Roles of Higher Plant K\textsuperscript{+} Channels\textsuperscript{1}

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Living organisms maintain a cellular solute composition very different from that of the external environment. This implicitly requires the transport of solutes across the cell membrane, and ion channels are integral membrane proteins that play indispensable roles in such transport. In the past dozen years, radical advances have aided in our understanding of ion channel function and regulation in higher plants. Nowhere are these advances more striking than with respect to K\textsuperscript{+} channels, where the synergistic application of electrophysiological, cell biological, physiological, and molecular techniques has demonstrated an array of channel types playing diverse but defined roles in plant physiology.

The major function of K\textsuperscript{+} channels in animal cells is that of membrane voltage control and short-term repolarization of the membrane. Although K\textsuperscript{+} channels in plants share similar roles in the regulation of the membrane voltage, early research on guard cells led to the model that shows that plant K\textsuperscript{+} channels in addition provide important pathways for long-term physiological K\textsuperscript{+} uptake and release. An extensive range of recent studies suggests diverse long-term transport functions of plant K\textsuperscript{+} channels, including participation in osmotically driven movements, solute loading into the xylem, cation nutrition, and, by virtue of the presence of K\textsuperscript{+} channels at endomembranes, intracellular solute redistribution and cytosolic volume control. Most plant K\textsuperscript{+} channels remain activated for long periods of time, which is critical for this proposed long-term transport function of K\textsuperscript{+} channels in plants. Because higher plant K\textsuperscript{+} channels are proposed to play a role in regulating both the influx and the efflux of K\textsuperscript{+} from cells, activity of these channels may impinge upon aspects of turgor and water relations of all plant cells. In this Update we focus on important principles of plant K\textsuperscript{+} channel function and on the proposed physiological roles of specific plant K\textsuperscript{+} channel types in the plasma membrane and tonoplast (Fig. 1A) of different plant cells.

ION CHANNELS DEFINED

Ion channels catalyze transport through membranes at rates between 10\textsuperscript{6} and 10\textsuperscript{9} per second per channel protein. Transport is “passive,” in other words, diffusion of ions through the channel is a function of both the membrane voltage and the concentration difference for the ion across the membrane and is not directly coupled to the input of other forms of free energy.

This simple picture of ions diffusing through a pore must be refined by consideration of two properties common to all ion channels. The first is selectivity. Selectivity implies the presence of binding sites for recognition of the ion during permeation, and channels are often named after the most permeant ion or after the ions of proposed physiological significance. However, it is important to recognize that selectivities are not absolute, and that many channels will conduct a range of ions to some extent. This property is reflected in the so-called ionic selectivity sequence for the channel. The selectivity of a channel can have great physiological significance. Thus, some K\textsuperscript{+} channels conduct Na\textsuperscript{+} to a finite extent, and this could have an impact on the degree to which plants can withstand salinity (e.g. Schachtman et al., 1991). The selectivity of ion channels can be derived either by measuring the conductance of different ions through the channel or by determining the reversal voltage of the current through the channel (this is the membrane voltage where the net current is 0 and reverses its sign). Selectivities derived from the two methods are not necessarily comparable, and in many physiological conditions they can yield different selectivity sequences (Hille, 1992).

The second universal property of ion channels is their ability to reside in “open” or “closed” conformational states, which, respectively, either permit or do not permit ion permeation. This conformational switching (Hille, 1992) can occur in response to ligands or to a change in membrane voltage after which channels activate (open) or deactivate (close). Inactivation, i.e. closing of open channels during continuous stimulation, is often observed in animal K\textsuperscript{+} channels and represents a form of desensitization. The control of activation by membrane voltage or by ligands (e.g. Ca\textsuperscript{2+}) holds a key to understanding the roles of ion

