Research Findings

Molecular Biology of K^+ Transport Across the Plant Cell Membrane: What Do we Learn from *Arabidopsis* and Rice Model Plants?

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Introduction

Potassium (K^+) can constitute up to 10 percent of total plant dry weight. It is the most abundant cation in the cytosol since it is compatible with protein structure even at high concentrations. At cellular level, this cation is used for basic functions, such as electrical neutralization of anionic groups, control of cell membrane polarization, osmoregulation and regulation of cell turgor (Clarkson and Hanson, 1980; Maathuis and Sanders, 1996). At whole plant level, K^+ is involved in highly complex and integrated functions. For instance, related to its involvement in turgor regulation, K^+ plays a role in tropisms, or guard cell movements, which allow the plant to regulate the aperture of the stomatal pores present at the leaf surface. The importance of these functions probably explains why membrane transport of K^+ has been studied more extensively than other nutrient ions, giving rise to breakthroughs and founding models, such as Epstein's dual mechanism, which proposes that membrane transport of K^+, as well as of most other ion nutrients, results from the activity of both high affinity and low affinity transport mechanisms.

Most of the present knowledge on K^+ transport in plants has been gained by studies using *Arabidopsis thaliana* as a model. It seems that the gene families involved in K^+ transport are strongly conserved across higher plant species, both in terms of family structure and gene numbers, with the exception of the HKT family, which may possess K^+-permeable members in monocots only. This difference suggests that graminaceous and dicotyledonous crops could behave differently regarding K^+ uptake and long distance transport within the plant, but the roles of K^+-permeable HKT transporters are still poorly understood.

In *Arabidopsis thaliana*, the genome encodes about 27,000 proteins. Based on current knowledge, at least 35 contribute to membrane K^+ transport (Mäser *et al.*, 2001). These membrane proteins form two families of K^+ channels, named Shaker and TPK, comprising nine and six members respectively, and two families of K^+ transporters, named HAK (or KT or Kup) and KEA, comprising 13 and six members respectively. In *Arabidopsis*, the single member from the HKT transporter family has been shown to be Na^+-selective (Uozumi *et al.*, 2000). In contrast, in rice, up to four transporters from the HKT family are permeable to K^+ (Corratgé-Faillie *et al.*, 2010). The Shaker and HKT transport systems, and at least part of the HAK and KEA systems, are located at the cell membrane. The TPK channels appear to play an essential role in K^+ transport across the vacuolar membrane. This review focuses on K^+ transport proteins active at the cell membrane and involved in K^+ uptake from the soil solution and transport within the plant.

Here we present the current model of membrane energization and ion transport in plants, and highlight some of the terminology used in this field of plant biology. Then we summarize what is currently known about molecular families of K^+ transport systems active at the plasma membrane, namely the Shaker K^+ channel family and the HAK, HKT and KEA transporter families. This is followed by a brief presentation of the roles that these systems play within the plant, in functions such as: K^+ uptake from the soil by roots; K^+ long distance transport in the xylem and phloem vasculatures; and K^+ accumulation and turgor-driven processes, like pollen tube elongation or guard cell movement, and regulation of transpirational water loss.

Energization of solute transport across the cell membrane in plants

Pioneering physiological analyses of solute uptake in roots in the 1960's and 70's led to the concept of passive and active transport (Maathuis and Sanders, 1996). Uptake of an electrically uncharged solute (e.g. glucose) across the cell membrane from the external solution into the cell is said to be active when it occurs against the concentration gradient of the solute, i.e. when the concentration of the solute is lower in the external solution than in the cytosol. Conversely, the uptake is said to be passive when it occurs down the concentration gradient, i.e. when the external concentration

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of the solute is higher than the internal concentration. From an energetics point of view, when the solute is electrically charged (e.g. mineral nutrient ions like K^+, SO_4^{2-} or NO_3^-), its transport across the membrane depends not only on its concentration gradient across the membrane but also on the electrical gradient, i.e. the difference in electrical potential between the soil solution and the cell cytoplasm.

