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Minireview

K^+ channel activity in plants: Genes, regulations and functions

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Abstract Potassium (K^+) is the most abundant cation in the cytosol, and plant growth requires that large amounts of K^+ are transported from the soil to the growing organs. K^+ uptake and fluxes within the plant are mediated by several families of transporters and channels. Here, we describe the different families of K^+-selective channels that have been identified in plants, the so-called Shaker, TPK and Kir-like channels, and what is known so far on their regulations and physiological functions in the plant.

Keywords: Plant potassium channels; Shaker; TPK; Kir-like

1. Introduction

Potassium (K^+) can comprise up to 10% of the total plant dry weight. It is the most abundant cation in the cytosol as it is compatible with protein structure even at high concentrations. The cell uses this cation in several important functions, such as electrical neutralization of anionic groups, control of membrane polarization and osmoregulation. Concerning the latter function, K^+ uptake or release is the usual way through which the cell controls its water potential and turgor. This function has been extended, very early in plant evolution, from a purely structural role to a motor function underpinning cell extension and movements. For instance, in the phloem vasculature, control of K^+ transport plays a role in building the osmotic gradient which drives the sugar sap flow from mature photosynthesizing leaves to sink tissues. Similarly, in the xylem vasculature, the so-called root pressure, which involves K^+ secretion into the vessels, drives the crude sap flow from roots to shoots in absence of transpiration. Also, potassium plays a role in tropisms and nasties, or specialized movements such as guard cell movements which allow the plant to regulate the aperture of the stomatal pores present at the leaf surface.

Physiological analyses indicate that a large set of transport systems, differing in their affinity for K^+, selectivity and energetic coupling, is involved in K^+ uptake from the soil, translocation and compartmentalization. At the molecular level, at least 35 genes code for K^+ transport systems in the model plant *Arabidopsis thaliana* [1]. They form 3 families of channels and 3 families of transporters, for a total of 15 and 20 genes, respectively. The present review focuses on K^+ channels.

2. Three families of K^+-selective channels identified in plants

K^+ channels are multimeric proteins. Their transmembrane subunits, named α-subunits, are characterized by the presence of either one or two pore (P) domains. In the functional multimeric protein, four P domains are associated to form part of the channel conduction pathway, including its selectivity filter. A hallmark motif GYGD/E is present in P domains of highly selective K^+ channels.

Three families of α-subunits forming selective K^+ channels have been identified in plants [2]. They are named in this review Shaker, TPK and Kir-like. They all have counterparts in animal cells. The name Shaker comes from that of the first member of this family, initially cloned in *Drosophila*. Members from the Shaker family were the first plant K^+ channels identified at the molecular level. This occurred in 1992, with the cloning of two inward *Shakers* from *Arabidopsis* by functional complementation of yeast mutant strains defective for K^+ uptake [3–5]. TPK channels (for Tandem-Pore K^+ Channels) are the plant counterparts of animal TWIK/TREK channels. The first plant *TPK* member was identified in 1997 by in silico searches, taking advantage of the *Arabidopsis* genome sequencing program [6]. Plant Kir-like channels are related to animal Kir (K^+ inward rectifier) channels. The first Kir-like channel was identified in *Arabidopsis* by searching for TPK1 related sequences in genome sequence databases [7]. The topology of these three types of α-subunits is depicted in Fig. 1. Shaker α-subunits display six transmembrane segments (TMS) and one P domain, present between the 5th and the 6th TMS. TPK α-subunits (formerly KCO#1, 2, 4, 5 and 6 in *Arabidopsis*) display a hydrophobic core composed of 4 TMS and 2 P domains in tandem. Kir-like subunits (formerly KCO3 in *Arabidopsis*) display 2 TMS and 1 P domain [7]. A functional channel should be built of 2 (TPK) or 4 (Shaker and Kir-like) α-subunits since 4 P domains are required for the formation of the pore. Major advances in elucidating the structure-function relationship of K^+ channels were the determination of the three-dimensional structure of a bacterial K^+ channel from the 2TMS-1P type [8] and that of an animal K^+ channel from the Shaker type [9].

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Abbreviation: ABA, abscisic acid

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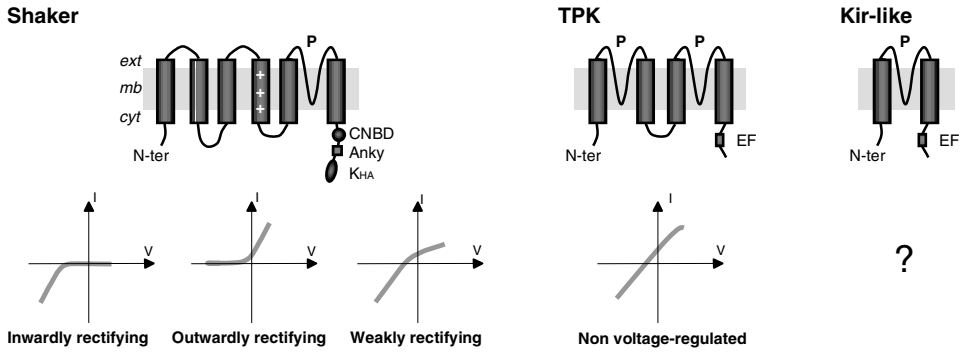


