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Identification and Disruption of a Plant Shaker-like Outward Channel Involved in K^+ Release into the Xylem Sap

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Summary

SKOR, a K^+ channel identified in *Arabidopsis*, displays the typical hydrophobic core of the Shaker channel superfamily, a cyclic nucleotide-binding domain, and an ankyrin domain. Expression in *Xenopus* oocytes identified SKOR as the first member of the Shaker family in plants to be endowed with outwardly rectifying properties. *SKOR* expression is localized in root stelar tissues. A knockout mutant shows both lower shoot K^+ content and lower xylem sap K^+ concentration, indicating that SKOR is involved in K^+ release into the xylem sap toward the shoots. *SKOR* expression is strongly inhibited by the stress phytohormone abscisic acid, supporting the hypothesis that control of K^+ translocation toward the shoots is part of the plant response to water stress.

Introduction

Potassium is a major inorganic constituent of the living cell and the most abundant cation in the cytosol. K^+ ions are involved in various functions such as neutralization of nondiffusible negative charges, regulation of the osmotic potential of the cell, and long- and short-term control of cell membrane polarization. For physico-chemical reasons (Eisenman, 1961), no other cation can replace K^+ in this set of functions.

In the trophic chain, plants play a key role in acquiring and accumulating potassium from the soil. The plant root is a complex organ that has evolved to exploit soil mineral resources, selectively taking up nutrient ions and translocating them toward the aerial parts. The endodermis, a ring of cells separating the cortex from the stele, plays a key role in controlling the flux of ions entering the xylem vessels for transport to the shoots. The radial- and cross-walls of the endodermal cells are impregnated with hydrophobic compounds offering a high resistance to ion diffusion. They thereby form a barrier that isolates the cortical cell wall continuum (cortical apoplast) from the stelar one, preventing free (i.e., uncontrolled) diffusion of ions between the former and the latter apoplast. The only pathway that circumvents this barrier, as soon as functional endodermis has differentiated, is the continuum of the cytoplasms linked by plasmodesmata (symplasm) extending from the epidermis to the parenchyma bordering the xylem vessels. The radial pathway of an ion from the soil solution to the xylem vessels, which form part of the stelar apoplast, therefore involves at least two transmembrane transport events: first in the epidermis or in the cortex, for entering the symplast upstream from the endodermal barrier, and second in the stele, for leaving the symplast downstream from this barrier.

Stelar cells are hardly accessible to current electrophysiological techniques, since they are buried in the heart of the root. Therefore, little is known on the K^+ transport systems operating on their membrane. However, in some species such as barley and maize, the cortex can be stripped off from the stele, enabling isolation of protoplasts from stelar cells, which can be used in patch clamp experiments. Outwardly rectifying, voltage-gated K^+ (K_{out}) channels were thereby shown to dominate the K^+ conductance of most stelar cells (Wegner and Raschke, 1994; Roberts and Tester, 1995). These channels are believed to drive K^+ release into the upward-flowing xylem sap. The ascent of sap is promoted by transpiration that takes place in the leaves through the stomatal pores. In guard cells, which edge stomatal pores, rapid efflux of K^+ ions through K_{out} channels results in decreased osmotic pressure, thereby causing reduction in the pore aperture and limiting transpiration (but also the rate of CO_2 fixation). The stress phytohormone abscisic acid (ABA), notably synthesized in case of water stress, has a positive control on guard cell K_{out} channels, a phenomenom that plays a key role in the plant response to drought (MacRobbie, 1997). Interestingly, ABA has been recently found to significantly reduce activity of stelar cell K_{out} channels in maize, supporting the hypothesis that control of K^+ translocation toward the shoots is also part of an important adaptation of the plant to survive drying soils (Roberts, 1998).

As mentioned above, K_{out} channels expressed in stelar tissues in roots and in guard cells in the aerial parts are inversely regulated by ABA and play key roles in the whole plant physiology by controlling the xylem sap loading and flowing. The molecular identification of these channels is therefore eagerly awaited. So far, the majority of knowledge at the molecular level concerns two inward K^+ channels (K_{in}) from *Arabidopsis thaliana*, AKT1 (Sentenac et al., 1992; Gaymard et al., 1996) and KAT1 (Anderson et al., 1992; Schachtman et al., 1992). These channels share structure and sequence homologies with animal K^+ channels of the Shaker superfamily (Jan and Jan, 1992). From its expression pattern, AKT1 was suggested to be involved in K^+ uptake from the soil solution (Lagarde et al., 1996), and this has just been

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confirmed using a knockout mutant (Hirsch et al., 1998). Expression data and functional analyses support the hypothesis that KAT1 mediates K^+ influx in guard cells (Nakamura et al., 1995; Ichida et al., 1997).