When measured in young root periphery cells, this electrical gradient is usually found to lie between -50 and -250 mV, depending on the external ionic conditions (mainly pH and K^+ concentration). Uptake is active, and thus must be directly fueled by membrane mechanisms when it occurs against the ion electrochemical potential μ, as described by the Nernst equation [$\mu = \mu_0 + RT\ln(C) + zF\Psi$, where C is the ion concentration, z the ion valency, Ψ the electrical potential in the cytosol, and μ_0, R, T and F have their usual meaning (standard electrochemical potential of the ion, ideal gas constant, temperature and Faraday constant, respectively). Conversely, the uptake is said to be passive when it occurs down the ion electrochemical potential. As indicated above, active transport has to be energized. This is achieved by H^+-excreting ATPases active at the cell membrane. H^+-pumping out of the cell by these enzymes, fueled by ATP hydrolysis, results in a gradient of electrical potential and pH across the cell membrane, and thus in an inwardly directed electrochemical gradient of H^+.

This electrochemical gradient, which renders "spontaneous" H^+ re-entry into the cell (i.e. exergonic), energizes the membrane and active transport activity via H^+-driven co-transport systems (as described by the Mitchell theory). Regarding K^+ uptake, this means that proteins named H^+-K^+ symporters, are active at the cell membrane, permeable to both H^+ and K^+ and couple the spontaneous re-entry of H^+ to K^+ uptake against the electrochemical potential of K^+. In other words, the spontaneous movement of H^+ within the H^+-K^+ symporter back to the cytosol is coupled to (energize) the movement of K^+ within the symporter, from the external medium to the cytosol against the K^+ electrochemical gradient. This process of membrane energization and of active K^+ uptake is depicted in Fig. 1. Of the four major cations taken up by plants (Ca^{2+}, Mg^{2+}, K^+ and Na^+), only K^+ requires high levels of active transport activity, in addition to passive transport activity depending on soil K^+ availability, with the three other cations being essentially taken up passively.

Besides this classification into passive and active transport mechanisms, mechanistic analyses have led to the division of transport proteins into two classes, channels and transporters (Stein, 1990) (in addition to pumps). When open, channels can be considered as selective pores through which substrate ions move, without inducing a change in the general conformation of the protein, while transporters undergo a cycle of conformational

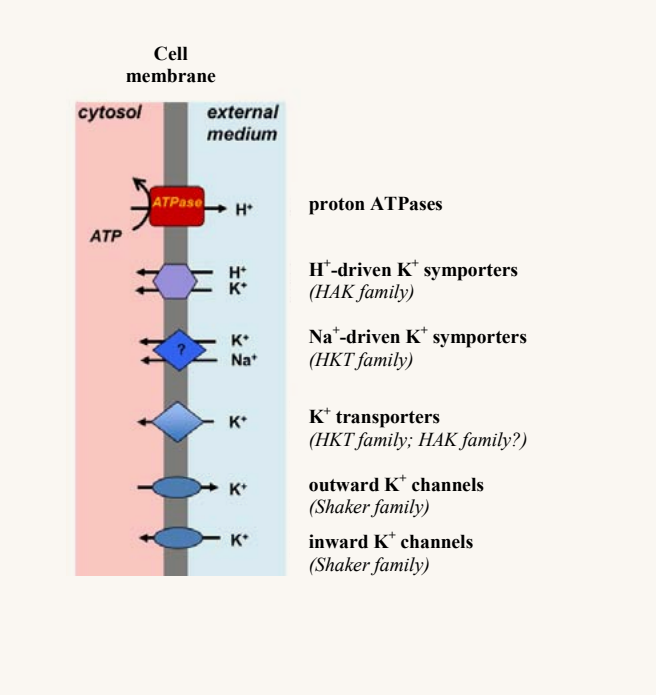

Fig. 1. Mechanisms of membrane energization and potassium transport across the plasma membrane.

changes for each solute they transport. In the classical model, a transporter binds its substrate(s) onto a site facing the external solution; then, a reorientation step allows the binding site and the substrate to have access to the cytosol, into which the substrate(s) diffuse(s); then, a new conformational change allows the binding site to move back to the other side of the membrane, facing the external solution again.