Fig. 1. Topology and functional types of plant K^+-selective channels. Three families of K^+-selective channels have been identified in plants: the Shaker (by analogy with the animal Shaker family), TPK (its animal counterpart is named KCNK), and Kir-like (by analogy with the animal Kir family). The secondary structures proposed for the plant channels are those currently given for their animal counterparts. Current–voltage (*I–V*) relationships illustrate the functional types encountered in the plant K^+ channel families. Channels forming inwardly-rectifying, outwardly-rectifying or weakly inwardly-rectifying conductances are found in the Shaker family. Only two TPK channels, AtTPK1 (Maathuis, F.J.M., personal communication) and AtTPK4 [33], have been functionally characterized so far. They have been shown to form non-voltage-regulated, leak-like, conductances. Abbreviations: ext/cyt, extracellular/cytoplasmic side; mb, membrane; P, CNBD, Anky, KHA, EF, respectively, pore domain, putative cyclic nucleotide-binding domain, ankyrin domain, domain rich in hydrophobic and acidic residues, and EF hand domains; +++, positively charged amino-acids in the channel voltage sensor.

The multimeric structure of the functional protein can potentially give rise to formation of heteromeric channels, associating α-subunits encoded by different genes, a process likely to lead to increased diversity in channel functional properties and regulation process. Heteromeric assembly does occur in the Shaker family [10] (see below), but probably not in the TPK and Kir-like families [11]. There is so far no evidence that α-subunits from different families could associate and form functional channels.

The present review focuses on Shaker, TPK and Kir-like channels (K^+-selective channels). Information on K^+-permeable poorly selective cation channels, such as TPC1 in *Arabidopsis* or the so-called Cyclic Nucleotide Gated Channels (CNGC), can be found in recent reports and other reviews [12,13].

3. Subcellular localization and trafficking

Integrated approaches developed in *Arabidopsis*, including subcellular localization of GFP constructs and electophysiological analyses on wild-type and mutant (KO) plants, support the hypothesis that all Shaker genes and one member of the TPK family, named *TPK4*, code for channels that are active at the plasmalemma. Kir-like channels and TPK channels other than TPK4 would be active at the tonoplast since they are found to be associated with this membrane when they are fused with GFP [11,14]. The molecular nature of K^+ channels active in other membrane systems is still unknown [15–17].

During stomatal movement, the variations in guard cell surface area that result from the changes in guard cell turgor [18] involve addition or retrieval of vesicular membrane into or from plasma membrane [19] carrying active inward and outward K^+ channels [20]. Electrophysiological analyses (of membrane capacitance and conductance) coupled to confocal microscopy (on guard cell protoplasts expressing the *Arabidopsis* Shaker channel KAT1 fused to GFP protein) indicate that exocitosis vesicles deliver clusters of K^+ channels to the plasma membrane during stomatal opening [21]. Upon stomatal closure, channels can be retrieved from plasma membrane via endocytosis even against high turgor [21,22]. Direct evidence has been obtained that SNARE proteins play a major role in trafficking of the inward Shaker channel KAT1 and in its distribution and anchoring within microdomains of plasma membrane [23]. A di-acidic motif DXE/DXD located in the C-terminal region of KAT1, downstream the channel hydrophobic core, in the putative cyclic nucleotide binding domain, has been shown to be involved in export of the functional channel from ER [24].

4. Channel functional types

4.1. Tools for electrophysiological analyses

Information on channel ionic selectivity, rectification and sensitivity to voltage, pH, K^+ or Ca^{2+} is a prerequisite to understand the physiological functions fulfilled by a given channel in the plant. In the case of channels active at the plasma membrane, such information has been gained, most often so far, by electrophysiological analyses after heterologous expression in animal systems. *Xenopus* oocytes provide a straightforward system for this purpose but a significant proportion of plant K^+ channels seems to be not functional when expressed in this system. For example, 4 from the 9 *Arabidopsis* Shaker genes do not produce any functional channel when directly expressed in *Xenopus* oocytes. In such cases, transfected insect or mammal cells (*Sf*9, COS, CHO or HEK cell cultures) can be used as an alternative to *Xenopus* oocytes. This has allowed to characterize, for example, 2 from the 4 *Arabidopsis* Shakers that are not functional in oocytes, AKT1 [25] and SPIK [26]. For AKT1, the reasons for the absence of activity in *Xenopus* oocytes have been recently made clear, in a pioneering study aiming at identifying regulators of K^+ nutrition in *Arabidopsis*. A network of interacting proteins, comprising a protein kinase from the CIPK family and 2 calcineurin B-like proteins, was shown to regulate AKT1 activity in *Arabidopsis* roots [27] (see below). Incidentally, this study revealed that lack of proper phosphorylation is responsible for the absence of activity of AKT1 when this channel is expressed alone in oocytes. It is however worth to know that the reasons why

some channel DNAs do not generate functional proteins in a given heterologous system cannot be anticipated. In this context, plant expression systems suitable for electrophysiological analyses have been developed [21,28,29]. Although they are less convenient than the oocyte system, they now appear as actually providing valuable ways for combining functional analyses and subcellular localization studies. They also offer a more physiologically relevant cellular environment than classically used animal expression systems [21,28–30].

