subunit AtKC1 co-assembles with the K_{in} α subunit AKT1 and contributes two essential features to the channel: (1) It shifts the activation voltage threshold to more negative values, and (2) it reduces the stability of the pore in low external K^+.[49] Both features appear to be physiologically significant, especially in conditions in which the external K^+ concentration is very low, such as in the soil solution surrounding roots. The presence of the AtKC1 subunit in the channel complex strongly reduces shunt outward K^+ fluxes under very extreme conditions (**Figure 8(b)**).

6.10.8 Outlook

This chapter summarized the current knowledge concerning (1) the similarity in the membrane structure and the ion-selective filter between K^+ channels and K^+ transporters (Trk/Ktr/HKT and Kdp), (2) the topogenesis of K^+ channels, (3) the rectification mechanisms of plant K^+ channels, and (4) the regulation of plant K^+ channel assembly and its physiological implications. The results obtained by very different technical approaches provide the fundamental backbone toward an understanding of K^+ transport in plants, but they also provide general hypotheses and clues for the understanding of nutrient and element transport in plants mediated by other channels and transporters. The gained experience from K^+ channel/transporter research teaches us that the intensive study of the structure–function correlates in plant transport systems will help to gain deep insights into the physiological role of the transmembrane transport. These insights are of enormous value because they may point to solutions for maintaining sustainable crop production in a changing global environment.

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