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Abbreviations: \( E_{\text{K}} \), equilibrium voltage for K\textsuperscript{+}; TEA, tetra-ethyl ammonium.
channels in cell biology. Two main types of K+ channels have been identified, each with a characteristic voltage dependence (Fig. 1B). One class of K+ channels opens in more hyperpolarizing conditions (i.e. at rather negative membrane voltages) and was found capable of facilitating K+ uptake based on its transport properties (Schroeder et al., 1987). These channels are therefore known as inward-rectifying K+ (K+in) channels (for review, see Schroeder et al., 1994). A second class of K+ channel opens in depolarizing conditions, and at these relatively positive voltages it will carry an outward K+ current. These are outward-rectifying K+ (K+out) channels, and they have been described in both algae and higher plants (for review, see Tester, 1990). Detailed studies established that inward- and outward-rectifying K+ currents in plant cells have different kinetics and pharmacological profiles, and it is generally accepted that the underlying channels are two different protein entities. K+ channels comprise the dominant class of channel observed in a wide variety of plant cell types, including guard cells, aleurone cells, leaf cells, stem tissue, mesophyll cells, cortical and stelar cells, root hairs, cortex, and stele (Schroeder et al., 1987; Bush et al., 1988; Spalding et al., 1992; Fairley-Grenot and Assmann, 1993; Elzenga and Van Volkenburgh, 1994; Findlay et al., 1994; Gassmann and Schroeder, 1994; for review, see Schroeder et al., 1994; Vogelzang and Prins, 1994; Wegner and Raschke, 1994; Maathuis and Sanders, 1995; Roberts and Tester, 1995; White and Lemtiri-Chlieh, 1995). Viewed in terms of both abundance and distribution, K+ channels must clearly play some fundamental physiological roles in plant biology.

**STRUCTURE OF PLANT K+ CHANNELS**

The first identification of K+ channel cDNAs from plants was achieved by functional complementation of yeast cells that were defective in K+ uptake. Two distinct Arabidopsis thaliana cDNAs, termed AKT1 and AKT1, were independently cloned (Anderson et al., 1992; Sentenac et al., 1992). Based on the levels of similarity in both amino acid sequence and protein structure, it was concluded that AKT1 and AKT1 proteins are members of the so-called “Shaker” superfamily, which comprises voltage-dependent, K+out channels from animal plasma membranes (Jan and Jan, 1992). Similar to Shaker-type channels, KAT1 and AKT1 hydrophy profiles predict six or seven membrane-spanning regions (Fig. 2A). Between the fifth and sixth membrane span, the P-domain is proposed to form the channel pore and contain binding sites for the permeating ions. The S4 transmembrane domain contains several basic residues spaced every third position. The positive charge on these residues comprises the voltage sensor, which is required for the channel to gate open in response to voltage changes over the membrane (Hille, 1992; Jan and Jan, 1992).

Functional channels probably consist of four a-subunits of 65 to 100 kD (Fig. 2B) with the P-domain from each subunit lining the channel pore (Hille, 1992; Jan and Jan, 1992). In animal systems β-subunits provide additional mechanisms to diversify K+ channel properties by either modifying inactivation properties or by acting as chaperones (Fink et al., 1996). In plant cells, similar regulatory functions could be fulfilled by hydrophilic subunits: an Arabidopsis homolog to animal K' channel β-subunits has been identified that may contribute to K+ channel functioning (Tang et al., 1995). Binding of β-subunits to a guard cell membrane protein that is recognized by KAT1 antibodies supports this hypothesis (Tang et al., 1996).

Despite the similarities to depolarization-activated K+ (K+out) channels in animal cells, voltage-clamp studies of KAT1 in Xenopus oocytes (Schachtman et al., 1992) and of AKT1 in yeast (Bertl et al., 1994) demonstrated that both behave as K+in channels. The functional properties of KAT1 and AKT1 correspond to hallmark characteristics of K+in channels described in patch-clamp studies of higher plant cells, including the time dependence, voltage dependence, K+ dependence, cation selectivity, lack of inactivation, and Ba2+ and TEA+ block (Schachtman et al., 1992; Bertl et al., 1994).

Homologous K+in channel cDNAs have been identified in A. thaliana (AKT2/AKT3) and in potato (KST1) (Cao et al., 1995; Müller-Röber et al., 1995; Ketchum and Slayman, 1997).
Roles of Higher Plant K⁺ Channels