The maximum velocity of transporters (up to 10^4 transport events per second per protein) is thus much lower than that of channels (up to 10^6 ions per second and protein). Taking into account energetic criteria, transporters can be divided into uniporters and cotransporters. Uniporters move their substrates down their electrochemical gradients and thus mediate passive transport, whereas cotransporters (symporters or antiporters) can move a substrate against its electrochemical gradient, by coupling this transport to another ion (e.g. H^+ in H^+-K^+ symporters).

In plants, the most extensively characterized proteins mediating K^+ transport across the plasma membrane are K^+ channels from the Shaker family. These channels mediate passive K^+ fluxes that dominate the membrane conductance to K^+ in most cell types (Lebaudy *et al.*, 2007). The HAK family, which is less characterized, comprises H^+-K^+ symporters, playing a crucial role in active K^+ uptake (Gierth and Mäser, 2007). The HKT family displays two types of transporters, the first one permeable to Na^+ only and present in both dicots and monocots, and the second

one permeable to both Na^+ and K^+ and present in monocots only (Corratgé-Faillie *et al.*, 2010). The KEA family, which is comprised of cotransporters that couple K^+ flux to anion (Cl^-) flux, is still poorly characterized (Gierth and Mäser, 2007). The information currently available on each of these four families is summarized below.

Shaker K^+ channels

Two Shaker K^+ channels from *Arabidopsis thaliana*, named AKT1 and KAT1 (for *Arabidopsis* K^+ transport and K^+ *Arabidopsis thaliana*, respectively), were the first nutrient ion transport systems to be identified in plants. They were cloned in 1992 by functional complementation of a mutant strain of yeast *(Saccharomyces cerevisiae)* which was defective in K^+ uptake and unable to grow on physiological concentrations of K^+ (Anderson *et al.*, 1992; Sentenac *et al.*, 1992). Mutant yeast cells were transformed with gene (cDNA) libraries and spread on agar plates displaying low K^+ concentrations. Colonies showing rapid growth were picked and their transforming construct extracted and sequenced. The deduced polypeptides were found to share similarities, both at the sequence and structure levels, with animal voltage-gated K^+ channels, forming the so-called Shaker superfamily (Jan and Jan, 1997).

Shaker genes encode polypeptides displaying six transmembrane segments, i.e. six hydrophobic segments (of about 20 amino acids), each one spanning the cell membrane (Fig. 2A). The fourth transmembrane segment harbors positively electrically-charged amino acids and acts as a voltage-sensor rendering the gating of the channel voltage-sensitive: movements of this segment within the membrane, in response to changes in the transmembrane electrical potential gradient, result in conformational changes of the protein that favors opening or closure of the channel pore. A highly conserved membranar loop, located between the fifth and the sixth transmembrane segment, and called the P (pore) domain, forms part of the selectivity filter of the ion-conducting pore. Downstream of this hydrophobic core, plant Shaker polypeptides harbor a large cytosolic region, which comprises several domains, including a putative cyclic nucleotide-binding domain. In most Shaker channels, this cytosolic region also comprises an ankyrin domain, which constitutes a site of interaction with regulatory proteins (e.g. kinases or phosphatases) (Fig. 2A). It should be noted that a Shaker polypeptide does not form a functional channel by itself. Indeed, the functional protein has a tetrameric structure associating four Shaker polypeptides (Fig. 2B). The channel can have a homotetrameric structure, associating four Shaker polypeptides encoded by the same gene, or a heterotetrameric, associating Shaker polypeptides encoded by different genes. The heteromerization process results in increased diversity in functional properties (e.g. sensitivity to the membrane potential). In other words, with a given number of Shaker genes, the plant can generate a larger number of channel types.

