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Topical Review

Properties of *Shaker*-type Potassium Channels in Higher Plants

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Abstract. Potassium (K^+), the most abundant cation in biological organisms, plays a crucial role in the survival and development of plant cells, modulation of basic mechanisms such as enzyme activity, electrical membrane potentials, plant turgor and cellular homeostasis. Due to the absence of a Na^+/K^+ exchanger, which widely exists in animal cells, K^+ channels and some type of K^+ transporters function as K^+ uptake systems in plants. Plant voltage-dependent K^+ channels, which display striking topological and functional similarities with the voltage-dependent six-transmembrane segment animal *Shaker*-type K^+ channels, have been found to play an important role in the plasma membrane of a variety of tissues and organs in higher plants. Outward-rectifying, inward-rectifying and weakly-rectifying K^+ channels have been identified and play a crucial role in K^+ homeostasis in plant cells. To adapt to the environmental conditions, plants must take advantage of the large variety of *Shaker*-type K^+ channels naturally present in the plant kingdom. This review summarizes the extensive data on the structure, function, membrane topogenesis, heteromerization, expression, localization, physiological roles and modulation of *Shaker*-type K^+ channels from various plant species. The accumulated results also help in understanding the similarities and differences in the properties of *Shaker*-type K^+ channels in plants in comparison to those of *Shaker* channels in animals and bacteria.

Key words: Ion channels — Shaker — Potassium — Channel topology — Plant cells
Abbreviations: ABA: abscisic acid; BA: benzyladenine; CDS: Coding Sequence; cNBD: cyclic nucleotide binding domain; 2,4-D: 2,4-dichlorophenoxyacetic acid; EAG: *ether a gogo*;

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Introduction

Among macronutrients, potassium (K^+) is the most important and abundant cation present in concentrations that may reach up to 10% of the plant dry weight. Potassium plays a crucial role in a series of basic mechanisms like enzyme-activity regulation and tuning of electrochemical membrane potential, which in turn controls several important physiological processes such as cell homeostasis, turgor adjustments, cell elongation, stomata and leaf movements. To fulfil these activities, potassium needs to be absorbed by the roots and readily transferred against its concentration gradient, which typically is much larger in the cytoplasm (100–200 mM) with respect to the soil (1–10 μM); then, potassium ions must be distributed to different plant aerial tissues. Ion transport is accomplished by various types of membrane transport systems, which allow the ions to selectively permeate according to their electrochemical gradients or at the expense of other energy sources (Epstein, Rains & Elzam 1963). To understand the mechanisms regulating K^+ distribution mediated by voltage–dependent channels, a large amount of data on plant K^+ channels has been accumulated, which helps to reveal the limiting steps in plant growth with a future prospect to improve crop productivity even under less favorable growth conditions.

This review focuses on plant potassium channels related to the *Shaker* superfamily initially identified in *Drosophila melanogaster* (Tempel et al., 1987; Lainè, Papazian & Roux, 2004). Cellular localization and comparison of the functional, biophysical and topological characteristics of the plant *Shaker*-type K^+ channels demonstrate that in vivo they are expressed at the plasma membrane, where they are activated by hyperpolarizing and depolarizing potentials (Schroeder, Raschke & Neher, 1987), dominating potassium fluxes (inward and outward of the cell) at millimolar K^+ concentrations in the many cell types

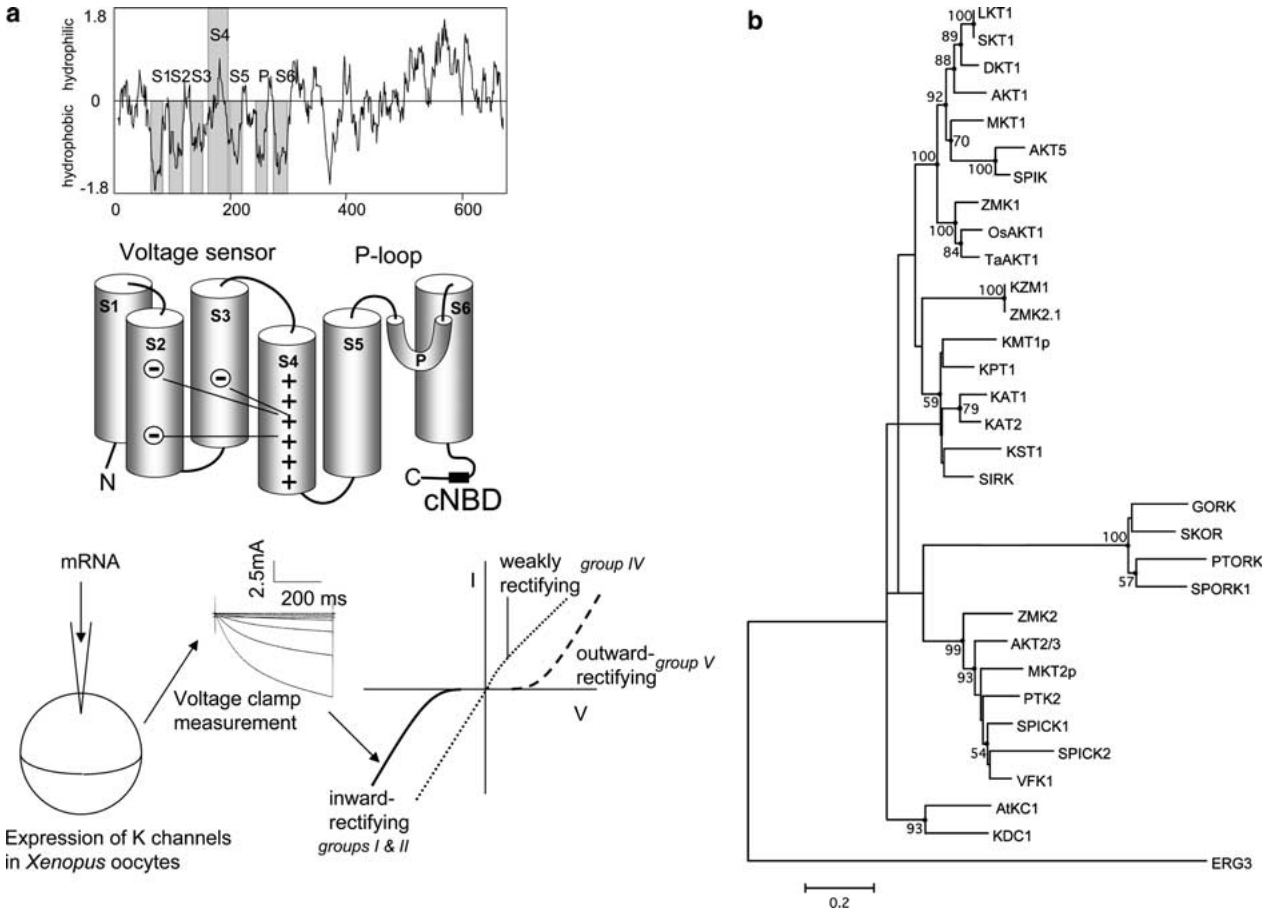

Fig. 1. (*a*) *Upper panel*: Hydrophobicity profile of KAT1. S4 shows low hydrophobicity, while the pore (P) segment is highly hydrophobic. The N and C terminals present more hydrophilic characteristics. *Middle panel*: schematic representation of the six transmembrane segments, the P-loop segment and the cNBD characterizing all the *Shaker*-type K^+ channel subunits in higher plants. Lower panel: Interactions among the S4 and S2 and S3 segments controlling channel activations are also schematically represented. (*b*) Phylogenetic trees of plant *Shaker*-type K^+ channels. Phylogenetic relationships of the 31 channels illustrated in this review, based on protein analysis. Accession numbers used to construct the tree are reported in the first column of Table 1. The alignment was performed using Vector NTI Advance 9.0 Suite (Informax, North Bethesda, MD) and the phylogenetic tree was constructed by Treeview. Bootstrap and consensus tree calculations were performed using the DAMBE and MEGA programs. Bootstrap values > 50 of 100 replicates are reported near the dotted nodes of the protein phylogram rooted by sequence of the ERG3 *Drosophila melanogaster* channel (CAA29917) (Pilot et al., 2003b). The branch length is proportional to the evolutionary distance between the channels. Group I comprises inward-rectifying K^+ channels with ankyrin domains, which are mainly expressed in the roots. Group II comprises inward-rectifying K^+ channels without ankyrin domains; however, recently characterized clones displaying ankyrin domains were also included in this group (*see* Table 1, column 9, notes, for details); channels of this group are mostly expressed in the leaves and specifically in guard cells. Group III includes weakly-rectifying K^+ channels; they are widely distributed in plant tissues but are primarily present in the phloem. K^+ channels, which modulate other inward-rectfying *Shaker*-type K^+ channels, belong to group IV and were identified mainly in the roots. While this subfamily includes only two members, other putative channels supporting this category can be identified, for example, in the rice genome (BAB68056). Finally, outward-rectifying K^+ channels belonging to group V are expressed in a variety of tissues in the whole plant.