CATION SELECTIVITY OF PLANT K⁺ CHANNELS

Cation selectivities for different K⁺ channels vary widely, from highly selective for K⁺ to a virtual absence of cation discrimination. Mutational analysis of the KAT1 K⁺ in channel supports the model derived from animal systems in which the P-domain determines cation selectivity and channel block (Anderson et al., 1995; Uozumi et al., 1995; Nakamura et al., 1997). The ability to complement K⁺ uptake-deficient yeast mutants with plant K⁺ in channels has added a powerful genetic approach for identifying amino acids and structures involved in K⁺ selectivity, which was not available in previous animal K⁺ channel studies. An elegant genetic selection of mutants in yeast has pointed to the importance of the amino acid stretch Gly-Tyr-Gly (GYG) for determining K⁺ selectivity (Anderson et al., 1995; Nakamura et al., 1997). Furthermore, mutations 5' of the GYG sequence (T256) were found to invert the specificity of KAT1 when comparing the K⁺ conductance with the Rb⁺ and NH₄⁺ conductances. Whereas the Rb⁺ and NH₄⁺ conductances of KAT1 expressed in oocytes are approximately 20 to 30% of the K⁺ conductance (Schachtman et al., 1992), the mutations T256D and T256G evoked Rb⁺ and NH₄⁺ conductances that were 10 times larger than the K⁺ conductance (Uozumi et al., 1995). However, this large inversion in selectivity, as measured by conductance ratios, did not apply to the selectivity as measured by reversal voltage analysis (Uozumi et al., 1995), emphasizing the fact that the two different types of analysis are not comparable (Hille, 1992; Very et al., 1995).

VOLTAGE-DEPENDENT GATING

K⁺ in and K⁺ out channels are increasingly activated at more negative and more positive membrane voltage, respectively. This voltage-dependent gating results in current "rectification" (i.e. transport in mainly one direction). Inward rectification of animal K⁺ in channels is achieved by the selective blocking of outward currents through these channels with cytoplasmic Mg²⁺ or polyanamines (Hille, 1992; Lopatin et al., 1994). Channel blockage by these compounds is voltage-dependent and becomes more pronounced at increasing membrane depolarization. However, studies of KAT1 expressed in Xenopus oocytes (Hoshi, 1995) and of Vicia faba guard cell K⁺ in channels have shown that in plants inward rectification of K⁺ in channels is not mediated by a similar mechanism, but is likely to be controlled directly by the membrane voltage via the S4 voltage sensor.

The voltage sensor contains positively charged residues that react to changes in the membrane voltage. The number of charges in the channel protein that is displaced during channel opening (the gating charge) can be estimated with biophysical analyses and was determined to be 1.3 to 1.8 for KAT1 (Hoshi, 1995; Very et al., 1995). The crucial role of the S4 domain in sensing the membrane voltage also became apparent from studies in which mutations in the S4

levels in whole leaves than those determined for KAT1 (Cao et al., 1995).

Figure 2. A, Generalized structure of voltage-gated channels with six membrane-spanning regions. A sensor in the S4 region reacts to the membrane voltage and contains several positively charged amino acids. The P-domain is thought to form part of the channel pore and is crucial in determining channel selectivity. At the COOH terminus, nucleotide binding sequences (NBS) and ankyrin-like structures (ANK) are present in AKT1 and AKT2 channels. B, K⁺ channels are thought to form tetramers of the main subunit (α), which contains the selectivity filter (SF) and a voltage sensor (VS). The voltage sensor or gate opens or closes the channel in response to alterations in the membrane voltage. Additional β-subunits may have regulatory functions.
domain converted the animal Shaker $K^+_{\text{out}}$ channel into a $K^+_{\text{in}}$ channel (Miller and Aldrich, 1996). Based on such observations, a model was derived for the Shaker $K^+_{\text{out}}$ channel in which mutations in the S4 domain can shift the voltage-dependence of parameters such as activation and inactivation to more negative values (for details, see Miller and Aldrich, 1996). Ultimately, this shift would result in S4-dependent channel opening by hyperpolarization instead of depolarization, and such a model could explain the high degree of similarity between KAT/AKT plant channels and Shaker-type channels, despite their opposite rectification characteristics.

Expression levels of plant $K^+$ channels can also shift the voltage-dependent parameters (Cao et al., 1995; Very et al., 1995), indicating that voltage sensing may also depend on the channel environment or local channel density, although the physiological significance of these observations remains unknown.

**K+ CHANNELS SENSE K+**

$K^+$ concentrations can affect the gating behavior of $K^+$ channels by modulating the threshold membrane voltage where channel opening starts, i.e. the activation voltage (Fig. 1B). In guard cells, relatively little change in the activation voltage was observed when $[K^+]_{\text{ext}}$ changed in the range from 10 to 100 mM $K^+$. However, in the range from 0 to 10 mM $K^+$, the activation voltage does respond to alterations in $[K^+]_{\text{ext}}$ (Schroeder and Fang, 1991). Similarly, in *A. thaliana* root cells the $K^+_{\text{in}}$ channel activation voltage always remained 20 to 30 mV negative of $E_k$ (Maathuis and Sanders, 1995). Therefore, channel opening only occurs whenever the $K^+$ gradient is inward, suggesting that $K^+_{\text{in}}$ channels function as a $K^+$-sensing valve, allowing $K^+$ uptake only.