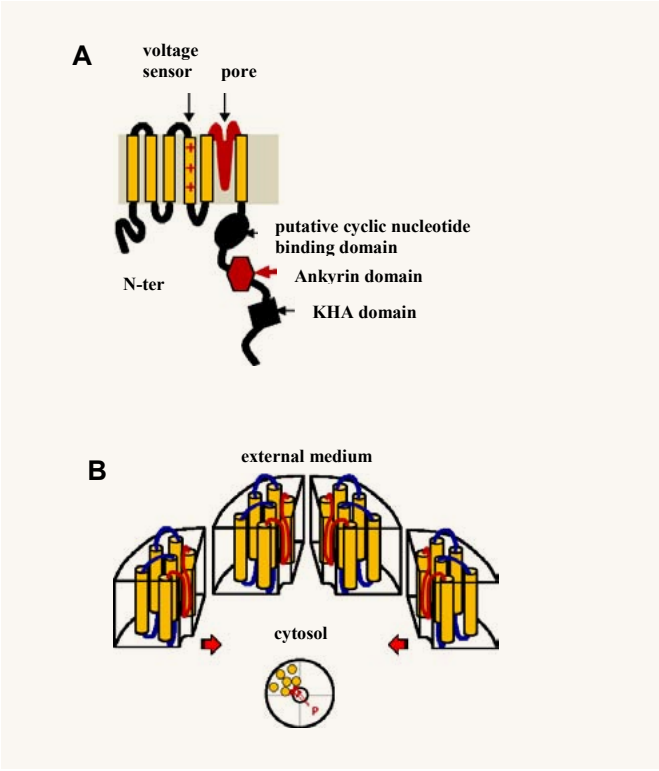

Fig. 2A. Shaker genes encode polypeptides displaying six hydrophobic transmembrane segments (displayed in yellow in the figure). The fourth transmembrane segment harbours positively charged amino acids and acts as a voltage sensor. A P domain is located (in red) between the fifth and the sixth transmembrane segment. The cytosolic region comprises several domains, including a putative cyclic nucleotide-binding domain (in black) and, in most Shaker channels, an ankyrin domain (in red), which constitutes a site of interaction with regulatory proteins. Downstream of the ankyrin domain, the KHA domain is involved in channel tetramerization (Daram *et al.*, 1997).
Fig. 2B. Tetrameric structure associating four Shaker polypeptides.

In the model plant *Arabidopsis thaliana*, nine Shaker genes are present. Five of them (KAT1, KAT2, AKT1, SPIK and AKT6) are dedicated to K^+ uptake across the cell membrane, two (SKOR and GORK) are dedicated to K^+ secretion across the cell membrane, one (AKT2) encodes a channel which allows both K^+ uptake and K^+ secretion, and one (AtKC1) encodes a channel regulatory subunit, affecting the sensitivity of heteromeric channels to voltage (i.e. to the transmembrane electrical potential gradient) (Lebaudy *et al.*, 2007). The direction of transport (uptake or secretion) through these Shaker channels depends on the channel sensitivity to voltage. Channels that activate upon hyperpolarization of the transmembrane electrical potential mediate K^+ uptake. They are called inward (or inwardly rectifying) channels in electrophysiological analyses. Conversely, channels that activate upon a depolarization of the transmembrane electrical potential mediate K^+ secretion. They are called outward (or outwardly rectifying) channels.

Comparison amongst species indicate that the structure of the Shaker gene family is strongly conserved in higher plants, in terms of gene number (e.g. nine genes in *Arabidopsis thaliana*, 11 genes in rice, ten genes in grapevine) as well as in terms of channel types (e.g. inward or outward types). Such conservation should result in easier transfer of the basic knowledge gained in classical plant models (*Arabidopsis* and, to a lower level at the present time, rice) to other plants and crop species.

HAK transporters

In plants, genes from the HAK family (also named KT or KUP by different authors) were identified by sequence homology with K^+ uptake transporters from bacteria (KUP) and high-affinity K^+ transporters (HAK) from fungi (Santa-María *et al.*, 1997; Rodríguez-Navarro, 2000; Gierth and Mäser, 2007).

Little is known about the structure of these transporters (Fig. 3). Hydrophobicity profiles suggest that they possess ten transmembrane segments and a long cytosolic loop between the second and third segment (Gierth and Mäser, 2007). So far, no region involved in ion conduction has been identified in these transporters.