investigated so far (Very & Sentenac, 2002; Véry & Sentenac, 2003; Cherel, 2004). These voltage-dependent K^+ channels in plants display high sequence homology with animal *Shaker* channels and particularly with channels belonging to the *ether a gogo* (EAG) subfamily (for an exhaustive review on the phylogenetic relationships of *Shaker*-type K^+ channels *see* Pilot et al., 2003b). Each subunit possesses a six-segment (S1–S6) membrane-spanning topology (Uozumi et al., 1998) (Fig. 1*a*). Functional channels comprise four subunits, each one characterized by intrinsic voltage sensors, which are composed primarily of charged amino acid residues in S2, S3 and S4 segments of the proteins (Latorre et al., 2003; Cherel, 2004). A pore (P) segment, linking the S5 and S6 segments, also present in Ktr/Trk/HKT transporters (Durell & Guy, 1999; Durell et al., 1999; Kato et al., 2001), forms the outer portion of the pore and confers the potassium selectivity characteristics to the channel (Fig. 2); the four pore (P) loop segments (from the four subunits), arranged in a fourfold symmetry, protrude from the extracellular side

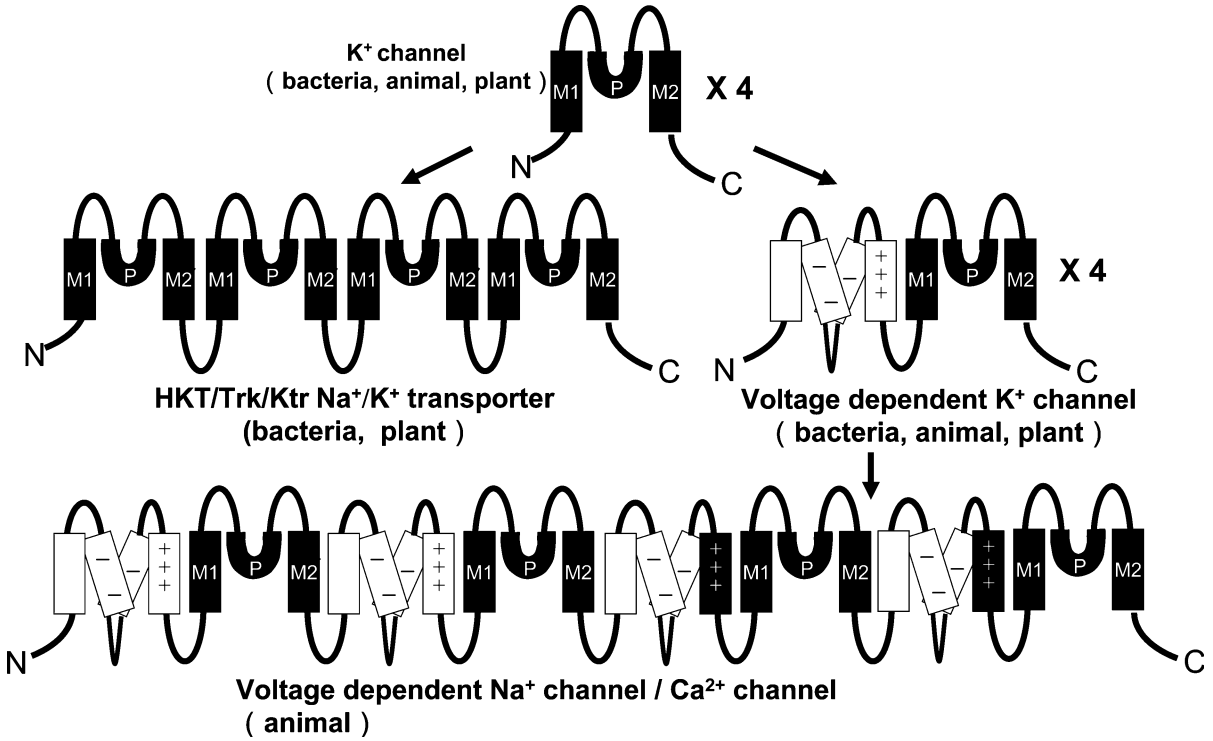

Fig. 2. Membrane structure of cation channels and cation transporters. Membrane-pore-membrane (MPM) structure forms the ion-conducting pore. The MPM is highly conserved among cation channels and transporters (HKT/Trk/Ktr-type) in prokaryotes and eukaryotes (Durell & Guy, 1999; Durell et al., 1999; Kato et al., 2001). The voltage-sensing region is fused to the N-terminus of MPM.

towards the membrane interior (Durell, Shrivastava & Guy, 2004; Shrivastava, Durell & Guy, 2004; Long, Campbell & Mackinnon, 2005a, b). The loop segment comprises the universal TXXTXGYG motif (Heginbotham, Abramson & MacKinnon, 1992; Hille, 2001), a hallmark for the majority of potassium-selective channels in plant and animal cells (Doyle et al., 1998), which is well conserved in the sequences of the plant *Shaker*-type K^+ channels illustrated in this review.

The closest relatives of the plant *Shaker*-type K^+ channels are the members of the animal EAG subfamily characterized by a cyclic nucleotide binding domain (cNBD) downstream the S6 segment (Pilot et al., 2003b; Cherel, 2004). Indeed, like the EAG channels, plant K^+ *Shaker* channels have a putative cNBD at the C-terminal region of the protein which has been proposed to be responsible for K^+ subunit interactions. Another motif, the ankyrin domain, is also found downstream of the cNBD in some types of plant K^+ channels (*see* Table 1). Indeed, all (completely sequenced) channels (illustrated in this review) of the AKT1 and the AKT2/3 subfamilies are characterized by an ankyrin domain (typically comprising 5–6 ankyrin repeats), which has been suggested to facilitate the binding of the channels to the cytoskeleton, or protein-protein interactions or modulation by cytosolic factors (Sentenac et al., 1992; Cao et al., 1995; Ketchum & Slayman, 1996; Zimmermann et al., 1998; Buschmann et al., 2000; Hartje et al., 2000; Schachtman, 2000; Su et al., 2001; Moshelion et al., 2002; Mouline et al., 2002; Cherel, 2004; Formentin et al., 2004). An ankyrin domain is also present in the outward-rectifying channels (Gaymard et al., 1998, Ache, 2000, Moshelion, 2002; Langer et al., 2004). The names of the plant *Shaker* channels, e.g., AKT1 and KAT1, were initially determined according to the classification based on the presence or absence of ankyrin domains at the C-terminal (Pratelli et al., 2002; Langer et al., 2004). Since recently an ankyrin domain was identified in the SIRK (Pratelli et al., 2002) and KPT1 (Langer et al., 2004) channels belonging to the KAT1 subfamily, previously considered to be devoid of ankyrin repeats, SIRK and KPT1 are not consistent with this previous classification.

Despite the structural and topological similarity with outward-rectifying animal K^+ channels, on the basis of their voltage-dependence the plant *Shaker*-type K^+ channels can be divided into three functionally distinct categories: i) hyperpolarization-activated inward-rectifying K^+ channels which typically mediate potassium uptake, ii) depolarization-activated outward-rectifying channels mediating potassium release, and iii) weakly-rectifying K^+ channels which mediate both uptake and release, depending on the electrochemical K^+ gradients.