Additional unequivocal evidence that $K^+_{\text{in}}$ channels actually do sense the external $K^+$ concentration originates from the observation that these channels no longer open when extracellular $K^+$ is removed (Schroeder and Fang, 1991; Gassmann and Schroeder, 1994; Maathuis and Sanders, 1997). If $K^+_{\text{in}}$ channels could not sense external $K^+$, a very large outward $K^+$ current would emanate in $K^+$-free external solutions because of the large cytosolic $K^+$ concentration.

The ability of $K^+_{\text{in}}$ channels to sense $[K^+]_{\text{ext}}$ is not absolutely $K^+$-specific. Thus, although KAT1 currents do vanish when $[K^+]_{\text{ext}}$ becomes 0 (Schachtman et al., 1992; Cao et al., 1995), after replacement of $K^+$ by Na$^+$ or Li$^+$, large outward $K^+$ currents can be generated by KAT1 (Cao et al., 1995; Very et al., 1995). Apparently, the $K^+_{\text{in}}$ channel sensor also responds to cations of limited permeability such as Li$^+$ and Na$^+$, and further research on its physiological role would be of interest.

**REGULATION OF MEMBRANE VOLTAGE**

In all biological membranes the membrane voltage must be regulated within rather tight limits for several reasons. First, the cytosol-negative membrane voltage provides the driving force for the electrophoretic transport of many solutes against their chemical gradients. This applies not just to important cationic nutrients such as NH$_4^+$, but also to neutral solutes such as sugars and amino acids, where transport is frequently coupled to that of H$^+$. Second, the membrane voltage is thought to play a key role in certain types of cellular signaling. For example, effectors can cause a membrane depolarization that in turn can trigger Ca$^{2+}$ channel activation as part of a signaling pathway (for review, see Ward et al., 1995). Third, the membrane voltage must normally be held in a range that facilitates activity of the primary electrogenic H$^+$-pumping ATPase. This enzyme generally functions continuously to remove excess metabolically produced H$^+$, and failure to short-circuit some of the ATPase current via ion channels would inevitably result in membrane damage and stalling of the pump at its highly negative equilibrium voltage of around $-450$ mV.

The manner in which $K^+$ channels maintain membrane voltage can be assessed by calculation of the $E_k$ from the Nernst equation:

$$E_k = 59 \log\left[\frac{[K^+]_{\text{ext}}}{[K^+]_{\text{cyt}}}\right]$$

in which $[K^+]_{\text{ext}}$ and $[K^+]_{\text{cyt}}$ are the $K^+$ activities of the extracytosolic and cytosolic compartments, respectively, and $E_k$ is in millivolts. If $K^+$ channels comprise the dominant electrical conductance, the membrane voltage will approximate $E_k$. Typically, in higher plants $[K^+]_{\text{cyt}}$ is of the order of 80 to 200 mM (Maathuis and Sanders, 1993; Walker et al., 1996). In contrast to cytoplasmic $K^+$ concentrations, which are tightly regulated, the $K^+$ content of soils varies highly, and this will affect $E_k$. For example, with values of $[K^+]$ in the soil solution and leaf apoplast ranging from around 500 $\mu$M to 10 mM, $E_k$ would be $-60$ to $-150$ mV for a typical plant cell.

Resting levels of the plasma membrane voltage can become more negative than $E_k$, mainly depending on the extent to which activity of the H$^+$-ATPase hyperpolarizes the membrane. $K^+_{\text{in}}$ channels exhibit a strongly increasing tendency to open as the pump drives the voltage more negative, and it is $K^+$ uptake through these channels that prevents the membrane voltage from becoming too negative.