The roles of these systems in plants are not yet fully understood, in particular because they do not seem to be functional at the cell membrane when heterologously expressed in *Xenopus* oocytes. Expression in yeast or *E. coli* mutant strains deficient for K^+ uptake has, however, allowed characterizing of the functional properties of some members from the HAK/KUP/KT family, belonging to groups I and II. It has been suggested that some transporters are devoted to high-affinity K^+ transport, from µM concentrations, whereas others play a preponderant role in the millimolar K^+ concentration range. *In planta*, there are indications that high affinity HAK/KUP/KT members are involved in root K^+ active uptake, in conditions of low K^+ availability, by mediating H^+-K^+ symport activity (Santa-María *et al.*, 1997 & 2000; Gierth and Mäser, 2007). This is very likely to be the case for HAK5 from *Arabidopsis thaliana* (Gierth *et al.*, 2005).

HKT transporters

Plant HKT transporters are related to the fungal Trk and prokaryote KtrB and TrkH transporters (Durell and Guy, 1999; Rodríguez-Navarro, 2000). Fungal and prokaryote systems of this superfamily are thought to work as H^+-K^+ or Na^+-K^+ symporters, or as K^+ uniporters. In fungi, Trk transporters are the major contributors to K^+ uptake at micromolar to submillimolar K^+ concentrations, at least at neutral and basic pH (Rodríguez-Navarro, 2000). Sequence analyses have led to the hypothesis that these transporters have evolved from bacterial K^+ channels and display a transmembrane hydrophobic core structure formed of four tandemly repeated domains, each of them comprising one transmembrane segment followed by one P domain and another transmembrane segment. The four P domains line a central pore (Durell and Guy, 1999) (Fig. 4).

Phylogenetic and functional analyses identify two subfamilies of HKT transporters in plants. The first one, present in both dicots and monocots, displays transporters permeable to Na^+ only. The second one, which has only been identified in monocots so far, comprises transporters permeable to both Na^+ and K^+. It should

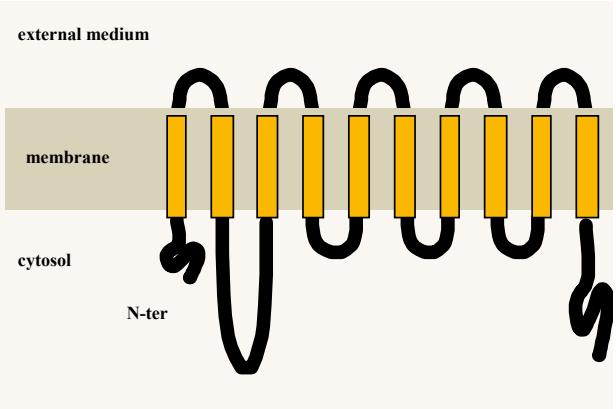

Fig. 3. Plant KUP/HAK/KT transporters were identified by sequence homology with K^+ uptake transporters from bacteria and high-affinity K^+ transporters from fungi. Based on hydrophobicity analysis, they would possess ten transmembrane segments (displayed in yellow) and a long cytosolic loop between the second and third segment. Based on genome sequence analysis, thirteen HAK genes are present in *Arabidopsis thaliana* and 26 in rice *(Oryza sativa)* (Mäser *et al.*, 2001; Amrutha *et al.*, 2007). Phylogenetic analyses indicate that four groups can be distinguished in the plant KUP/HAK/KT family (Bañuelos *et al.*, 2002).

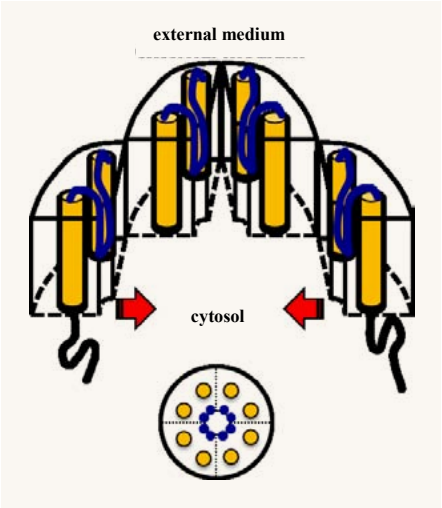

Fig. 4. The proposed topology of HKT transporters is that suggested for the whole (plant) HKT/(fungal and bacterial)Trk/(bacterial) KtrB superfamily. They display four successively arranged MPM domains, each one comprising a transmembrane segment (displayed in yellow), then a P domain (in blue), and another transmembrane segment (in yellow). The four P domains line a central pore.

also be noted that the size of the HKT family appears to be much smaller in dicots than in monocots: e.g. a single HKT gene in *Arabidopsis* or poplar, compared to ten genes in rice or each wheat genome (Corratgé-Faillie *et al.*, 2010).