Table 1. Localization and functional properties of *Shaker*-type K^+ channels in higher plants

Channel/ Accession code	Plant species	Organ/Tissue	Function	Modulators	Single channel conductance	Voltage dependence	Heterologous expression system	Notes	Reference
AKT1 subfamily / GROUP I									
AKT1 At2g26650	*Arabidopsis thaliana*	Peripheral root cell layers, root hairs, hydathodes, leaf primordia, hypocotyls, guard cells	K^+ uptake into the roots	2,4-D and BA reduce transcript accumulation in the roots		Inward rectifying	Yeasts, SF9 insect cells	Ankyrin Het	Basset et al., 1995; Dennison et al., 2001; Gaymard et al., 1996; Hirsch et al., 1998; Ivashikina et al., 2001; Lacombe et al., 2000b; Lagarde et al., 1996; Pilot et al., 2003a; Sentenac et al., 1992; Szyroki et al., 2001
AKT5 At4g32500	*Arabidopsis thaliana*	Flower and developing siliques						Ankyrin	Lacombe et al., 2000b
SPIK At2g25600	*Arabidopsis thaliana*	Pollen tube	Contributes to pollen tube growth	Blocked by Cs^+ (≈100 mM) Activated by H^+_{ext}	14 pS in 50 mM KCl_{ext}	Inward rectifying	COS cells	Ankyrin	Mouline et al., 2002
DKT1 AJ697979	*Daucus carota*	Apex, cotyledon, leaf (also guard cells), root, stem, shoot apical merystem, embryos	K^+ uptake and membrane potential regulation. Stomatal regulation?	Blocked by Cs^+_{ext} (≈5 mM)		Inward rectifying Het	*Xenopus* oocytes	Ankyrin. Coexpression in oocytes by co-injection with KDC1	Formentin et al., 2004
LKT1 X96390	*Lycospersicon esculentum*	Roots (hairs)	K^+ absorption into root hairs	Blocked by Cs^+_{ext} (≈ μM); Activated by H^+_{ext}		Inward rectifying	*Xenopus* oocytes	Ankyrin	Hartje et al., 2000
MKT1 AF267753	*Mesembryanthemum crystallinum*	Root (epidermis, cortex and stele) Leaf (mainly vasculature)	K^+ uptake into the roots	Down-regulated during salt stress				Ankyrin	Su et al., 2001
OsAKT1 AK120308	*Oryza sativa*	Roots (epidermis, endodermis), coleoptile, leaf (xylem parenchyma, phloem, mesophyll)	K^+ uptake system into the roots and coleoptile	Blocked by Cs^+ (1 mM). Activated by $H^+_{ext.}$ Downregulated during salt stress. Inhibited by Ca^{2+}_{ext}.	25 pS in 100 mM KCl_{ex}	Inward rectifying	HEK293	Ankyrin	Fuchs et al., 2005; Golldack et al., 2003
SKT1 T07651	*Solanum tuberosum*	Roots, abaxial guard cells		Blocked by Cs^+ in 100 μM range. Activated by $H^+_{ext.}$		Inward rectifying	*Xenopus* oocytes; insect cells	Ankyrin Het	Zimmermann et al., 2001; Zimmermann et al., 1998

TaAKT1 AF207745	*Triticum aestivum* L.	Roots	Constitutive and inducible K^+ uptake in wheat roots?	Blocked by Cs^+_{ext} (≈10 mM). Upregulated by K^+-starvation		Inward rectifying (experiments done on protoplasts)		Ankyrin	Buschmann et al., 2000
ZMK1 Y07632	*Zea mays*	Coleoptile (vascular? and non-vascular tissues) and mesocotyl	Participates in coleoptile growth	Blocked by Cs^+_{ext} (≈1 mM). Activated by H^+_{ext} Upregulated by IAA treatment		Inward rectifying	*Xenopus* oocytes	Ankyrin	Philippar et al., 1999
KAT1 subfamily / GROUP II									
KAT1 At5g46240	*Arabidopsis thaliana*	Guard cells	Stomata regulation	Activated by H^+_{ext} and H^+_{in}. Inhibited by TEA^+ (10 mM), Ba^{2+}_{ext} (10 mM), dendrotoxin (50 nM). Blocked by Cs^+_{ext} (1 mM)	5–6 pS (100 mM KCl_{ext})	Inward rectifying	*Xenopus* oocyte; Sf9 insect cells; CHO cells; *E. coli;* yeasts	Regulated by cytoplasmic ATP and cGMP. Unaffected by internal TEA^+ and Cs^+ Het	Anderson et al., 1992; Baizabal-Aguirre et al., 1999; Becker et al., 1996; Bertl et al., 1995; Gaymard et al., 1996; Hedrich et al., 1995; Hoshi, 1995; Ichida et al., 1997; Ichida & Schroeder, 1996; Kwak et al., 2001; Lai et al., 2005; Marten et al., 1996; Sato et al., 2002; Sato et al., 2003; Nakamura et al., 1995; Sato et al., 2003; Schachtman et al., 1992; Szabo et al., 2000; Szyroki et al., 2001; Uozumi et al., 1995; Uozumi et al., 1998; Véry et al., 1995
KAT2 At4g18290	*Arabidopsis thaliana*	Leaf (guard cells, minor veins, phloem); flower	Stomata regulation	Activated by H^+_{ext} Inhibited by Cs^+_{ext} (0.5 mM), Ba^{2+}_{ext} (5 mM) and TEA^+ (10 mM)	6.7 pS (100 mM KCl_{ext})	Inward rectifying	*Xenopus* oocytes, HEK293 cells.	Het	Ivashikina et al., 2001; Pilot et al., 2001
KMT1 AF267754	*Mesembryanthemum crystallinum*	Leaf, seedpod	Osmotic regulation; support to sustained respiration under stress?	Transiently upregulated during salt stress;				Partial CDS	Su et al., 2001
KPT1 AJ344623	*Populus tremula*	Leaf (guard cells), leaf buds (epidermis)	Associated with K^+ dependent leaf development. Major K^+-uptake channel in guard cells				*Escherichia coli*	Ankyrin Het	Langer et al., 2002; Langer et al., 2004

Table 1. Continued

Channel/ Accession code	Plant species	Organ/Tissue	Function	Modulators	Single channel conductance	Voltage dependence	Heterologous expression system	Notes	Reference
KST1 X79779	*Solanum tuberosum*	Guard cells, leaf, flower	Stomatal regulation.	Activated by Mg-ATP$_{in}$ and H_{ext} Blocked by $Cs^{+}{}_{ext}$ in the mM range. Down regulated by drought	7 pS in 100 mM KCl_{ext}	Inward rectifying	*Xenopus* oocytes;	Het	Hoth et al., 1997; Hoth et al., 2001; Kopka, Provart & Müller-Röber, 1997; Müller-Röber et al., 1995
KZM1 AJ421640	Zea mays	Leaf (vascular strands and epidermis), node and silk	Mediating bulk potassium flow into the phloem	Activated by $H^{+}{}_{in}$. Insensitive to variation of external pH	20 pS in 30 mM KCl_{ext}	Inward rectifying	*Xenopus* oocytes	Possible allele of ZmK2.1?	Philippar et al., 2003; Su et al., 2005
SIRK AF359521	*Vitis vinifera*	Leaf (guard cells), berry (guard cells and xylem)	Regulation of K^{+} loading and/or water loss in berry	Activated by $H^{+}{}_{in}$ and $H^{+}{}_{ext}$	13 pS in 100 mM KCl_{ext}	Inward rectifying	*Xenopus* oocytes	Ankyrin (single repeat)	Pratelli et al., 2002
ZmK2.1 AY461583	*Zea mays*	Leaf (vascular bundle, sheath strands, epidermis)	Contribution to K^{+}-dependent inward conductance (also in guard cells).	Activated by $K^{+}{}_{ext}$, less by $Rb^{+}{}_{ext.}$ Weakly activated by $H^{+}{}_{ext.}$ Slightly blocked by $Ca^{2+}{}_{ext}$ (1–10 mM)	14 pS in 50 mM KCl_{ext}	Inward rectifying	*Xenopus* oocytes, yeasts, COS cells	Possible allele of KZM1?	Su et al., 2005
AKT2 subfamily / GROUP III									
AKT2/3 At4g22200	*Arabidopsis thaliana*	Leaf (epidermis, guard cells, mesophyll, phloem), root (phloem), flower (sepal), stem	Plant response to drought. Phloem loading/ unloading. Calcium sensor for other inward K^{+} channels in guard cells.	Outward current modulated by $K^{+}{}_{ext}$ Phosphatase AtPP2CA, ABA-inducible Inhibited by $H^{+}{}_{ext.}$ Inhibited by $Ca^{2+}{}_{ext}$		Weakly rectifying	*Xenopus* oocytes, *E. coli*, Yeasts, HEK293 and COS cells	Ankyrin. Inward rectifying at acidic internal pH Het	Baizabal-Aguirre et al., 1999; Cao et al., 1995; Cherel et al., 2002; Deeken et al., 2003; Deeken et al., 2000; Dennison et al., 2001; Dreyer et al., 2001; Geiger et al., 2002; Hoth et al., 2001; Ivashikina et al., 2005; Ketchum & Slayman, 1996; Lacombe et al., 2000b; Marten et al., 1999; Michard et al., 2005a; Michard et al., 2005b; Szyroki et al., 2001; Uozumi et al., 1998
MKT2 AF267755	*Mesembryanthemum crystallinum*	Roots, leaves, stems, flowers, seed capsule	Responsible for K^{+} homeostasis in flowers?	Slightly downregulated by salt stress				Partial CDS	Su et al., 2001