4.2. Channel sensitivity to voltage

The activity of a given channel can be dependent on, or insensitive to, membrane polarization. Shaker channels are voltage sensitive. The 4th TMS of the α-subunit hydrophobic core bears basic amino acid residues (R and K) which allow it to act as a voltage sensor. Its movements in response to changes in transmembrane potential are thought to result in conformational changes in the channel protein, leading to its activation (opening of the aqueous pore favored). When individually expressed in heterologous systems, most plant Shaker α-subunits form channels that are strongly voltage sensitive (Fig. 1). Some are activated by membrane hyperpolarization and others by membrane depolarization (i.e., inwardly- and outwardly-rectifying channels, respectively). For instance, in *Arabidopsis*, 6 from the 9 Shaker members are involved in formation of hyperpolarization activated channels, and 2 in formation of depolarization activated channels. The last one, named AKT2, gives rise to weakly inwardly-rectifying currents [31,32]. In silico analyses of plant TPK and Kir-like α-subunits do not reveal any TMS that might be expected to behave as voltage sensor. Consistently, two members from the TPK family have been characterized so far (in *Arabidopsis*) and they have been found to be poorly sensitive to voltage ([33] and Maathuis, F.J.M., personal communication) (Fig. 1).

4.3. Major K^+ conductances characterized in planta and their encoding genes

In most tissues and cell types (root cortex, root hairs, xylem parenchyma, guard cells, mesophyll, pollen ...), voltage-gated highly selective K^+ conductances dominate the plasma membrane permeability to K^+ upon membrane hyperpolarization or depolarization, at millimolar K^+ concentrations [34–39]. Based on their sensitivity to voltage, these conductances can be sorted into two types, named IRK and ORK (for inwardly and outwardly-rectifying K^+ conductance, respectively). The first one is activated by membrane hyperpolarization and allows K^+ uptake into the cell, while the other one is activated by membrane depolarization and is involved in K^+ secretion. It is very likely that, in most tissues, the IRK and ORK conductances are encoded by Shaker channel genes. Direct support to this hypothesis has been provided by genetics analyses in *Arabidopsis* for several tissues/cell types, e.g., root cortex, mesophyll, guard cell or pollen [2]. A voltage-insensitive or weakly sensitive background conductance has been shown to be active at the plasma membrane in some cell types, contributing along with the IRK and ORK conductances to the membrane permeability to K^+. In *Arabidopsis* mesophyll cells, this background conductance might be formed by AKT2 Shaker α-subunits [40]. In pollen, TKP α-subunits encoded by *AtTPK4* gene contribute to the background conductance [33].

Regarding K^+ channels active in the tonoplast, patch clamp analyses on vacuoles have revealed three major types of cation conductance, named FV, SV and VK, for Fast Vacuolar, Slow Vacuolar and Vacuolar K^+, respectively. FV and SV channels are not within the main focus of this review since they are not characterized as K^+ selective. Briefly, both FV and SV conductances mediate currents carried by different cations, K^+, Ca^{2+} and Na^+. FV channels are inhibited by cytosolic Ca^{2+} concentrations higher than 0.1 μM [41], whereas SV channels are inactive at low Ca^{2+} concentrations and activated at Ca^{2+} concentrations higher than 0.5 μM [42,43]. FV channels have not been associated with any gene so far and their roles in the plant are still largely unknown. They have been suggested to play a role in vacuolar K^+ release at resting cytosolic Ca^{2+} concentrations, e.g. upon Ca^{2+}-independent abscisic acid (ABA) mediated stomatal closure [44,45]. SV channels are ubiquitous in vacuoles and dominate the tonoplast conductance at μM cytosolic Ca^{2+} concentrations. Their existence has recently been shown to require expression of the two pore cation channel gene *TPC1* (in *Arabidopsis*). The SV conductance is likely to play a role in vacuolar Ca^{2+} release and thereby in Ca^{2+} signaling events that are however still rather poorly understood [13]. It has also been suggested to play a role in cation homeostasis and osmoregulation processes, e.g., in vacuolar K^+ release during stomatal closure [13,44]. However, this hypothesis is questioned by the recent finding that *tpc1* KO mutant plants do not seem to be impaired in stomatal closure [13]. Unlike FV and SV channels, VK channels are highly selective for K^+ [46]. They are non-rectifying and thus appear as leaks that can mediate K^+ release or uptake [43,47]. Evidence has been obtained that the *TPK1* gene (in *Arabidopsis*) codes for these channels. Vacuolar localization of TPK1 channels has been demonstrated by GFP imaging and biochemical analyses [11,14]. Also, these channels exhibited (when heterologously expressed in yeast) the hallmark properties of the vacuolar VK conductance in terms of activation properties, ion selectivity and Ca^{2+} sensitivity [46–48]. Finally, patch-clamp analyses using wild-type *Arabidopsis*, *tpk1* loss of function mutants and TPK1 overexpressors, confirmed that *TPK1* encodes the VK current. Phenotypic characterization of the three genotypes showed that ABA dependent stomatal closure and overall K^+ homeostasis are influenced by *TPK1* expression levels (Maathuis, F.J.M., personal communication).