Conversely, physiological stimuli induce rapid membrane depolarizations, for example, by anion and Ca$^{2+}$ channel activation (for review, see Ward et al., 1995). Depolarizations positive of $E_k$ will stimulate opening of $K^+_{\text{out}}$ channels, and the resulting efflux of $K^+$ will tend to limit the extent of the depolarization. Subsequent additional activation of $K^+_{\text{out}}$ channels gradually restores the membrane potential to a more negative value. These properties of $K^+$ channels in stabilizing membrane voltages are universal in eukaryotes and constitute the principal role of $K^+$ channels in animals, in which the repolarizing phase of action potentials is generated through opening of $K^+$ channels.
K+ CHANNELS AND K+ NUTRITION

K+ is the most abundant cation in most nonhalophytic higher plant cells. Extensive studies on the kinetics of K+ uptake into roots showed that K+ uptake is mediated by at least two mechanisms, with high and low affinities for K+, respectively (for review, see Maathuis and Sanders, 1996). The ability of K+ in channels to sense K+ concentration is of major significance to the process of K+ nutrition, and K+ in channels have been described in different root cells including cortical, root hair, stelar, and xylem parenchyma cells (Fig. 3; Findlay et al., 1994; Gassmann and Schroeder, 1994; Vogelzang and Prins, 1994; Wegner and Raschke, 1994; Maathuis and Sanders, 1995; Roberts and Tester, 1995; White and Lemtiri-Chieh, 1995).

In A. thaliana root cells, two types of K+ in channel have been observed (Maathuis and Sanders, 1995). Interestingly, activity of the predominant K+ in channel (conductance 6 pS at physiological K+ concentrations) was found to increase when K+ levels in the growth medium were lowered to micromolar values.

Hallmarks of the Arabidopsis K+ in channels are channel blockage by TEA, which has been shown specifically to inhibit low-affinity unidirectional K+ fluxes in corn roots, a selectivity (K+ ≈ Rb+ > Na+ > Cs+) that resembles selectivities for unidirectional fluxes in whole tissue, and enough K+ -conducting capacity to explain the influx observed in intact plants. In wheat root hairs and cortical cells, typical K+ in channel currents were identified that show a similar K+ selectivity (Findlay et al., 1994; Gassmann and Schroeder, 1994). Furthermore, wheat root hair K+ in channels were inhibited by AP+ (K0.5 = 8 μM) (Gassmann and Schroeder, 1994), which agrees with a previously established AP+ sensitivity of K+ uptake in intact plants. In both wheat and A. thaliana root cells, the K+ in channel-mediated currents saturate as a function of [K+]ext with an apparent Km in the range of 8 to 19 mM, values that correlate well with those determined for low-affinity K+ absorption using tracers.

Two major overlapping functions have been proposed for these K+ in channels: (a) they provide a pathway for low-affinity K+ uptake, which is driven by the H+ pump-established membrane voltage, and (b) they contribute to membrane voltage control by modulating the membrane conductance and by sensing the soil-to-cell K+ gradient, similar to a K+ electrode (see above). The latter function in particular could influence nutrient uptake by other transporters (Schroeder et al., 1994).

Channel-mediated K+ absorption can only proceed when the electrochemical gradient for K+ is inward. However, generally, the K+ gradient becomes outward when [K+]ext drops below 0.2 to 0.5 mM (see Eq. 1). In these conditions K+ uptake must be active and is proposed to be mediated by symporters that act in parallel with channel-mediated K+ transport. Therefore, any K+ conductance forms a potential shunt pathway through which symport-accumulated K+ can leak out of the cells, and it is imperative that K+ channel conductance is minimized. As described above, K+ in channels will be deactivated in these circumstances as a result of their K+-sensing characteristics. However, activity of K+ out channels is not necessarily 0 at low [K+]ext and overall conductance via this pathway is probably restricted by a dramatic reduction of the K+ out open-channel conductance (Maathuis and Sanders, 1997).

STOMATAL MOVEMENTS

Changes in cell turgor are responsible for the movement of cells and organs. Examples include phototropic responses and the fTytrap closure of insect-eating plants such as Didea muscipula, which is mediated by large and very rapid turgor changes in the trap lobe cells. A further example is the opening and closing of stomata, which is a well-characterized event. Patch-clamp studies on V. faba guard cells demonstrated the abundance of K+ -selective single channels in the plasma membrane (Schroeder et al., 1984). The early finding that these K+ channels remained active during long recordings, together with their sufficient K+ transport activity, led to the hypothesis that plant K+ channels provide pathways for K+ uptake and release, required in guard cells to osmotically drive opening and closing of stomata, respectively (Fig. 3; Schroeder et al., 1984). Two main types of K+ channels were identified in guard cells of both V. faba and maize: the hyperpolarization-activated K+ in channels proposed to mediate K+ uptake, and the depolarization-activated K+ out channels proposed to mediate K+ release (Schroeder et al., 1987; Fairley-Grenot and Assmann, 1993). Biophysical studies on the cation selectivity, voltage, and time dependencies, transport rates, lack of inactivation, and channel block by AI3+ supported the model that these channels provide important pathways for K+ uptake and release during stomatal movements (Schroeder et al., 1987).