PTK2 AJ271447	*Populus tremula*	Vascular-rich petiole, phloem	Seasonal wood production	Inhibited by Ca^{2+}_{ext} and H^{+}_{in}	Weakly rectifying	*Xenopus* oocytes	Ankyrin Het	Langer et al., 2002
SPICK1 AF099095	*Samanea saman*	Pulvinar tissues (extensor, rachis)	Light and clock regulated pulvinar movements				Ankyrin. Max trancript in the night.	Moshelion et al., 2002
SPICK2 AF145272	*Samanea saman*	Pulvinar tissues (extensor, flexor)	Light and clock regulated pulvinar movements				Ankyrin. Max trancript in the morning.	Moshelion et al., 2002
VFK1 Y10579	*Vicia faba*	Sink tissues & stem; phloem	K^+ loading in the phloem	Inhibited by H^{+}_{ext}	Weakly rectifying	*Xenopus* oocytes	Ankyrin Expression in oocytes by coinjection with mutated KAT1 Het	Ache et al., 2001
ZMK2 AJ132686	*Zea mays*	Coleoptile (vascular bundles); primary leaves		Inhibited by H^{+}_{ext} (from pH 7 to 4.5)	Weakly rectifying Fast activation kinetics	*Xenopus* oocytes	Ankyrin	Philippar et al., 1999
AtKC1 subfamily / GROUP IV								
AtKC1 At4g32650	*Arabidopsis thaliana*	Roots (hair, epidermal and cortical cells); leaf (trichomes, hydathodes, guard cells)	Participates in K^+-uptake as a modulatory subunit. Contributes to adaptation to salt stress	ABA, BA and 2,4-D reduce transcript accumulation		Heteromeric channels together with KAT1	Modulator/helper α-subunit of other inward K channels. Transcripts depend on Na^{+}_{ext}? Het	Dreyer et al., 1997; Ivashikina et al., 2001; Pilot et al., 2003a; Reintanz et al., 2002
KDC1 AJ249962	*Daucus carota*	Root (hairs, epidermal cells)	Modulator alfa-subunits of other K channels. Participates in K^+ uptake and modulation of membrane potential?	Potentiated by Zn^{2+}	Inward rectifying?	*Xenopus* oocytes, CHO cells	Expression in oocytes by co-injection with DKT1 and KAT1 Het	Downey et al., 2000; Paganetto et al., 2001; Formentin et al., 2004; Picco et al., 2004
SKOR subfamily / GROUP V								
SKOR At3g02850	*Arabidopsis thaliana*	Root stellar tissues. Low expression also in pollen grain	K^+ release into xylem sap towards the shoots	Inhibited by 4-amino pyridine (10 mM), Verapamil (10 μM), Ba^{2+} (5 mM), TEA^+ (10 mM). Allosteric regulated by K^+. Inhibited by H^{+}_{in} and H^{+}_{ext}. Regulated by K^{+}_{ext}.	23 pS (10 mM KCl)	Outward rectifying	*Xenopus* oocytes	Ankyrin ABA (100 μM), BA (50 μM) and 2,4-D (10 nM) inhibit transcript in roots Het	Gaymard et al., 1998; Lacombe et al., 2000a; Mouline et al., 2002; Pilot et al., 2003a

Table 1. Continued

Channel/ Accession code	Plant species	Organ/Tissue	Function	Modulators	Single channel conductance	Voltage dependence	Heterologous expression system	Notes	Reference
GORK At5g37500	*Arabidopsis thaliana*	Guard cells, root epidermal cells, root hairs	Potassium release from guard cells	Inhibited by Ba^{2+} (5-10 mM) and TEA^+ (10 mM) and H^+_{ext} Regulated by K^+_{ext}	In guard cells 20 pS $(30\ mM)_{out}/(150mM)_{in}$	Outward rectifying	*Xenopus* oocytes	Ankyrin Het	Ache et al., 2000; Ivashikina et al., 2001
PTORK AJ271446	*Populus tremula*	Vascular-rich petiole, phloem	Seasonal wood production	Activation decreases increasing K^+_{ext}		Outward rectifying	*Xenopus* oocytes	Ankyrin Het	Langer et al., 2002; Langer et al., 2004
SPORK1 AJ299019	*Samanea saman*	Pulvinar tissues (extensor, flexor)	Light and clock regulated pulvinar movements					Ankyrin. Max transcript in the morning.	Moshelion et al., 2002

Denomination and accession codes of *Arabidopsis* genes are from Aramemnon: http://aramemnon.botanik.uni-koeln.de/ and (Pilot et al., 2003b); for other genes they were derived from the original papers. The same codes were used for the construction of the phylogenetic tree in Fig. 1*B*. Note the partial coding sequences of KMT1 and MKT2. *Ankyrin*: means presence of an ankyrin domain; *ext*: external, *in*: internal/cytoplasmatic; *Het*: proven heteromerization capability with some other *Shaker*-type channel. The properties of 31 *Shaker*-type channels, or putative *Shaker*-type channels, which have been genetically identified in 12 different plant species and at least partly localized in plants and/or functionally characterized, are illustrated in this Table. For recent reviews *see* Cherel, 2004; Dreyer, Müller-Röber & Köhler, 2004a; Fan, Zhao & Assmann, 2004; Latorre et al., 2003; Maser et al., 2001; Pilot et al., 2003b; Véry & Sentenac, 2003.

In *Arabidopsis thaliana*, the gene encoding K^+ channels described above as category i) "the inward-rectifying potassium channels, KAT1 and AKT1, activated by hyperpolarizing membrane potentials", were first isolated in 1992 by functional complementation of yeast mutants defective for K^+ uptake (Anderson et al., 1992; Sentenac et al., 1992). Since then, other inward-rectifying K^+ channels from various plant origins have been identified, functionally expressed and characterized in yeasts (Bertl et al., 1995; Nakamura et al., 1995; Uozumi et al., 1995; Véry et al., 1995; Dreyer et al., 2004b; Lai et al., 2005; Su et al., 2005; Becker et al., 1996) as well as in *Xenopus laevis* oocytes (Schachtman et al., 1992; Hedrich et al., 1995; Müller-Röber et al., 1995; Philippar et al., 1999; Hartje et al., 2000; Paganetto et al., 2001; Zimmermann et al., 2001; Langer et al., 2002; Pratelli et al., 2002; Dreyer et al., 2004b; Formentin et al., 2004; Su et al., 2005;) and in a variety of other heterologous systems such as cultured animal cells (Szabo et al., 2000; Dreyer et al., 2001; Ivashikina et al., 2001; Mouline et al., 2002; Su et al., 2005), insect cells (Gaymard et al., 1996; Marten et al., 1996; Zimmermann et al., 1998; Baizabal-Aguirre et al., 1999) and bacteria (Uozumi et al., 1998; Uozumi, 2001; Langer et al., 2004). Thereafter (1995), the gene encoding for the weak inward-rectifying K^+ channel AKT2 (Cao et al., 1995) (and its truncated version AKT3; Ketchum & Slayman, 1996) were identified in *Arabidopsis* phloem and characterized (Uozumi et al., 1998; Baizabal-Aguire et al., 1999; Marten et al., 1999; Lacombe et al., 2000b). This channel protein is also referred to with the term AKT2/3 or AKT2/AKT3 (*see* http://aramemnon.botanik.uni-koeln.de/). AKT3 is shorter than AKT2 and lacks the first 15 amino acids of AKT2. In this review we refer to AKT2/3 using the nomenclature adopted by the article(s) quoted. The weak rectification of AKT2/3 is caused by the possible interaction of positive residues in the voltage-sensing segment and phosphorylated residues in the cytosolic space (Cherel et al., 2002; Michard et al., 2005a, b).