HKT transporters, permeable to Na^+ only, have been shown to contribute to plant adaptation to salinity constraint. Expressed in the plant vasculature, the transporter's activity results in decreased Na^+ translocation from roots to leaves and reduced leaf Na^+ contents. Such contribution to plant tolerance to saline conditions has been evidenced both in dicots and monocots (Corratgé-Faillie *et al.*, 2010).

The role of HKT transporters permeable to both Na^+ and K^+ is still poorly understood and, thus, the physiological significance of the fact that such transporters are present in monocots only is unclear. HKT transporters from this type have been shown to be able to behave as Na^+-K^+ symporters when expressed in heterologous systems (Rubio *et al.*, 1995; Haro *et al.*, 2005) and could thus mediate active high affinity K^+ uptake. The fact that reduced availability of K^+ in the external (soil) solution results in increased expression of such HKT transporters in roots, provides further support to the hypothesis that these systems are involved in high affinity active K^+ uptake. However, all attempts to identify Na^+-K^+ symport activity in plants seem to have failed so far. Studies concerning the actual activity of these systems - and those relating to the difference in number and type of members in the HKT family between monocots and dicots - are thus highly exciting and at the forefront of scientific research.

KEA antiporters

Plant KEA (potassium exchange antiporters) are thought to function as H^+/K^+ antiporters, given their homology to proteins with this function in bacteria (Yao *et al.*, 1997). Six KEA are present in *Arabidopsis thaliana* (Mäser *et al.*, 2001), but their physiological role is so far largely unknown. In *E. coli*, KefB and KefC-mediated K^+ efflux is negatively regulated by glutathione. In the plant KEA1–3 transporters, the glutathione-binding pocket is not clearly conserved suggesting that plant KEA1–3 is regulated in a different way (Gierth and Mäser, 2007). It has been suggested that H^+/K^+ antiporters might play important roles at the plasma membrane by contributing to active K^+ secretion in the xylem sap, or regulating K^+ homeostasis by loading K^+ into vacuoles or other acidic compartments.

Root K^+ uptake from the soil solution

Information available in *Arabidopsis* identifies two systems involved in root K^+ uptake: the AKT1 Shaker channel, probably under control of the Shaker AtKC1 channel subunit and the AtHAK5 transporter (Fig. 5). Close homologs of AKT1 and AtHAK5 have been identified in other species, for instance in rice (Bañuelos *et al.*, 2002; Fuchs *et al.*, 2005; Amrutha *et al.*, 2007).

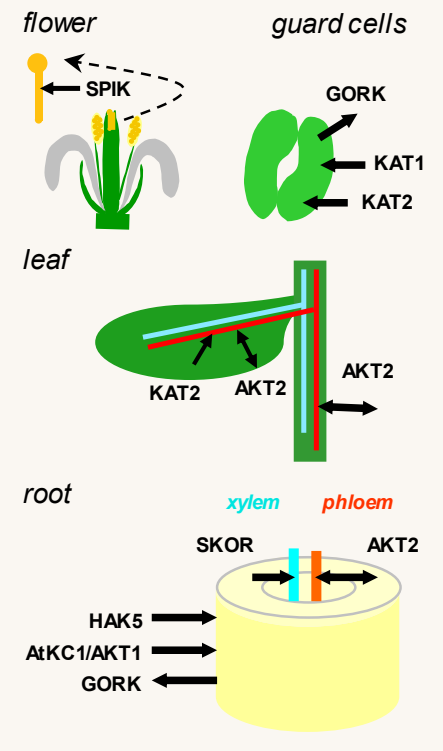

Fig. 5. Expression patterns and roles of K^+ channels and transporters in *Arabidopsis*.