Studies on the modulation of guard cell K+ channels by cytosolic factors have shown correlations between second messenger levels and regulation of stomatal movements. These cellular regulation mechanisms of guard cell K+ channels and their physiological implications for stomatal movements are reviewed in more detail elsewhere (Assmann, 1993; Ward et al., 1995), but examples are: (a) ABA-induced Ca2+ elevation and the inhibition of stomatal opening by Ca2+, which correlates with a down-regulation of K+ in channels by elevated Ca2+ concentrations (Schroeder and Hagiwara, 1989); (b) enhancement of K+ out channel activity by cytosolic alkalinization can be triggered by ABA-induced alkalinization (Irving et al., 1992; Blatt and Armstrong, 1993); and (c) the activity of K+ in channels is increased by extracellular acidification, which can provide an additional mechanism, enhancing proton pump-induced stomatal opening (Blatt, 1992).

In addition, several studies suggest that protein phosphatases play important roles in K+ channel regulation. Calcineurin (PP2B) inhibitors disrupt the Ca2+-dependent down-regulation of guard cell K+ in channels (Luan et al., 1993). K+ in channel down-regulation by the protein phosphatase inhibitor okadaic acid suggests that other protein phosphatases produce the opposite effect to that proposed for calcineurin (Thiel and Blatt, 1994; Li et al., 1994). Furthermore, the characterization of the ABI1 gene as a protein phosphatase 2C homolog (Meyer et al., 1994; Leung et al.,
brane (e.g. through anion channel activation) to enhance models in which $K^{\text{in}}$ channels are essential but not rate limiting during stomatal movements (Schroeder et al., 1987). Indeed, a 90% block of $K^{+}$ channel current in $V. faba$ guard cells range from 100 to 500 pA for a membrane voltage of $-150 \text{ mV}$. However, the average absolute $K^{+}$ fluxes during stomatal opening amount to only 8 pA in $V. faba$ (Schroeder et al., 1987). Indeed, a 90% block of $K^{+}$ channel current in $V. faba$ guard cells only slightly slowed but did not inhibit light-induced stomatal opening, providing support for models in which $K^{\text{in}}$ channels are essential but not rate limiting during stomatal movements (Schroeder et al., 1987; Kelly et al., 1995). This observation holds for $K^{\text{in}}$ channels exclusively and not for $K^{\text{out}}$ channels (Kelly et al., 1995). In the case of $K^{\text{in}}$ channels, proton pump-mediated membrane hyperpolarization can compensate for partially blocked $K^{\text{in}}$ channel currents by increasing the driving force for $K^{+}$ simultaneously increasing channel activity. By contrast, the ability to depolarize the membrane (e.g. through anion channel activation) to enhance $K^{\text{out}}$ channel activity is more restricted. One reason why $K^{+}$ channel activities are large under most conditions may lie in other essential roles they play. Functions such as membrane voltage control and cellular homeostasis (see above) may all require some level of constitutive $K^{+}$ channel activity.

Another question relating to $K^{+}$ channel overcapacity concerns the possible existence of two types of $K^{\text{in}}$ channels in both laser-ablated Commelina communis guard cells (Henriksen et al., 1996) and in patch-clamped $V. faba$ guard cells (Wu, 1995). Both these channel types may be involved in stomatal control. This seeming surplus in $K^{+}$ channel capacity is also observed in other cells (e.g. in root cells) and its biological significance remains to be determined. Molecular physiological analyses of plant $K^{\text{in}}$ channels will allow questions of possible redundancies in $K^{+}$ transport pathways to be addressed.

**LEAF MOVEMENTS**

In nyctinastic, or night-closing, plants, rapid leaf movements occur in response to day-night changes. Night closing is under the control of both light and internal clock stimuli, and is driven by the motor cells at the base of the leaf. Flexor cells shrink during leaf opening, whereas opposite extensor cells swell. The reverse happens during leaf closure. Cell swelling and cell shrinkage involve the uptake and release of large amounts of $K^{+}$, $Cl^{-}$, and other solutes.