The outward-rectifying K^+ channels, activated by depolarizing membrane potentials, were cloned in 1998 with the identification of SKOR from *Arabidopsis thaliana* stelar tissues (Gaymard et al., 1998). This finding is consistent with ionic transport already identified in stelar protoplasts where outwardly rectifying voltage-dependent channels dominate the K^+ transport (Wegner & Raschke, 1994; Roberts & Tester, 1995; Wegner & De Boer, 1997) driving K^+ release into the xylem sap. Identification of the SKOR gene and characterization were soon followed by the cloning and expression of GORK from *Arabidopsis* guard cells (Ache et al., 2000), which also displays outward-rectifying channel characteristics.

Here, we illustrate the properties of 31 *Shaker*-type channels, or putative *Shaker*-type channels, which have been genetically identified in 12 different plant species (*Arabidopsis thaliana, Daucus carota, Lycospersicon esculentum, Mesembryanthemum crystallinum, Oryza sativa, Populus tremula, Samanea saman, Solanum tuberosum, Triticum aestivum, Vicia faba, Vitis vinifera* and *Zea mays*), at least partly localized in *planta* and/or functionally characterized. The analysis of *plant Shaker*-type K^+ channel genes reveals that they can be classified into 5 groups (Pilot et al., 2003b) (or subfamilies, named after the first identified *Arabidopsis* channel of the subfamily) by sequence similarities and common functional properties (Table 1 and Fig. 1*b*). Several other genes have been identified in sequences available from plant sequencing projects (e.g., rice). Among the 31 channels reported in Table 1, 25 are inward-rectifying (group I, II) or weakly inward-rectifying (group III), 2 belong to the helper channel family (group IV) while 4 genes encode for outward-rectifying channels comprised in the SKOR subfamily (group V). In *Arabidopsis*, 9 genes have been identified (Maser et al., 2001; Pilot et al., 2003b) (KAT1, KAT2, AKT1, AKT5, SPIK, AKT2/3, AtKC1, SKOR and GORK) and, with the exception of AKT5 (occasionally also denominated AKT6 (Maser et al., 2001; Pratelli et al., 2002; Véry & Sentenac, 2003)) and AtKC1, expressed as homomeric functional channels in different expression systems (Véry & Sentenac, 2002; Véry & Sentenac, 2003; Dreyer et al., 2004b). The crucial functional properties of the channels belonging to these five groups and their tissue localization are described in the following paragraphs and summarized in Table 1.

Tissue Localization and Physiological Roles of *Shaker*-type K^+ Channels in Plants

Some plant *Shaker*-type channels are present only in some specific tissues and others are widely distributed in several plant tissues. Generally, channels of group II were identified in plant leaves, primarily in guard cells, channels of group I and IV are mostly present in the root, channels of group III are widely distributed but mainly expressed in the phloem, while the outward channels of group V were identified in plant roots, as well as in guard cells and phloem (for details *see* Table 1).

AKT1, AtKC1 and other members of groups I and IV are mainly expressed in plant roots (Sentenac et al., 1992; Basset et al., 1995; Lagarde et al., 1996; Hirsch et al., 1998; Philippar et al., 1999; Buschmann et al., 2000; Downey et al., 2000; Hartje et al., 2000; Ivashikina et al., 2001; Su et al., 2001; Zimmermann et al., 2001; Reintanz et al., 2002; Golldack et al., 2003; Pilot et al., 2003a; Formentin et al., 2004; Fuchs et al., 2005) where they seem to play an important role in potassium uptake from soil. Indeed, contrary to early interpretations, it has been demonstrated that K^+ channels might be involved in K^+ transport not only in the low affinity ($\approx$0.2–15 mM) but also in the high affinity ($\approx$1–200 μM) range in plant roots (Epstein et al., 1963; Schroeder, Ward & Gassmann, 1994; Fox & Guerinot, 1998; Latorre et al., 2003). Indeed, in highly hyperpolarized cells (i.e., at membrane potentials more negative than −200 mV), K^+ uptake in the low concentration range can be also mediated by passive transporters like channels. This has been demonstrated for the inward-rectifying K^+ channel AKT1, which has the capability to mediate potassium accumulation in plant cells at external potassium concentrations as low as 10 μM (Hirsch et al., 1998). Other inward and outward K^+ currents identified in plant roots (Lew, 1991; Schachtman, Tyerman & Terry, 1991; Findlay et al., 1994; Gassmann & Schroeder, 1994; Roberts & Tester, 1995; Bregante et al., 1997) may also play a significant role in potassium uptake in the roots. Keeping in mind the contribution of high-affinity K^+ uptake transporters, like AtHAK5, which are induced by potassium starvation, the contribution of K^+ channels on potassium uptake at the micromolar level of K^+ is likely to be limited (Gierth, et al., 2005). An uptake, sustained by very negative membrane potentials in the low micromolar potassium range, has been suggested for the SPIK channel, too, which in *Arabidopsis* pollen contributes to pollen growth and development (Mouline et al., 2002). The outward-rectifying K^+ channel, SKOR, is specifically and predominantly expressed in the root stele and is involved in K^+ release into xylem toward the shoots (Gaymard et al., 1998). Silent helper channel subunits (Pilot et al., 2003b) (which do not form functional homotetramer channels in heterologous systems) like AtKC1 (Dreyer et al., 1997; Reintanz et al., 2002) or helper channels like KDC1 (which forms channels in heterologous cells only under specific conditions (Downey et al., 2000; Paganetto et al., 2001; Picco et al., 2004)) may play a modulatory role in K^+ uptake in the roots. For example, they may modify the activation threshold and the kinetic properties of their companion subunits, i.e., AKT1 in *Arabidopsis* (Reintanz et al., 2002) and DKT1 in carrots (Costa et al., 2004; Formentin et al., 2004). The hypothesis that AtKC1 is a silent modulatory subunit, unable to form functional channels, deserves further investigation, as this appears contradictory to the observation that AtKC1 seems to be the sole member, or the member expressed at a much higher level than the other *Shaker*-type K^+ channel genes present in some specific cells like trichomes and hydathodes (Pilot et al., 2003a).

Some of the inward-rectifying K^+ channels listed above are expressed in specific tissues; for example,

SKT1, AKT1 and AtKC1 in guard cells as well as in hypocotyl and tumor tissues (Zimmermann et al., 1998; Ivashikina et al., 2001; Szyroki et al., 2001; Deeken et al., 2003; Philippar et al., 2004). Besides being expressed in roots, the transcripts of ZMK1 (Philippar et al., 1999; Fuchs et al., 2003) and DKT1 (Formentin et al., 2004) have also been localized in maize coleoptile and in several other carrot tissues, respectively (like leaves and shoot apex as well as cotyledons and stems).

The inward-rectifying K^+ channels of group II, e.g., KAT1 and KAT2 (like KST1 in potato), are mainly expressed in guard cells (Nakamura et al., 1995; Kwak et al., 2001; Pilot et al., 2001) and are involved in the control of stomatal movements with the outward-rectifying K^+ channel GORK (Table 1). KAT1 is phosphorylated in respose to treatment of stomatal guard cells with abscisic acid (Li & Assmann, 1996; Li, Lee & Assmann, 1998; Li et al., 2000; Mori, Uozumi & Muto, 2000). The relevance of the role played by inward-rectifying K^+ channels in the regulation of stomatal opening is still an open question (Ichida et al., 1997; Kwak et al., 2001; Szyroki et al., 2001). On the contrary, GORK seems to be the only outward-rectifying channel responsible for potassium efflux in *Arabidopsis* guard cells. This is certified by the strong reduction of the outward-rectifying K^+ channel activity in transgenic *Arabidopsis* plants expressing a dominant negative GORK mutant and by the concomitant impaired stomatal closure (Hosy et al., 2003). Also, knockout mutants of the *GORK* gene fully suppressed outward-rectifying K^+ channel activity (Hosy et al., 2003). Notably, GORK has been demonstrated to be present also in root hair cells where it may behave as a potassium sensor and cooperate in regulating the membrane potential of root hairs (Ivashikina et al., 2001).

After K^+ is uploaded into the roots, it is transferred to the root xylem to be delivered to the shoots by the outward-rectifying stelar channel SKOR, which seems to be the unique *Shaker*-type channel responsible for K^+-secretion in the xylem sap (Gaymard et al., 1998). AKT2/3 has been suggested to be responsible for K^+ transport into the phloem tissues and the maintenance of membrane potential (in the phloem) which, in turn, affects the regulation of sucrose loading/unloading into and from the phloem sap (Cherel et al., 2002; Deeken et al., 2000; Lacombe et al., 2000b; Marten et al., 1999). Similar roles could possibly be played by other inward-rectifying K^+ channels belonging to either group I or III, like VFK1, OsAKT1, PTK2 in the phloem of *Vicia faba*, *Oryza sativa* and *Populus tremula*, respectively. Moreover, AKT2/3 has been suggested to be also responsible for K^+ permeability in *Arabidopsis* mesophyll cells (Dennison et al., 2001) and calcium sensitivity in guard cells (Ivashikina et al., 2005).