5. Regulation of K^+ channel activity in planta

Various major functions at the cell or whole-plant levels, such as guard cell movements or cell growth and elongation (see below), involve regulation of K^+ channel activity. Molecular identification of α-subunits has opened the way for molecular analysis of these regulations. Most information available so far concerns the Shaker family. A large variety of processes, including sensitivity to voltage (see above), K^+ [49], pH [50] and Ca^{2+} [30], along with transcriptional and post-translational mechanisms [51] (for review, see [2]), contribute to tune K^+ channel activity in the plant. Progresses in identifying post-translational mechanisms are summarized below.

5.1. Shaker α-subunit heteromerization results in increased diversity in functional properties

Formation of heteromeric channels associating different α-subunits from the plant Shaker family was initially observed in heterologous systems (*Xenopus* oocytes). New types of

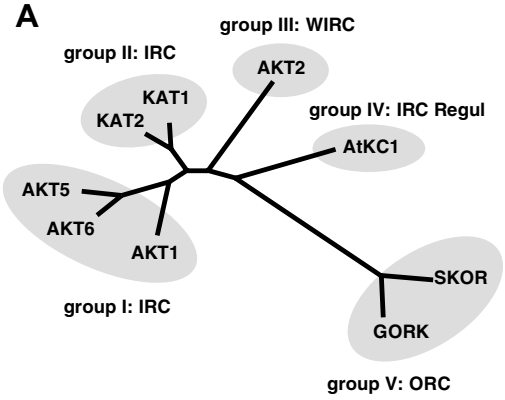
conductances, displaying unique features, not yet associated to any α-subunit when individually expressed, were observed upon co-expression of α-subunits [10,51]. Furthermore, mutated α-subunits displaying a dominant negative behavior (due to a mutation in the pore domain) were shown to functionally interact not only with the corresponding wild-type subunits but also with subunits encoded by other members of the family [52–54]. First evidence that heterotetramerization of Shaker α-subunits does occur in planta, giving rise to increased diversity in functional properties, was obtained in *Arabidopsis* root hairs. These cells express (at least) two Shaker genes, *AKT1* and *AtKC1*. Patch-clamp studies on root hair protoplasts revealed that, in absence of AKT1 (in mutant plants disrupted in *AKT1*), AtKC1 did not form inward channels whereas expression of AKT1 in absence of AtKC1 (in mutant plants disrupted in *AtKC1*) gave rise to such channels [55]. Interestingly, the latter channels differed from the inward K^+ channels observed in the presence of both AtKC1 and AKT1 (in wild-type plants), in terms of activation threshold and sensitivity to external pH and Ca^{2+}. Thus, the whole set of data indicated that AtKC1 formed with AKT1 functional heterotetrameric channels endowed with own functional features [55].

Based on sequence similarities, gene structure and functional analyses, the plant Shaker family can be divided into five groups (Fig. 2A). All Shaker subunits forming outward conductances belong to group V. The other four groups comprise α-subunits that can contribute to formation of inward conductances. Subunits forming strongly inwardly-rectifying conductances belong to groups I and II, and weakly inwardly-rectifying conductances to group III. Regulatory subunits of inward channels (see above for AtKC1) are found in group IV. Evidence is available that formation of heteromeric channels can occur between members of group I and II, I and III, I and IV, II and III, and III and IV [51,56,57] (Fig. 2B). Recently, association of α-subunits from group II and group III (KAT2 and AKT2 from *Arabidopsis*) has been shown to provide channels endowed with functional properties inherited from both α-subunit types, e.g., weak rectification inherited from the group III subunit associated to activation by external acidification, inherited from the group II subunit [58]. Preferential heteromerization would occur between these two subunits and, besides resulting in increased diversity in channel functional properties, the heteromerization process could contribute to regulation of channel trafficking and targeting to the cell membrane [58]. In conclusion, this survey indicates that a greater structural and functional diversity exists amongst inward than outward conductances in the cell membrane. It would be very interesting to understand the reasons for this difference.

Regarding the vacuolar K^+ channels, TPK α-subunits would not form heteromeric channels, based on results from FRET experiments and gene expression pattern analysis [11].

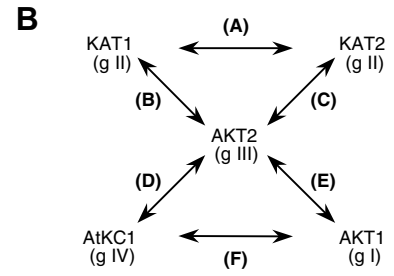
5.2. Regulatory proteins

Once the α-subunits are assembled, the resulting channels are the targets of signaling cascades leading to their activation or inactivation in response to different stimuli. Regulation of channel activity by interactions with protein partners is a highly active and fruitful field of research in the animal field. Less is known in plant cells [51]. Furthermore, most studies



Channel type

IRC:	Inwardly rectifying conductance
WIRC:	Weakly inwardly rectifying conductance
ORC:	Outwardly rectifying conductance
IRC Regul:	Regulatory subunit involved in IRC formation



Interactions	References
(A)	[53]
(B)	[52] [57]
(C)	[58]
(D)	[56]
(E)	[56]
(F)	[56] [57]