Here, we report the identification, functional characterization, expression, and function of a K^+ channel from *Arabidopsis*. This channel belongs to the same family as AKT1 and KAT1 but, in contrast to the other plant Shaker-like channels functionally characterized to date, it displays outwardly rectifying properties. The corresponding gene is specifically expressed in the root stele (pericycle and stelar parenchyma). This channel has therefore been named SKOR, for *s*telar K^+ *o*utward *r*ectifier. Both the functional properties and the expression pattern of this channel suggest that it is involved in K^+ release into the xylem sap. This hypothesis has been confirmed using a reverse genetic approach. Interestingly, ABA rapidly and strongly represses *SKOR* gene expression.

Results

Molecular Cloning of SKOR

Pore-forming domains of cloned *Arabidopsis* K^+ channels were used to search related sequences in the GenBank database. An *Arabidopsis* expressed sequence tag (EST Z33794) was identified, corresponding to a partial copy (1.8 kb; incomplete at the 5′) of a mRNA coding for a K^+ channel belonging to the *Arabidopsis* KAT1/AKT1 K^+ channel family (Chérel et al., 1996). A DNA probe was derived from this sequence and used to screen both a genomic library (Voytas et al., 1990) and a cDNA library (Newman et al., 1994, provided by the ABRC). A genomic clone (5.7 kb; 1.8 kb of promoter region) was obtained. Since screening of the cDNA library resulted in the isolation of partial cDNA, the full-length sequence (2.6 kb) was determined by 5′-RACE. The full-length cDNA was reconstituted by PCR. Sequence comparison of the genomic clone with the cDNA (accession numbers: AJ223357 and AJ223358) indicates that the coding sequence is interrupted by ten introns (Figure 1).

Primary Structure

SKOR-deduced polypeptide is 828 amino acids long, with a predicted molecular mass of 93.8 kDa (Figure 1A). Sequence comparisons with known plant K^+ channels (not shown) identified typical domains (Chérel et al., 1996): a hydrophobic core with six transmembrane segments named S1 to S6, a putative cyclic nucleotide-binding domain, and an ankyrin domain (Figure 1A). This sequence and structural pattern indicate that SKOR belongs to the AKT1/KAT1 family and, hence, to the Shaker superfamily (Jan and Jan, 1992). In the SKOR channel, as in Shaker channels, S4 contains positively charged amino acids (Figure 2A). This segment has been shown to be a voltage-sensing domain in voltage-gated channels (Jan and Jan, 1997). The highest degree of sequence identity among Shaker channels is found in a domain named P (for Pore), located between S5 and S6, which has been shown to be involved in the formation of the aqueous pore and to control the ionic selectivity of