Topogenesis of Plant *Shaker*-type K^+ Channels

KAT1 shares similarities with other animal and plant K^+ channels in the negatively charged residue located in the middle of S3 segment and several positively charged residues of S4 which serves as a weakly hydrophobic voltage sensor (Fig. 1*A*), yet S4 is integrated into the membrane. The electrostatic interaction between negatively charged residues in S2 and S3, and positively charged residues in S4 is crucial for the efficiency of the *Shaker* voltage sensor (Papazian et al., 1995; Larsson et al., 1996; Seoh et al., 1996). Mutational analysis has identified some of the charged residues, which are considered to be components of the voltage sensor, as demonstrated in animal K^+ channels (Papazian et al., 1995; Larsson et al., 1996; Seoh et al., 1996). In KAT1, several lines of evidence achieved by electrophysiological measurements strongly suggest that S4 is responsible for the voltage dependence of channel activation. The relevant gating differences of inward-, outward- and weakly-rectifying plant K^+ channels, displaying the same putative membrane topology but different voltage dependence, may account for different coupling of the voltage sensor S4 and pore opening mechanisms (for review *see* Latorre et al., 2003). For example, recently it has also been demonstrated that in KAT1 the S4 segment is tightly packed with the pore domain interacting in a very specific manner with the S5 segment (Lai et al., 2005).

In general, a series of hydrophobic domains in polytopic membrane proteins are sequentially integrated by means of the translocon into the endoplasmic reticulum (ER) membrane. It is interesting to investigate how these voltage-sensing regions are integrated into the membrane (Fig. 3). Using KAT1 as a model channel, each topogenic function was evaluated: S1 inserts into the membrane and remains in the membrane as a signal anchor, and the following S2 is integrated as a stop-transfer sequence (Sato et al., 2002). The S3 helix alone lacks the ability to integrate into the membrane. The poor translocation efficiency for S3 is consistent with the results observed in the *E. coli* system (Uozumi et al., 1995). Masking the charge of the residue by pairing with a positive residue in S4 enables the S3 insertion into the membrane (Sato et al., 2002). For the membrane integration of the voltage-sensing segments, specific pairing of the positive residues in S4 with the negative residues in S2 and S3 contributes to the insertion and retention of S4 in the membrane (Sato et al., 2003). S5-P-S6 can be arranged into a proper topology without the preceding segments S1–S4 (Sato et al., 2002). This supports the notion that the S5-P-S6 structure in KAT1 corresponds to a membrane-pore-membrane structure in an ancestral K^+ channel structure (Durell & Guy, 1999; Durell et al., 1999). The pore segment between S5 and S6 in the KAT1

pore region, like the two α-helix membrane-spanning animal K^+ channels Kir 2.1 and bacterial KcsA, shows no topogenic function despite its high hydrophobicity and a high prediction score for being a transmembrane segment and evidence for being embedded in the membrane in the final structure (Umigai et al., 2003). Notably, the non-topogenic function of the hydrophobic pore segment is one of the determinants contributing to the final membrane structure of the K^+ channel (Fig. 3).

Formation of Heteromeric K^+ Channels May Confer Further Flexibility to Plants

While the *Shaker*-type plant K^+ channels display striking topological similarities, they show diverse voltage dependencies (inward-rectifying, weakly inward-rectifying and outward-rectifying characteristics), kinetic properties (slow or very fast activation) as well as different susceptibilities to diverse ions, exogenous and endogenous modulators. This functional flexibility can be further fine-tuned by the non-covalent tetrameric organization of *Shaker*–type K^+ channels and by the capability of different subunits to form heteromeric complexes. Indeed, it is well known that animal potassium channels can form heterotetrameric complexes, which display characteristics significantly different from those of the corresponding homomeric complexes (Salinas et al., 1997; Stocker, Hellwig & Kerschensteiner, 1999; Ottschytsch et al., 2002). It has been suggested that channel heteromerization might confer further flexibility to living cells which, by transcription and assembly of a variety of heteromeric channels, may respond to a wider class of external stimuli (Isacoff, Jan & Jan, 1990; Post, Kirsch & Brown, 1996; Dreyer et al., 1997; Ottschytsch et al., 2002).

Together with post-translational modifications specific to the cell-type, the formation of K^+ channels comprising heterogenous subunits may generate further diversity in plant K^+ channels, too, and explain some differences observed in the characteristics of plant K^+ channels measured *in vivo* and expressed in heterologous systems (Bruggemann et al., 1999; Dreyer et al., 1997). This mechanism might be particularly important for plants, which generally do not need to use fast signal transmission. Besides the fine-tuning of plant-response to external stimuli, the presence of a variety of composite channels also gives the possibility to reduce the plant sensitivity to external disturbances. In turn, the stoichiometry and the extent of the heteromeric structures composed in the different tissues may be subjected to further modulation by parameters that regulate the transcription of diverse subunits (as demonstrated for changes in the transcriptional level of AKT1, AKT2/3 and AtKC1, which in mesophyll cells display a different sensitivity to various hormonal factors; Pilot et al., 2003a; Roberts, 1998).

While in animals a barrier to heteromerization seems to exist, a less selective assembly capability characterizes the interaction among channel subunits belonging to different subfamilies and even different plant species (Dreyer et al., 1997). For example, co-expression in *Xenopus* oocytes and protein interaction detected by yeast two-hybrid system experiments suggests that KAT1 and KAT2 (which belong to the same subfamily and are localized in *Arabidopsis* guard cells) can form functional heteromultimeric channels (Pilot et al., 2001). On the other hand, the heteromerization capability of channels belonging to different subfamilies, like KAT1 and AKT2, has also been demonstrated by using the dominant negative strategy, where negative mutations introduced in one mutant decrease the current density of coexpressed channels (Baizabal-Aguirre et al., 1999). Similarly, KST1 (from potato guard cells) co-assemble with SKT1 (from abaxial leaves and guard cells) via their conserved C-terminus K_T domains (Zimmermann et al., 2001).

Interestingly, the two carrot channel subunits, KDC1 and DKT1 (colocalized in some common tissues, i.e., roots and root hairs) are not able to form functional channels when injected by themselves in *Xenopus* oocytes; on the contrary, when the mRNA of these two subunits are co-injected in the same oocyte, robust and stable inward-rectifying K^+ currents can be observed (Formentin et al., 2004). Also, two other channels, SKT1 (from potato) and AKT1 (from *Arabidopsis*), which do not form channels in oocytes when injected alone, when co-injected in oocytes, elicit inward-rectifying K^+ currents (Dreyer et al., 1997). This is not surprising as, also in animal cells, it has been demonstrated that silent subunits, which do not generate currents by themselves, might be efficient partners in the formation of heteromeric K^+ channels (Isacoff et al., 1990; Post et al., 1996; Ottschytsch et al., 2002). It has also been shown that coexpression can alter the protein targeting and modify the intracellular destination of the subunits. For example, the inward-rectifying animal K^+ channel Kir3.1 remains localized in the ER when injected alone, while it is correctly targeted to the plasma membrane when coexpressed together with another channel, Kir3.4 (Ma et al., 2002). Similar mechanisms could be responsible for the functional expression of complementary subunits in plants.