Fig. 2. Phylogenetic groups and physical interactions between subunits within the *Arabidopsis* Shaker family. (A) Phylogenetic tree based on amino acid sequence analysis distinguishing five groups. Group I and group II contain channels forming inwardly-rectifying conductances, group III contains one channel forming weakly inwardly-rectifying conductance, group IV contains one regulatory subunit and group V, channels forming outwardly-rectifying conductances. (B) Physical interactions between Shaker subunits revealed by yeast two hybrid tests (A), (B), (C), (D), (E), (F), split ubiquitin system (B), (E) or functional analyses (A), (B), (C), (F). Groups I, II, III, IV, V: gI, gII, gIII, gIV, gV, respectively.

do not establish whether the functional interaction involves physical interactions between the regulatory protein and the channel, or whether the effect is indirect, the actual target being a protein that interacts with the channel through more or less complex networks. The available information indicates however that basic types of regulation are conserved between plants and animals [51]. For example, conserved actors in the plant and animal regulatory networks are β-subunits [59,60], actin skeleton proteins [61], G proteins [35,62,63], syntaxins [23,64], 14.3.3 proteins [65,66], kinases and phosphatases [67–69]. As summarized below, evidence for direct interactions with the target channel has been obtained in four cases: for β-subunits [59] and 14.3.3 proteins with Shaker KAT1 [66], a phosphatase (AtPP2CA) with Shaker AKT2 [70,71], and a kinase (CIPK23) with Shaker AKT1 [27,72].

In animal cells, oxidoreductases named β-subunits can associate with cytoplasmic regions of K^+ Shaker α-subunits to form macromolecular complexes. These auxiliary subunits affect the channel activity. Homologues of these proteins have been identified in plants and shown to actually interact with plant Shaker channels. Co-expression of the *Arabidopsis* KAB1 β-subunit with KAT1 in *Xenopus* oocytes resulted in increased current levels with no change in gating properties [60], suggesting that the interaction would stabilize the channel in plasma membrane. Intriguingly, Shaker channels would not be the single targets of the β-subunits in the cell since immuno-cytochemical analyses indicate that KAB1 is present in different membrane types, plasma membrane, tonoplast, chloroplast inner membrane, mitochondrial inner envelope.

14-3-3 proteins are involved in control of ion transport across plasma and vacuolar membrane through interaction with H^+-ATPase and V-ATPase [73–75]. They have also been shown to functionally interact with SV conductances [76] and with Shaker-type outward and inward conductances in the plasma membrane [65,77]. Biochemical and electrophysiological evidence for direct and functional interactions between 14-3-3 proteins and the *Arabidopsis* KAT1 channel has recently been obtained, by using recombinant plant 14-3-3 proteins and *Xenopus* oocytes to express KAT1 [66]. The interaction resulted in increased KAT1 currents by inducing a positive shift in the voltage dependence of the channel [66].

The *Arabidopsis* phosphatase AtPP2CA, a close relative of the ABI1 phosphatase known to affect ABA-induced responses of guard cell K^+ currents [78], was initially identified as a potential partner of the Shaker channel AKT2 by yeast two-hybrid screens [70,71]. In vitro binding assays have provided further evidence for direct interaction between the two proteins [71]. The interaction involves the cytoplasmic C-terminal region of the channel and the C-terminal (catalytic) domain of the phosphatase. Co-expression of the two partners in *Xenopus* oocytes has revealed that their physical interactions result in functional interactions, modulating the channel activity. The level of AKT2 current was decreased upon co-expression with AtPP2CA, and the level of rectification increased. It has been suggested that AtPP2CA-mediated conversion of AKT2 from a leak-like conductance into an inwardly-rectifying one, plays a role in the regulation of the membrane potential [71].

Recently, a protein network comprising the two Ca^{2+} sensors CBL1 and CBL9 (for Calcineurin B-Like protein 1 and 9) from *Arabidopsis* and their target protein kinase CIPK23 (for CBL-Interacting Protein Kinase 23) has been shown to control the activity of the AKT1 K^+ channel and its contribution to K^+ uptake from the soil solution [27] (see below). CIPK23 is activated by the binding of CBL1 or CBL9 and directly phosphorylates AKT1 [27,72] (see below).

6. Physiological functions

6.1. Root K^+ uptake

The *Arabidopsis* Shaker gene *AKT1* [5] is expressed in root epidermis and cortex [79] and encodes α-subunits that can form homotetrameric inwardly-rectifying K^+ channel when expressed in heterologous systems [25]. Direct evidence for AKT1 contribution to K^+ channel activity in root periphery cells and K^+ uptake from the soil solution was obtained by using a mutant line disrupted in the encoding gene [80]. *AKT1* is required for expression of the dominant inward (voltage-gated) K^+ conductance of the plasma membrane in root periphery cells (Fig. 3). Surprisingly, this study revealed that *AKT1* dependent channel activity, supported by strongly hyperpolarized membrane potentials, can significantly contribute to root K^+ uptake even at low external K^+, in the concentration range corresponding to the high affinity mechanism in Epstein formalism. Another interesting finding was that AKT1 contribution to root K^+ uptake is essential for plant development only when NH_4^+ is present in the external medium. In absence of NH_4^+, the *akt1* mutant does not display any phenotype while, in the presence of this cation, it displays decreased ($^{86}Rb^+$)K^+ fluxes into roots, impaired seed germination and reduced growth rate. The whole set of data has led to distinguish 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 presence of NH_4^+. The non-AKT1 component is NH_4^+-sensitive, and can suffice for root K^+ uptake in absence of NH_4^+. K^+ transporters from the KUP/HAK family have been shown to be inhibited by external NH_4^+ [81]. In *Arabidopsis*, *AtHAK5* is expressed in root epidermis and contributes to K^+ deprivation-induced high-affinity K^+ uptake [82]. It could thus form part of the non-AKT1 component of K^+ uptake.