Direct evidence for AKT1 contribution to K^+ uptake from the soil was obtained by using an *Arabidopsis* mutant line disrupted in the encoding gene (Hirsch *et al.*, 1998). Interestingly, AKT1 contribution to root K^+ uptake appeared to be essential for plant development only when NH_4^+ was present in the external medium. In the absence of NH_4^+, the AKT1 mutant does not display any phenotype while, in the presence of this cation, it displays decreased K^+ uptake capacity, impaired seed germination and reduced growth rate. These results led to the distinction of two components in root K^+ uptake activity, based on sensitivity to NH_4^+. The AKT1 component plays an essential role in root K^+ uptake, even from low K^+ media in the presence of NH_4^+. The non-AKT1 component is NH_4^+-sensitive, and can suffice for root K^+ uptake in the absence of NH_4^+.

K^+ transporters from the KUP/HAK family have been shown to be inhibited by external NH_4^+ (Santa María *et al.*, 2000). In *Arabidopsis*, AtHAK5 is expressed in the root epidermis and contributes to K^+ deprivation-induced high-affinity K^+ uptake (Gierth *et al.*, 2005). It could thus form part of the non-AKT1 component of K^+ uptake. It should also be mentioned that HKT transporters permeable to both K^+ and Na^+ (in monocots) are expressed in root periphery cells and could thus contribute to root K^+ uptake. However, as indicated above, the actual role of these systems is still unclear.

Long-distance transport of K^+ in plants

Reverse genetic approaches in *Arabidopsis* have revealed that activity of the Shaker K^+ channel SKOR, which is expressed in pericycle and xylem parenchyma, contributes to about 50 percent of K^+ translocation toward the shoot (Gaymard *et al.*, 1998) (Fig. 5). The other systems involved in K^+ loading into the xylem sap are still unknown.

Work on the mechanisms of K^+ transport in phloem tissues has been focused on two K^+ channels from the Shaker family, AKT2 and KAT2. KAT2 is an inward channel but AKT2 is able to mediate both K^+ uptake and K^+ secretion. This functional plasticity, and the fact that AKT2 is expressed in the phloem vasculature, both in leaves and roots, has led to the hypothesis that this channel plays a role in K^+ loading in source leaves and unloading in sink organs (Marten *et al.*, 1999; Lacombe *et al.*, 2000) (Fig. 5). At the transcriptional level, AKT2 displays CO_2-dependent light induction, suggesting that AKT2 expression in phloem tissues is regulated by photosynthates (Deeken *et al.*, 2000). Analysis of the phenotype of an AKT2 loss-of-function mutant revealed a delay in plant development and, interestingly, a 50 percent reduction in the sucrose content of phloem sap (Deeken *et al.*, 2002).

HKT transporters permeable to K^+ (and Na^+) are expressed in phloem tissues (Kader *et al.*, 2006) but their actual capacity to transport K^+ in situ in plant cells is not demonstrated, as indicated above. HKT transporters permeable to Na^+ only are expressed in plant vascular tissues and play a role in controlling/reducing Na^+ translocation towards the shoots and accumulation in leaves.

Pollen tube elongation

Although K^+ plays a major role in inducing cell turgor, only a few studies so far directly support the hypothesis that K^+ channels or transporters are involved in the control of cell growth. In *Arabidopsis*, disruption of the gene encoding the inward Shaker channel SPIK strongly impaired pollen tube development (Mouline *et al.*, 2002) (Fig. 5). SPIK mutant pollen germinated normally but elongation of the pollen tube most often aborted quickly. Tubes that succeeded in developing grew more slowly than in wild-type pollen. Since SPIK is a major component of the K^+ inward conductance in pollen, the impairment of tube development in the knock-out mutant is probably due to a deficit in K^+ uptake. This defect was shown to result in a strong decrease of pollen fitness and fertilization capacity.