The functional co-assembly in *Xenopus* oocytes of subunits localized in different tissues in plant and plant species, like KST1 and KAT1, SKT1 and KAT1, KST1 and AKT1 or AtKC1 (from potato and *Arabidopsis*, respectively) (Dreyer et al., 1997), and finally VFK1 and KAT1 (from *Vitis vinifera* and *Arabidopsis*, respectively) (Ache et al., 2001) are definitely striking results (Dreyer et al., 1997). In the

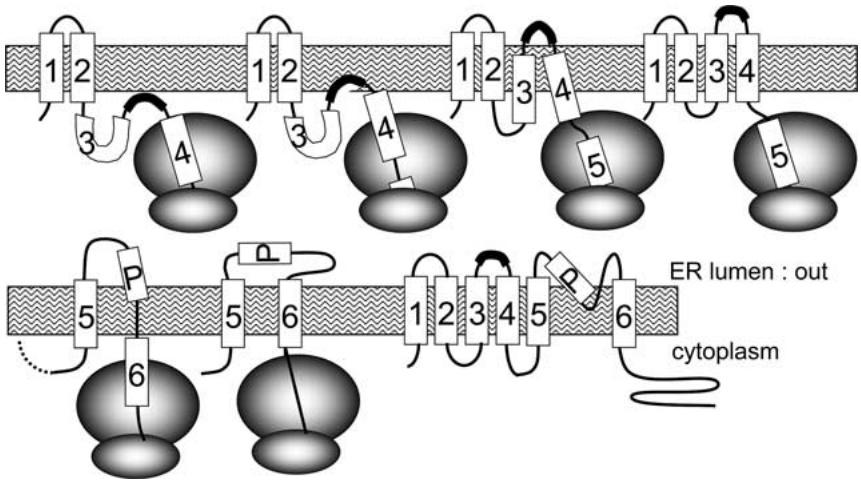

Fig. 3. Integration of voltage-dependent K^+ channel (KAT1) into the ER membrane. The insertion process of KAT1 S3-S4 into the membrane involves the post-translational peptide binding of transmembrane segments, a newly recognized second type of membrane protein integration process. Electrostatic interactions between charged residues in S2, S3 and S4 were found to be required to achieve the correct voltage-sensor topology in the ER membrane. S1–S4 can be integrated independently of S5-P-S6 into the ER membrane. The hydrophobic pore segment (*P*) shows no topogenic function, which is one of the determinants contributing to the pore-forming structure of K^+ channels (Sato et al., 2002; Sato et al., 2003; Umigai et al., 2003).

animal kingdom this would be equivalent to "heteromultimerization of a *Shaker*-type subunit from rat brain and *Shal*-type subunit from human heart" (Dreyer et al., 1997). Consistently, KDC1 from *Daucus carota* forms heteromeric channels mediating inward-rectifying currents when coinjected in oocytes together with the *Arabidopsis* guard cell channel KAT1 (Paganetto et al., 2001; Picco et al., 2004). Experiments performed with a dimeric KDC1-KAT1 construct provided further information on the stoichiometry of the subunits participating in the channel, suggesting a preferred (2KDC1 + 2KAT1) stoichiometric composition of this heteromer (Picco et al., 2004).

The tetramerization of different subunits in EAG and plant voltage-dependent *Shaker*-type K^+ channels seems to involve the C-terminal cytoplasmic region (Daram et al., 1997; Ehrhardt, Zimmermann & Muller-Rober, 1997; Ludwig, Owen & Pongs, 1997; Marten & Hoshi, 1997; Urbach et al., 2000; Zhong et al., 2002; Zagotta et al., 2003; Dreyer et al., 2004b; Bentley et al., 1999) and the cyclic-nucleotide binding domain (cNBD) which is present in all plant *Shaker*-type K^+ channels (Daram et al., 1997; Urbach et al., 2000). The deletion of the C-terminus downstream of the cNBD does not affect the functional channel assembly, while further deletions towards the N-terminus prevent the formation of functional channels (Marten & Hoshi, 1997). Notably, in SKOR channels cNBD is essential for SKOR assembly, but cNBD itself does not determine the specificity of the assembly as cNBDs of other *Shaker*-type K^+ channels (e.g., KAT1) also support formation of homomeric SKOR channels (Dreyer et al., 2004b). The region between the cNBD and the C-terminal of S6 (Daram et al., 1997; Zagotta et al., 2003; Dreyer et al., 2004b) and the region at the C-terminal far end of potato inward-rectifying K^+ channels seem to be important in the assembly of these channels (Ehrhardt et al., 1997). Finally, from the favorable interaction of KPT1 with PTK2 and PTORK and missing interaction with KAT1, it has been suggested that species-specific interactions and ankyrin domains (present both in PTORK and KPT1) may play an important role and favor the interaction of these different subunits (Langer et al., 2004). It was also reported that two domains located at the C-terminus of SKOR, a proximal region containing the cNBD and a distal region which resembles the K_T domain (located directly upstream of the far end of the C-terminus of KAT1), are important for the assembly of outward-rectifying K^+ channels (Dreyer et al., 2004b). While both interactions seem to be necessary for formation of outward-rectifying K^+ channels, the formation of heteromeric channels consisting of outward- and inward-rectifying K^+ channel subunits has not been observed by these authors (Dreyer et al., 2004b). Other data suggest that inward- and outward-rectifying K^+ channel may coassemble, giving rise to the formation of non-functional channels. Indeed, KPT1 seems to interact not only with the weakly-inwardly rectifying poplar channel PTK2, but also with the outward-rectifying K^+ channel PTORK, as shown by the decrease of the number of active outward-rectifying K^+ channels in *Xenopus* oocytes (Langer et al., 2004).

Nevertheless, as many K^+ channel subunits are not able to coassemble, the capability to form functional heteromeric channels is not an indiscriminate property of all *Shaker*-type K^+ channels (Dreyer et al.,

2004b). In fact, AKT1 and VFK1 when injected in oocytes do not elicit currents (Gaymard et al., 1996; Ache et al., 2001) and similarly AtKC1 and SKT1 or AKT1 (Dreyer et al., 1997) as well as KPT1 and KAT1 (Langer et al., 2004) are unable to form functional heteromeric channels in oocytes. Also heteromerization between AKT1 and KAT1 (observed in oocytes; Dreyer et al., 1997) is controversial: the same subunits failed to form heteromeric complexes when co-expressed in insect cells or in yeasts, according to some authors (Urbach et al., 2000), while other authors confirmed in yeasts the KAT1-AKT1 heteromerization by using the mating-based Split Ubiquitin System (mbSUS) (Obrdlik et al., 2004).

These results suggest that other parameters affecting subunit coassembly deserve to be investigated further: it cannot be excluded that the membrane composition of the host organisms might give a contribution to subunit interactions.

Channel Modulation by Monovalent and Divalent Cations

The potassium selectivity characteristics of plant *Shaker*-type K^+ channels have been evaluated by numerous papers (*see*, for example, Uozumi et al., 1995, 2001; Becker et al., 1996; Ichida & Schroeder, 1996; Nakamura, Anderson & Gaber, 1997; Dreyer et al., 1998; Lacombe & Thibaud, 1998; Moroni et al., 2000). Here, we will shortly review only the effects mediated by some monovalent (H^+, Cs^+) and divalent cations (Ca^{2+}, Ba^{2+}), which behave as modulators of the *Shaker*-type K^+ channels. These studies are important not only for the obviously relevant physiological roles of these ions in plants but also because their interactions with specific binding sites provide useful information on the structure and biophysical mechanisms of *Shaker*-type K^+ channel functioning.

Reversible block by external Cs^+ is a classical feature of inward-rectifiying K^+ plant channels (Pilot et al., 2001; Véry & Sentenac, 2003; Cherel, 2004) (*see* Table 1) and is typically due to cesium interaction within the pore; consistently, it has been demonstrated that cesium block of KAT1 is strongly dependent on the applied transmembrane potential, as the blocking efficiency is progressively increased by more negative membrane potentials (Véry et al., 1994; Hedrich et al., 1995; Ichida & Schroeder, 1996). The blocking mechanism induced by Cs^+ and the interaction of Cs^+ in the permeation pathway of KAT1 was certified by site-directed mutagenesis of amino acids located inside the pore (Becker et al., 1996; Ichida & Schroeder, 1996; Ichida et al., 1997). The majority of plant inward-rectifiying K^+ channels are typically blocked by millimolar concentrations of external Cs^+; however, channels like LKT1 and SKT1 display a peculiar hypersensitivity to Cs^+, as submillimolar (10–100 μM) concentrations of Cs^+ are sufficient to inhibit 50% of the initial current (Zimmermann et al., 1998; Hartje et al., 2000). Conversely, SPIK is blocked in a voltage-independent manner only by cesium concentrations as high as 50–100 mM (Mouline et al., 2002). The different sensitivity of inward-rectifying K^+ channels to Cs^+ is important, as cesium is a competitive inhibitor for K^+ uptake over a wide range of K^+ concentrations, preventing seed germination (Sheahan, Ribeiro-Neto & Sussman, 1993). On the other hand, outward-rectifying K^+ channels in *Arabidopsis* root hair protoplasts are not blocked by external cesium. Presumably, positive transmembrane potentials applied to open outward-rectifying K^+ channels push away Cs^+ from the pore and avoid pore blocking (Ivashikina et al., 2001).