K^+ deprivation is known to enhance root K^+ transport capacity in *Arabidopsis* [82] like in other species [83,84]. At the functional level, both high-affinity K^+ uptake [82] and inward K^+ channel activity [85] have been reported to increase upon K^+ deprivation in *Arabidopsis*. At the molecular level, in this species, K^+ deprivation has been shown to result in up-regulation of genes encoding K^+ transporters [82–84] but does not affect the expression (transcript accumulation) of *AKT1* [56]. Thus, the increase in inward K^+ channel activity upon K^+ deprivation should mainly involve post-translational mechanisms.

The *Arabidopsis* Shaker gene *AtKC1* encodes a regulatory α-subunit that interacts with AKT1 α-subunits in root periphery cells (Fig. 3), giving rise to heteromeric channels with own functional features. In particular, the heteromeric channels display a more negative activation potential, by about −50 mV when compared with AKT1 homomeric channels [55]. It is tempting to speculate that this shift in activation threshold towards more negative values allows the cell to prevent AKT1 from mediating K^+ efflux at low external concentrations.

Search for mutant plants impaired in development at low external K^+ concentrations has recently revealed that the two calcineurin B-like protein CBL1 and CBL9 and their target

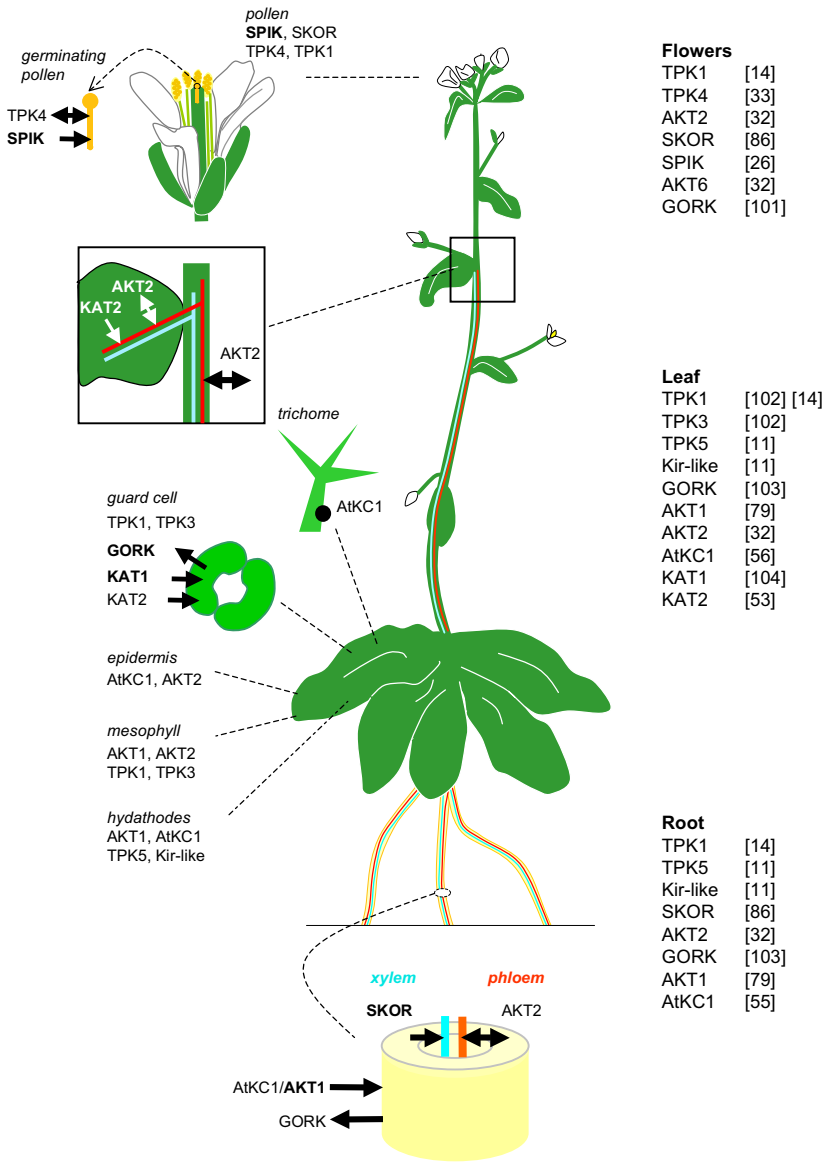


Fig. 3. Expression patterns of *Arabidopsis* K^+-selective channels. In bold are channels whose roles in planta have been determined based on complementary approaches, including localization at the tissue/cell level, functional characterization of K^+ transport activity in heterologous systems and reverse genetics analyses. In roots, AKT1 is involved in K^+ uptake from the soil solution [80] and the outward channel SKOR in K^+ secretion into the xylem sap [86]. In phloem, AKT2 has been proposed to be involved in both K^+ loading in sources and unloading in sinks. It would also control the membrane potential, favoring sugar loading in source leaves [31,32,91]. In guard cells, KAT1 plays a role in K^+ influx during stomatal opening [54,98], whereas GORK mediates most K^+ efflux during stomatal closure [97]. In pollen, SPIK mediates K^+ influx into the growing tube which plays a role in tube development [26]. See the references mentioned in the figure for more details.