A depolarization-activated $K^{+}$ channel in protoplasts derived from motor cells is capable of carrying the large $K^{+}$ currents that flow from shrinking cells, is sensitive to inhibition by TEA and quinine, and conducts several monovalent cations with a selectivity sequence $K^{+} > Rb^{+} > Na^{+} > Cs^{+} > Li^{+}$ (Moran et al., 1988). As explained above, to maintain a significant $K^{+}$ efflux the membrane voltage must be more positive than $E_{K}$. Therefore, an anion channel activation may be a feasible mechanism to sustain $K^{\text{out}}$ channel-mediated $K^{+}$ release in motor cells.

Motor cell swelling requires $K^{+}$ uptake. Stimulation of the $H^{+}$-ATPase will hyperpolarize the membrane and direct the $K^{+}$ gradient inward. Preliminary results indicate the presence of inward-rectifying channels in motor cells (Moran, 1990). However, it has not yet been established to what extent these channels are $K^{+}$ selective. Opening of these channels would lead to $K^{+}$ uptake, but involvement of other (active) transport systems may be necessary to explain $K^{+}$ uptake in its entirety.

How environmental signals such as light are translated into a change in $K^{+}$ uptake remains unclear. Modulation of the $H^{+}$-ATPase activity will play a crucial role in this process. It is interesting that $H^{+}$-ATPases in opposite motor cells react to light in either a stimulatory or inhibitory manner and this differential response would explain the necessary polarity in swelling and shrinking. Studies on how signals such as light are transduced into a change in $K^{+}$ transport have identified second-messenger signaling mechanisms that coordinately regulate $H^{+}$ pumps and $K^{+}$ channels (for review, see Coté, 1995).

**LOADING/UNLOADING OF THE XYLEM**

Nutrients taken up by roots need to be transported to the xylem before they reach aerial parts of the plant. It was long thought that the stele did not actively participate in this process because of a lack of oxygen. However, it is now established that xylem parenchyma cells, which surround the xylem vessels, contain proton pumps, water channels, and ion channels, pointing to an active role of these cells in xylem loading/unloading (De Boer and Wegner, 1997).

In barley xylem parenchyma cells three types of cation channels have been identified (Fig. 3; Wegner and Raschke, 1994). Two channels called KORC ($K^{+}$ outward-rectifying conductance; equivalent to $K^{\text{out}}$) and NORC (nonselective outward-rectifying conductance) become active at membrane voltages more positive than $-50$ and $+30 \text{ mV}$, respectively. NORC channels are nonselective among cations and therefore are similar to outward-rectifying channels described in endosperm cells (Stoeckel and Takeda, 1989). KORC and NORC can reside in the same cell and their respective activation depends on the cytoplasmic $Ca^{2+}$ level. In roots, the $K^{\text{out}}$ channels are probably involved in release of $K^{+}$ into the xylem. Channels would operate whenever the cell becomes depolarized as a result of re-
transport through K+; and K+-,, channels, respectively. The transpiration rate via adjustment of stomatal aperture crucially depends on transporting large amounts of K+ across the guard cell plasma membrane. Opening and closing of stomata involves K+ transport through K+; and K+-, channels, respectively. Loading of the xylem vessel may depend on channels such as KORC, which release K+ into the vessel, and may also play a role in the process of cation destination. In more mature parts of the xylem, resorption of specific ions may be mediated by KIRC-like channels and the non-selective channel NORC is believed to play a role in membrane voltage regulation. C, Low-affinity uptake of the important nutrient K+ takes place at the root-soil solution interface and probably involves both epidermal and cortical root cells. Specific K+-, channels have now been identified that form a pathway for such K+ uptake, provided the external K+ concentration is sufficiently high to create an inward K+ gradient.

\[ K_\text{in} \rightarrow K_\text{out} \]

\[ \mathbf{K}^+ \text{-selective inward-rectifying channels in barley xylem parenchyma cells, named KIRC (equivalent to K'+,,), activate whenever the membrane voltage is more negative than } -110 \text{ mV (Wegner and Raschke, 1994). These channels potentially mediate resorption from the xylem into the xylem parenchyma rather than release of salts. Growing parts higher up in the plant form a sink for ions that have to be extracted from the xylem. Similarly, a role in the resorption of harmful ions into specialized root cells is possible. A hyperpolarization of the membrane by the H+-ATPase in these cell types would stimulate opening of KIRC and increase the driving force for passive cation resorption. Interestingly, KIRC showed a high permeability for Cs+, an ion that normally blocks K+ channels.}

\[ \mathbf{VACUOLAR K^+ CHANNELS} \]

Nonwoody plants derive their rigidity from cell turgor, which is also the driving force for cell expansion. Accumulation of K+ is a major contributor to the intracellular osmotic pressure, and with vacuolar K+ activities normally comparable to those in the cytosol, up to 95% of cellular K+ could reside in the vacuole. In most conditions vacuolar K+ uptake requires active transport, whereas vacuolar K+ release has been proposed to be mediated by K+ channels.