It has been reported that many plant *Shaker*-type K^+ channels are strongly regulated by internal and external pH. This is particularly important in guard cell (Blatt, 1992; Miedema & Assmann, 1996) channels (group II), as acid-induced K^+ uptake through potassium channels is a prerequisite for stomatal opening. KAT1 and KST1 are activated by acidification of both cytosolic and extracellular solutions (Hedrich et al., 1995; Hoshi, 1995; Hoth et al., 1997; Hoth et al., 2001; Véry et al., 1995). In KAT1 the internal pH sensor has been identified with a histidine residue at position 118 in the loop between S2 and S3 (Tang et al., 2000). On the other hand, histidines in KST1, located in the S3-S4 linker and within the pore, function as a part of the external pH sensor (Hoth et al., 1997) which favors KST1 opening by shifting the activation curve to less negative membrane potentials, without any effect on the amplitude of the single-channel conductance. As far as KAT1 is concerned, it has been demonstrated that the molecular basis of the external pH sensor of KAT1 is different from that identified in KST1 but still located inside the channel pore (Hoth et al., 2001; Hoth & Hedrich, 1999).

Like in KAT1 and KST1, SIRK, belonging to the KAT1 subfamily, displays an increase of the current by acidification of both the intracellular and extracellular compartments (Pratelli et al., 2002); KZM1 is activated by internal acidic pH (Philippar et al., 2003) while KAT2 and ZmK2.1, as well as some inward-rectifying K^+ channels of group I (like LKT1, SKT1, SPIK, ZMK1 and OsAKT1), are activated by external acidic pHs (Philippar et al., 1999; Hartje et al., 2000; Mouline et al., 2002; Fuchs et al., 2003). Unlike the channels of the KAT1 and AKT1 subfamilies, channels of the AKT2 subfamily (group III) are inhibited by an increase of proton concentrations. In particular, AKT2/3, ZMK2 and VFK1 (Marten et al., 1999; Philippar et al., 1999, 2003; Lacombe et al., 2000b; Ache et al., 2001;

Langer et al., 2002) are inhibited by external and PTK2 by cytosolic acidification, respectively. Interestingly, the decrease in the acid-induced AKT2/3 current seems to be associated with channel-blocking by protons, as demonstrated by the decrease of the single-channel conductance (Marten et al., 1999); precisely, His^{228} as well as Ser^{271} in the channel pore have been identified as responsible for the proton (and K^+) sensitivity of this channel (Geiger et al., 2002). The outward-rectifying K^+ channel SKOR is blocked by both internal and external acidification (Lacombe et al., 2000a) while for GORK there is only evidence of inhibition by external acidification (Ache et al., 2000); the decrease of the current observed at more acidic extracellular pHs depends on an increase in the number of SKOR channels available for voltage activation, while the single-channel conductance remains practically unaltered (Lacombe et al., 2000a). The pH dependence of the outward-rectifying channels has been correlated with ABA-induced cytosolic acidification and extracellular alkalinization in guard cells with consequent inhibition and up-regulation of SKOR (Lacombe et al., 2000a).

Calcium (Ca^{2+}) and barium (Ba^{2+}) effects have been frequently investigated in plant *Shaker*-type K^+ channels because of their physiological implications (Schroeder et al., 1987; Thiel et al., 1992). In addition, calcium and barium are potent tools to investigate the structure of the pore of *Shaker*-type K^+ channels. It has been demonstrated that barium typically inhibits both KAT1 and KAT2, but to a different extent, as KAT1 displays a much higher sensitivity to Ba^{2+} than KAT2 (Pilot et al., 2001). Calcium inhibits plant *Shaker*-type K^+ channels almost independently of their group. For example, Ca^{2+} inhibits the *Zea mays* leaf inward-rectifying K^+ channels ZmK2.1 (group II) (Su et al., 2005), the rice root inward-rectifying K^+ channels OsAKT1 (group I) (Fuchs et al., 2005), the poplar inward-rectifying K^+ channel PTK2 (group III) (Langer et al., 2002) and, only slightly and in a voltage-dependent manner, KAT1 (group II) (Dreyer et al., 1998). Calcium can access the pore of SKOR, with a permeability ratio of calcium to potassium, $P_{Ca}/P_K \approx 0.9$ (Gaymard et al., 1998). Barium inhibition of both inward and outward currents in guard cell protoplasts (Schroeder et al., 1987) and the accessibility of the SKOR pore to both cations (Ca^{2+} and Ba^{2+}) has been confirmed by the blocking of this channel by Ba^{2+} (a typical inhibitor of K^+ channels, like the outward-rectifying K^+ channels in tobacco protoplasts) and verapamil (which inhibits L-type calcium channels in animal cells) (Thomine et al., 1994; Gaymard et al., 1998). Similarly, the inhibition of GORK by millimolar barium concentrations, both in guard cells (Ache et al., 2000) and in root hair protoplasts (Ivashikina et al., 2001), confirm the characteristic of ion selectivity of this channel, which is similar to that of SKOR. AKT2/3 has been suggested to play a crucial role as regulator of calcium sensitivity of other channels, as inward-rectifying K^+ channels in guard cells, are blocked by Ca^{2+} in the presence of AKT2/3 while they are not affected in *akt2/3* loss-of-function mutants. Thus, it has been proposed that AKT2/3 might be the calcium sensor for guard cell K^+ uptake channels acting as modulatory alpha subunits in calcium-sensitive heteromeric K^+ channels (Ivashikina et al., 2005).

In some animal outward-rectifying K^+ channels (Pardo et al., 1992; Gomez-Lagunas, 1997) the presence of the permeant ion K^+ is required to favor the activation or to avoid the collapse of outward-rectifying K^+ channels. In plants, the activation of outward-rectifying K^+ channels in guard cells (Blatt & Gradmann, 1997), as well as SKOR (Gaymard et al., 1998) and GORK in *Xenopus* oocytes (Ache et al., 2000; Ivashikina et al., 2001), is also allosterically regulated by external K^+. Moreover, the conductance characteristic shifts towards more positive voltages with E_k; this indicates a K^+ interaction, which complements the channel dependence on the membrane potential. Indeed, both GORK (Ache et al., 2000) and SKOR (Gaymard et al., 1998), as well as outward-rectifying channels in plant protoplasts (Blatt, 1991; Blatt, 1992), seem to operate as voltage-sensor able to adjust their transport characteristics as a function of the external K^+ concentrations.

The presence of K^+ is also required as an allosteric regulator of outward currents through the weakly-rectifying AKT2/3 channel from *Arabidopsis* (Geiger et al., 2002) and ZmK2.1 from maize (Su et al., 2005). Specifically, the potassium dependence of the AKT2/3 channel involves amino acid residues (His^{228} and Ser^{271}) in the outer mouth of the pore, which enables a fine-tuning of the channel and possibly represents an important element in the control of membrane potential and sugar loading into the phloem. On the other hand, ZmK2.1 becomes a totally non-conducting channel in the absence of K^+. As the potassium concentrations at which the channel is closed are within the physiological external K^+ range, the role of this maize channel seems to be restricted to low-affinity potassium uptake (Su et al., 2005). As demonstrated for animal *Shaker* B K^+ channels (Gomez-Lagunas, 1997), where monovalent cations, like Rb^+, Cs^+ and NH_4^+ may substitute K^+ to preserve or maintain channel conductivity, also in ZmK2.1 Rb^+ partially prevents channel closure in absence of K^+ (Su et al., 2005).

Conclusions

Since the first cloning of *Shaker*-type K^+ channels in *Arabidopsis thaliana* in 1992, many genes encoding

Shaker-type K^+ channels have been identified from various plant species. These studies provided evidences that *Shaker*-type K^+ channels also play a crucial role in plants. As described in this review article, the extensive characterization of plant channels, accompanied by the development of new expression systems, have led to new technical approaches for channel investigation. The modulation of the channel properties by chemico-physical parameters, and different endogenous and external compounds, as well as the interaction and cooperation of different K^+ channel subunits has been found. Even though the *Shaker* channel was originally isolated in *Drosophila* nerve cells, some properties and structures of animal channels were found in plant *Shaker*-type channels too. This study sheds light on the new aspect of *Shaker*-type channels in whole organisms. Taking into consideration the distribution of *Shaker*-type channels in bacteria, animals and plants, more knowledge on the diverse function and physiological role of these channels can be expected from future studies on plant membrane transport.

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