protein kinase CIPK23 form a regulatory network targeting AKT1-dependent K^+ channel activity in roots. Evidence has been obtained that CIPK23 behaves as a positive regulator of the channel AKT1 and the two Ca^{2+} sensors CBL1 and CBL9 act as upstream regulators of CIPK23. Co-expression of the different partners in oocytes has provided support to the hypothesis that activation of AKT1 by CBL-CIPK is dependent on cytoplasmic Ca^{2+} concentration [72]. It has been suggested that ROS production in response to low K^+ environment results in Ca^{2+} signals in the cytosol. The Ca^{2+} sensors CBL1 and CBL9 would be consequently activated and interact with their target protein CIPK23, which in turn would phosphorylate AKT1. This would lead to increased K^+ channel activity, favoring K^+ uptake under low K^+ conditions [72]. The discovery of this CBL-CIPK pathway clearly provides stimulating working hypotheses regarding regulation of K^+ channel activity and more generally of membrane transport [27,72].

6.2. K^+ long distance transport

Direct evidence that channels contribute to K^+ loading of the xylem sap was obtained in 1998 by reverse genetics in *Arabidopsis*: activity of the outward Shaker SKOR, which is expressed in pericycle and xylem parenchyma, was shown to contribute to ~50% of K^+ translocation toward the shoots [86] (Fig. 3). SKOR activity is modulated by external K^+ due to K^+-dependent gating [87]. This regulation allows the channel to sense the extracellular K^+ concentration and to open

only when the driving force for net K^+ flux is directed outward. A major molecular determinant of the K^+-dependent gating resides in a region adjacent to the pore [87].

Concerning phloem K^+ transport, two *Shaker* subunits have been localized in phloem tissues in *Arabidopsis* based on *GUS* reporter gene analyses, *AKT2* and *KAT2* (Fig. 3). AKT2, as homotetramer, forms a weakly inwardly-rectifying K^+ conductance, whose level of rectification is controlled by phosphorylation [71,88]. This functional plasticity and the fact that *AKT2* is expressed in the phloem vasculature both in leaves and roots have led to the hypothesis that AKT2 plays a role both in K^+ loading in source leaves and unloading in sink organs [31,32] (Fig. 3). *KAT2* is expressed in the phloem vasculature of the leaf only [53]. When expressed alone, KAT2 α-subunits form inwardly-rectifying channels, suggesting a role in K^+ loading in source leaves. Recent experiments performed heterologously have indicated that AKT2 and KAT2 subunits can interact to form heteromeric channels with own functional properties [58]. Heteromeric assembly appeared to be preferential. Heteromers were endowed with weak inward rectification properties, a feature inherited from the AKT2 subunit. Intriguingly, at variance with the data obtained using *GUS* reporter genes, Q-RT-PCR experiments on *Arabidopsis* leaf phloem companion cells have revealed low levels of *KAT2* transcripts and high ones of *KAT1* and *AKT2* [89]. Interestingly, the K^+ inward conductance identified in these cells [89] is different from what has been shown in oocytes for KAT1 and KAT2 (in terms of pH sensitivity), AKT2 (in terms of rectification and unitary conductance) and heteromers AKT2-KAT2 (in terms of rectification and pH sensitivity). It could correspond to heteromeric AKT2-KAT1 channels [52].

At the transcriptional level, *AKT2* displays CO_2-dependent light induction, suggesting that *AKT2* transcription is regulated by photosynthates [90]. Analysis of the phenotype of an AKT2 loss-of-function mutant showed a slight delay in plant development and, interestingly, a 50% reduction in the sucrose content of the phloem sap [91]. Based on simulations in oocytes AKT2 and a sucrose transporter, it has been proposed that AKT2 indirectly controls sugar loading of the phloem sap by controlling the phloem electrical potential [91].

Large quantities of K^+ are recirculated from the shoots to the roots via the phloem, and subsequently returned to the shoots via the xylem [92]. The magnitude of the K^+ flux recirculated from the shoots to the roots would constitute a message by which the growing shoots would communicate to the roots their K^+ requirement and regulate K^+ secretion into the xylem sap (and, finally, root K^+ uptake) [92]. Physiological studies have also provided evidence that K^+ secretion into the xylem sap is under hormonal control. Information at the molecular level on the mechanisms underlying this complex network of interactions is now available. *SKOR* transcript accumulation has been found to be decreased upon K^+ starvation [56], providing evidence that the root K^+ status regulate K^+ xylem transport via SKOR. Also, based on experiments in oocytes, low cytosolic K^+ could decrease SKOR channel activity [93], but this result is controversial [87]. Concerning hormonal control, treatments with auxin or cytokinins (which would tend to promote root growth) quickly and strongly decreased *SKOR* transcript accumulation [56]. ABA decreased the expression of *SKOR* in roots and, simultaneously, increased that of *AKT2* in shoots. This information is consistent with the hypothesis that regulation of AKT2 and SKOR channel activity upon K^+ starvation or drought stress results in decreased K^+ secretion into the xylem sap and increased K^+ recirculation in the phloem vasculature, favoring K^+ accumulation in roots, osmotic adjustment and finally root growth.