Three major types of tonoplast K+ channels have been characterized. A ubiquitous type is the SV (slow-activated vacuolar) channel, which conducts K+ into the vacuole (Hedrich and Neher, 1987; Colombo et al., 1988). At normally observed tonoplast voltages and cytoplasmic Ca2+ concentrations, its open probability is extremely low. Consequently, a rise in cytoplasmic Ca2+, which is frequently observed in stress conditions, could promote SV channel activity. Originally, SV channels were believed to show considerable anion permeability, but subsequent studies revealed that they function as nonsel ective monovalent cation channels without a measurable anion permeability (e.g. Colombo et al., 1988). Apart from conducting monovalent cations, these channels also allow Mg2+ permeation (Allen and Sanders, 1996; Gambale et al., 1996) and have a significant Ca2+ permeability with tight Ca2+-binding properties (Ward and Schroeder, 1994). SV channels were therefore proposed to contribute to Ca2+-induced Ca2+ release from the vacuole (Ward and Schroeder, 1994). Furthermore, it was shown that SV channels function by binding multiple ions, which are then transported through the pore in a single file (Allen and Sanders, 1996; Gambale et al., 1996). The multi-ion, single-file-pore mechanism of SV channels renders the proposed transport of anions across the membrane in the opposite direction (Schulz-Lessdorf and Hedrich, 1995) biophysically and thermodynamically unlikely.

A second type of K+ channel has been found in V. faba guard cells, which is highly selective for K+ and shows a cation selectivity sequence K+ >> Rb+ > NH4+ > Na+ > Li+ ~ Cs+ (Ward and Schroeder, 1994). These vacuolar K+ (VK) channels can be further distinguished from other tonoplast K+ channels in that they are voltage independent and activate at cytosolic Ca2+ elevations in the physiological range (Ward and Schroeder, 1994; Allen and Sanders,
1996). VK channels can conduct large K+ fluxes from guard cell vacuoles to the cytosol and have been proposed to provide a pathway for vacuolar, Ca2+-dependent K+ release during stomatal closing. In this model, vacuolar H+ ATPases would hyperpolarize the tonoplast to drive channel-mediated vacuolar K+ release (Ward and Schroeder, 1994) and simultaneously contribute to the cytosolic alkalization that occurs in response to ABA during stomatal closing (Irving et al., 1992; Blatt and Armstrong, 1993).

A third channel type is the fast vacuolar (FV) channel, which is, like the SV channel, relatively nonsel ective among cations (Hedrich and Neher, 1987) but highly selective for cations versus anions. This channel opens at low cytoplasmic Ca2+ levels and is active at physiological tonoplast voltages. It mainly conducts cations from the vacuole to the cytoplasm and could therefore increase the osmolarity in that compartment (Hedrich and Neher, 1987; Allen and Sanders, 1996). In guard cell vacuoles a recent study has demonstrated that FV channels could provide the pathway for vacuolar K+ release during Ca2+-independent stomatal closing (Allen and Sanders, 1996).

CONCLUSION

It is now abundantly clear that K+ channels are involved in a range of physiological processes in higher plants. For the K+in channel these include: (a) K+ uptake into guard cells and into various plant cell types during cell expansion, movements, and growth; (b) K+ nutrition and transport, by forming a low-affinity uptake pathway in root cells; (c) a possible role in xylem unloading by conducting cations from xylem to symplast; and (d) membrane voltage regulation, for example, by preventing excessive hyperpolarization.

Outward-rectifying channels function in: (a) membrane voltage regulation, by resetting the membrane potential after depolarizing stimuli or by preventing excessive depolarization; (b) solute release, for example, to promote stomatal closure, tissue movements, and osmoregulation; and (c) xylem loading, by transporting cations from symplast to the xylem vessel.

Clearly, these processes are under cellular control and are likely to be affected by the membrane voltage, levels of K+ and Ca2+, and phosphorylation. In spite of such proposed diversity in physiological functions, more specific identification of the roles of individual K+ channels is now becoming possible by cDNA isolation, functional characterization in heterologous systems, and molecular physiological studies.

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