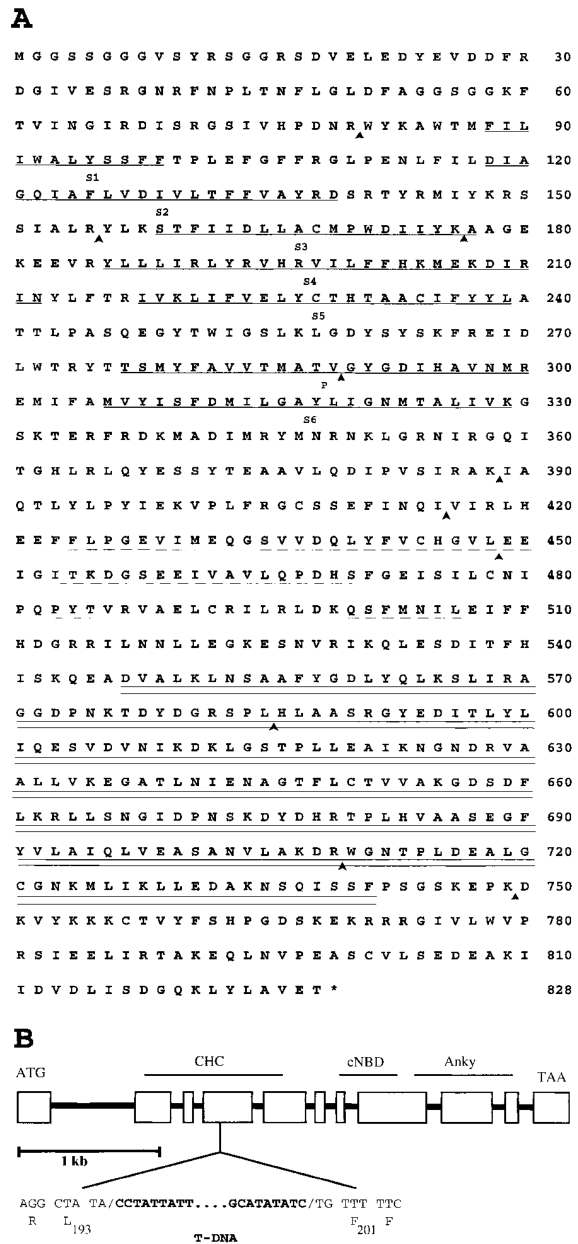

Figure 1. Deduced Amino Acid Sequence Encoded by *SKOR* cDNA, Gene Structure and Position of the T-DNA Insertion in the Disrupted Gene

(A) Sequence analysis of SKOR-predicted polypeptide (93.8 kDa) reveals the presence of a channel hydrophobic core typical of the K^+ channel Shaker superfamily, with six putative transmembrane segments (S1 to S6) and a pore-forming domain (P) between S5 and S6 (single line underlined). As in the *Arabidopsis* AKT1 channel, the channel core is followed by a putative cyclic nucleotide-binding domain and an ankyrin domain (dashed line and double line underlined, respectively). The arrowheads mark the positions of the ten introns identified in the genomic clone by comparison with the cDNA sequence.

(B) Schematic diagram of the genomic clone indicating the site of insertion of the disrupting T-DNA and the gene/T-DNA junction sequences. Boxes, exons; CHC, channel hydrophobic core; cNBD, putative cyclic nucleotide-binding domain; and Anky, ankyrin domain.

A S4 segment

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SKOR   L L L I R L Y R V H R V I L F F H K M E K D I R I N   212
AKT1   F N M L R L W R L R R V G A L F A R L E K D R N F N   185
AKT2   L G L L R F W R L R R V K H L F T R L E K D I R Y S   210
KAT1   L S M L R L W R L R R V S S L F A R L E K D I R F N   192
Sha    L A I L R V I R L V R V F R I F K L S R H S K G L Q   431
Eag    F S A L K V V R L L R L G R V V R K L D R Y L E Y G   368
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B P domain

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SKOR   T S M Y F A V V T M A T V G Y G D I H A V N M R E M   302
AKT1   T S M Y W S I T T L T T V G Y G D L H P V N T K E M   266
AKT2   A A I Y W S I T T M T T V G Y G D L H A S N T I E M   291
KAT1   T A L Y W S I T T L T T T G Y G D F H A E N P R E M   274
Sha    D A F W W A V V T M T T V G Y G D M T P V G F W G K   456
Eag    T A L Y F T M T C M T S V G F G N V A A E T D N E K   467
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C S5-P interdomain

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SKOR   T L P A S Q E G Y T W I G S L K L G D Y S Y S K F R E   268
AKT1   R N S N - - P A K T W I G A N - - - - - - - V A N F L E   233
AKT2   R Y P H - - Q G K T W T D A I - - - - - - - - P N F T E   257
AtKC1  H Y P R - - P T D T W I G S Q - - - - - - - V E D F K E   269
KAT1   R Y P N - - P R K T W I G A V - - - - - - - Y P N F K E   240
KAT2   Q Y H D - - P T K T W I G A V - - - - - - - Y P N F K E   108
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Figure 2. Analysis of the SKOR Polypeptide Sequence

Amino acids are numbered from the initiator ATG. Residues identical to SKOR are boxed in black, and homologous residues are in gray.