6.3. Cell growth

Although K^+ has a major role in building plant cell turgor, only few studies so far directly support the hypothesis that K^+ channels are involved in the control of cell growth. In pollen in *Arabidopsis*, disruption of the inward Shaker *SPIK* strongly impaired the pollen tube development [26] (Fig. 3). The knock-out pollen germinated normally but elongation of the pollen tube most often aborted quickly. Tubes that succeeded to develop grew more slowly than in wild-type pollen, whatever the external K^+ concentration in the range 5 μM–1 mM. 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. The effect of the disruption of another K^+ channel gene, *AtTPK4*, was also investigated in pollen. *AtTPK4* encodes a non-voltage-regulated K^+ channel active at the pollen membrane [33] (Fig. 3). In contrast to the disruption of *SPIK*, that of *AtTPK4* had no effect on pollen tube development [33].

Plant tumours can be induced by *Agrobacterium tumefaciens*. Integration of the bacterial oncogenes results in important biosynthesis of auxin and cytokinins which induces plant cell proliferation. Evidence that K^+ uptake through the inward Shaker AKT1 plays an important role in tumour cell proliferation has been obtained [94]. K^+ channel gene expression profile was compared between rapidly growing tumour cells and non-infected leaf tissues. Little difference was observed in *TPK* channel expression. In contrast, the level of expression of several *Shaker* channels changed: transcript accumulation of the inward rectifier *AKT1* strongly increased, that of the weak rectifier *AKT2* and the outward rectifier *GORK* decreased. In the *AKT1* knock-out mutant, tumour development was shown to be reduced.

Finally, correlation between growth peaks or auxin production and elevated expression of inward Shakers has been reported in a few studies, e.g., in maize coleoptile during the gravitropic response [95], and in *Arabidopsis* etiolated hypocotyls [96].

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

K^+ channels allowing rapid fluxes of this cation across the cell membrane and tonoplast are masterpieces of the molecular machinery that enables the guard cell osmocontractility and regulation of stomatal aperture. Most information available so far at the molecular level concerns channel activity at the cell membrane. However, characterization of vacuolar K^+ channels is in progress, as indicated above (see paragraph "Major K^+ conductances characterized in planta and their encoding genes").

Shaker K^+ channels dominate the guard cell membrane conductance. A single Shaker gene in *Arabidopsis*, *GORK*, encodes the outward conductance [97] (Fig. 3). Disruption of this gene resulted in impaired stomatal closure in response to darkness or ABA, both in terms of kinetics and steady states. Consistently, transpirational water loss was more important in *gork* mutant than in wild-type plants, from 10% up to 50%, depending on the soil water availability, the disadvantage of the

mutants being more important upon water stress [97]. Concerning the inward K^+ conductance, RT-PCR experiments indicate that *Arabidopsis* guard cells express at least five genes coding for Shaker α-subunits involved in formation of inward channels, *KAT1*, *KAT2*, *AKT1*, *AKT2* and *AtKC1* [98] (Fig. 3). Thus, regulation of the guard cell inward conductance might involve a larger set of mechanisms than that of the outward conductance. Quantitative RT-PCR data indicate that the levels of *KAT1* transcripts would be about 3 times higher than those of *KAT2* and *AKT1*, and more than 10 times higher than those of *AKT2* and *AtKC1* [98]. Consistently, disruption of the *KAT1* gene has been found to cause a strong reduction, by more than 50%, in the inward K^+ conductance (electrophysiological recordings performed on guard cell protoplasts) [98]. Such an important decrease was however without any significant effect on stomatal movements (steady-state aperture under light, and kinetics of CO_2 and ABA induced movements) [98]. Absence of defect in stomatal opening (steady-state aperture under light) was also observed in transgenic plants overexpressing a *kat1* dominant negative construct and displaying a reduction in guard cell inward conductance by about 60% [54]. Interestingly, a stronger reduction in inward conductance, by about 70–80%, observed in a few transgenic lines, was found to affect steady-state stomatal aperture [54]. Collectively, these data provide support to the early hypothesis that inward K^+ channel activity in guard cells is much larger than what would be predicted to be sufficient for stomatal opening [99]. Regulation of inward K^+ channel activity by ABA, pH, or Ca^{2+} has been discussed within the framework of this hypothesis [54]. Ca^{2+} sensitivity of the inward conductance has been recently shown to be provided by AKT2 α-subunits, probably via formation of heteromeric channels [100].

7. Conclusion

Characterization of loss-of-function *Arabidopsis* mutants has allowed considerable progress during the last few years in our understanding of the roles of K^+ channels in plant physiology. An exciting backdrop of information is now available regarding K^+ channels active at the plasma membrane and involved in root K^+ uptake and long distance transport, cell elongation, or K^+ transport in guard cells during stomatal movements. Identification of K^+ channels active at the tonoplast is in progress. Furthermore, a new field of research has been opened, aiming at identifying regulatory mechanisms, channel protein partners and interacting networks. A highly illustrative example in this domain 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. It is clear that the identification of such regulatory mechanisms constitutes a major objective for the coming years.

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