K^+ fluxes in guard cells and control of stomatal aperture

Guard cell movements at the leaf surface, allowing regulation of stomatal aperture and control of water transpirational loss, are osmotically driven. An increase or decrease in turgor of the guard cells lining the pore, opens or closes the stoma respectively. K^+ and accompanying anions (NO_3^-, Cl^- and malate) are among the major solutes involved in this osmotically driven process (Talbot and Zeiger, 1996, Schroeder *et al.*, 2001). In summary, activation (by light or other signals) of H^+-excreting ATPases, active at the guard cell membrane, results in membrane hyperpolarization and thereby in activation of inward K^+ channels. This leads to K^+ influx, increased K^+ accumulation and, finally, stomatal opening. Conversely, inhibition (e.g. by the stress hormone ABA) of H^+-excreting ATPases results in membrane depolarization (further supported by activation of anion channels), leading to activation of outward K^+ channels, K^+ efflux and, finally, stomatal closure. In *Arabidopsis*, the time-constant of stomatal closure and opening are close to about ten and twenty minutes, respectively.

A single Shaker gene, GORK, encodes the outward K^+ channels active at the cell membrane in *Arabidopsis*, while five genes (KAT1, KAT2, AKT1, AKT2 and AtKC1) code for the inward channels (Fig. 5). This suggests that tight control of K^+ influx during stomatal opening is more complex and crucial than control of K^+ efflux during stomatal closure. Disruption of guard cell outward or inward K^+ channel activity (by reverse genetics approaches in *Arabidopsis*) has been shown to increase the time-constant of stomatal closure or opening by about 50 percent and 400 percent respectively (Hosy *et al.*, 2003). As expected, disruption of the outward channel activity results in increased plant transpirational loss. Disruption of the inward K^+ channel activity results in strongly reduced reactivity to fluctuations in environmental conditions, like changes in relative humidity or in internal CO_2 availability, as well as in impaired control of stomatal movements in the presence of Na^+ (even at physiological concentrations) (Lebaudy *et al.*, 2008).

Conclusion and perspectives

Considerable progress in the analysis of K^+ transport across the cell membrane in plants has been made since the initial cloning of the Shaker K^+ channels (AKT1 and KAT1) from *Arabidopsis thaliana* in 1992. Several families of K^+ channels and transporters have been identified, revealing a much more complex situation than initially thought. At the present time, the Shaker K^+ channel family has been extensively characterized, highlighting the roles of members from this family in functions such as root K^+ uptake, and K^+ secretion into the xylem sap towards the shoots or guard cell movements. Much less is known, however, about other families of K^+ transport systems. Obtaining an integrated view of K^+ transport in plants will require more effort in the analysis of functional properties and roles *in planta* of transporters from the HAK, HKT and KEA families, as well as systems active at the tonoplast, such as TPK channels.

Most studies have focused on *Arabidopsis thaliana* as a model plant but information has also been gained in other species such as rice or grapevine. In the light of known available data, knowledge obtained on the Shaker family in *Arabidopsis* is likely to be significant in helping to investigate the roles of members of

this family in other species. This is particularly true as the Shaker family appears to be strongly conserved in plants, in terms of gene number, channel functional properties, as well as channel expression patterns, which are probably more complex than in other families. For instance, the HKT transporter family is very different, in terms of transporter number and functional subtypes, between monocots and dicots. Understanding the physiological meaning of such differences is a highly exciting and fundamental objective in analyzing K^+ transport in plants, especially in relation to plant tolerance to salinity.

Another major objective for future researches is to decipher the processes, at the gene or protein levels for instance, that contribute to controlling and integrating the activities of the various K^+ transport systems that the plant can express. For instance, K^+ deficiency or salinity constraint has been shown to strongly modulate the expression (transcript levels) of members from the Shaker, HAK or HKT families. At the protein level, a highly illustrative example is the identification of a CIP-Kinase and its two CBL partners that place K^+ channel activity involved in root K^+ uptake under control of cytosolic Ca^{2+} signals (Xu *et al.*, 2006).

In conclusion, although the Shaker K^+ channel family can be considered the best characterized family of nutrient ion transport systems across the plant cell membrane, important efforts are still to be made to enable further progress in this field of plant biology and to obtain an integrated view of the mechanisms and regulation of K^+ transport activities in plants. Such efforts, however, are clearly worthwhile because of the current status of the knowledge in this field, the available working hypotheses and exciting perspectives. The work is also worthwhile because of the importance of K^+ nutrition, both in terms of plant adaptation to biotic and abiotic stresses, including salinity, and biomass production.

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