(A and B) Sequence alignment of the putative voltage sensor segment, S4, and of the pore-forming domain, P, of SKOR with the corresponding domain of inwardly rectifying K^+ channels from *Arabidopsis* (AKT1, AKT2, KAT1) and outwardly rectifying K^+ channels from *Drosophila* (Sha, Eag): AKT1 (Sentenac et al., 1992), AKT2 (Cao et al., 1995), KAT1 (Anderson et al., 1992), Sha (for Shaker; Papazian et al., 1987), Eag (Warmke et al., 1991).

(C) Sequence alignment of the S5-P interdomain of every *Arabidopsis* Shaker-like K^+ channel described to date. AtKC1 (EMBL accession number: Z83202; Dreyer et al., 1997) and KAT2 (partial cDNA; EMBL accession number: U25694; Butt et al., 1997) are as yet uncharacterized.

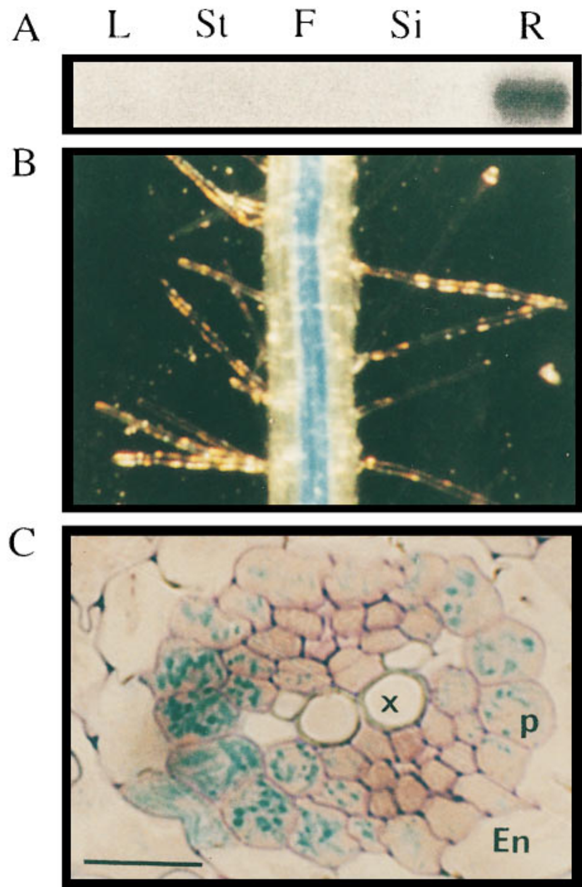


Figure 3. Expression Pattern of the *SKOR* Gene in *Arabidopsis*

(A) Northern blot analysis indicates that *SKOR* is expressed only in roots. Plants were grown for 40 days in soil. Total RNA was isolated from leaves (lane L), stems (lane St), flowers (lane F), siliques (lane Si), and roots (lane R). Ten micrograms total RNA per lane, hybridized with ^{32}P-labeled *SKOR* cDNA probe.

(B and C) GUS activity in transgenic *Arabidopsis* expressing the *GUS* reporter gene under the control of the *SKOR* gene promoter region. The cross section (C: bar: 50 μm) was 3 μm thick. En, endodermis; P, pericycle; X, xylem vessel.

the channel. A hallmark GYGD motif is present in the C-terminal half of the P domain in highly K^+ selective channels (Heginbotham et al., 1992) and is also present in SKOR (Figure 2B). Evidence has been obtained indicating that S4 and P domains in KAT1 do play the same role as their counterparts in the *Drosophila* Shaker channel (Uozumi et al., 1995; Dreyer et al., 1997). Shaker channels consist of four subunits arranged around a central pore (MacKinnon, 1991). AKT1 polypeptides also have been found to assemble into tetramers (Daram et al., 1997). A first interaction within the AKT1 tetrameric structure involves contact between the putative cyclic nucleotide domains. A second interaction has been found, involving the 80 last amino acids of the polypeptide, the region lying between the channel hydrophobic core, and the putative cyclic nucleotide-binding domain (Daram et al., 1997). As these interacting regions are highly conserved between AKT1 and SKOR (not shown), the structural organization of AKT1 probably extends to SKOR.

The highest degree of similarity between SKOR, other plant K^+ channels of the AKT1/KAT1 family, and animal K^+ channels of the Shaker superfamily are found in the region extending from S4 to the end of the S6 segment. In this region, SKOR shares about 63% similarity (48% identity) with AKT1 and KAT1, and 46% similarity (27% identity) with the *Drosophila* Shaker channel (Papazian et al., 1987). A phylogenetic tree derived using the S1-S6 hydrophobic core of the plant K^+ channels from the Shaker family presently cloned indicates that SKOR is the most divergent member (data not shown). The presence of additional residues in this channel just upstream from the P domain is the most distinctive difference from the other plant K^+ channels (Figure 2C). No counterpart for these additional residues was found in animal Shaker K^+ channels (Figure 2C).

Expression of *SKOR* Gene in *Arabidopsis*

Southern blot analysis of *Arabidopsis* DNA indicated that SKOR is encoded by a single gene (not shown). The *SKOR* locus is localized on chromosome 3, between nga32 and nga172 (marker called AKT4; Camilleri et al., 1998). Northern blot experiments detected *SKOR* mRNA in roots only (Figure 3A). RT-PCR experiments indicated that *SKOR* expression is restricted to this organ (not shown). In situ hybridization experiments performed to locate the expression of *SKOR* in roots failed to detect clearly the transcript, probably due to the low level of

expression of the gene. Localization of *SKOR* expression was therefore investigated using transgenic plants carrying the *E. coli* β-glucuronidase gene (*GUS*) under the control of the *SKOR* promoter region (1.8 kb). Reporter gene activity was analyzed on the F1 progeny of five independent transgenic plants. GUS activity was only detected in the inner tissues of the root (Figure 3B). Cross-section analysis reveals that GUS activity was mainly localized in the pericycle cells and in the parenchyma cells surrounding the xylem vessels (Figure 3C). Treatments which modulate *SKOR* mRNA accumulation (e.g. abscisic acid, see Figure 7) also affected *GUS* gene activity in the same manner in the transgenic plants, supporting the hypothesis that the chimeric reporter gene was indeed a good marker of *SKOR* expression and localization (not shown).

Functional Characterization

In *Xenopus* oocytes injected with *SKOR* cRNA, depolarization of the membrane elicited an outward current (Figure 4A) that was not recorded in control oocytes injected with water (data not shown). The exogenous macroscopic current displayed a slow sigmoidal activation kinetic and reached a steady-state value within 2 s. No inactivation could be seen even during depolarizing pulses up to 10 s (not shown). The steady-state I-V plots show a strong outward rectification and reveal a positive shift of the threshold activation potential toward the K^+ equilibrium potential (E_K) upon increasing the external concentration of K^+ in the 10 to 100 mM range (Figure 4B). The latter phenomenon has already been reported for both plant (Thiel and Wolf, 1997) and animal (Kv1.4 and Eag; Pardo et al., 1992; Brüggemann et al., 1993) voltage-gated K^+ channels.

The reversal potential (E_{rev}) of SKOR current was determined at different external K^+ concentrations (Figures 4C and 4D). Following a change in the external K^+ concentration from 10 to 100 mM, E_{rev} shifted by 56 mV, remaining close to E_K (Figure 4D, inset) indicating that the outward currents shown in Figure 4A were mainly carried by K^+ ions. Determination of reversal potential under pseudo-bi-ionic conditions allowed relative permeability ratios to be derived, which indicate that SKOR channel displays the following permeability sequence (Eisenman's series IV): K (1) > Rb (0.58 ± 0.08)> Cs (0.15 ± 0.05)> Na (0.046 ± 0.009)> Li (0.033 ± 0.010) (ion symbol followed by the corresponding permeability coefficient relative to that for K^+, mean ± SD, n=5).

We also investigated SKOR channel permeability to Ca^{2+}. The endogenous Ca^{2+}-activated Cl^- current was prevented by chelating the cytoplasmic Ca^{2+} with BAPTA injected into the oocyte (Galzi et al., 1992). In these conditions, we recorded SKOR current in solutions containing either 1 mM or 90 mM Ca^{2+}, together with 10 mM K^+. The presence of K^+ was required because of the allosteric regulation of SKOR activity by external K^+ (see below), and it should be noted that such regulation hinders electrophysiological analysis of Ca^{2+} influx through K^+ channels. The reversal potential of the current shifted toward a more positive value (from −56.3 to −41.1 mV, shift of 15.2 ± 1.9 mV, n=4) upon increasing the Ca^{2+} concentration from 1 to 90 mM (not shown),

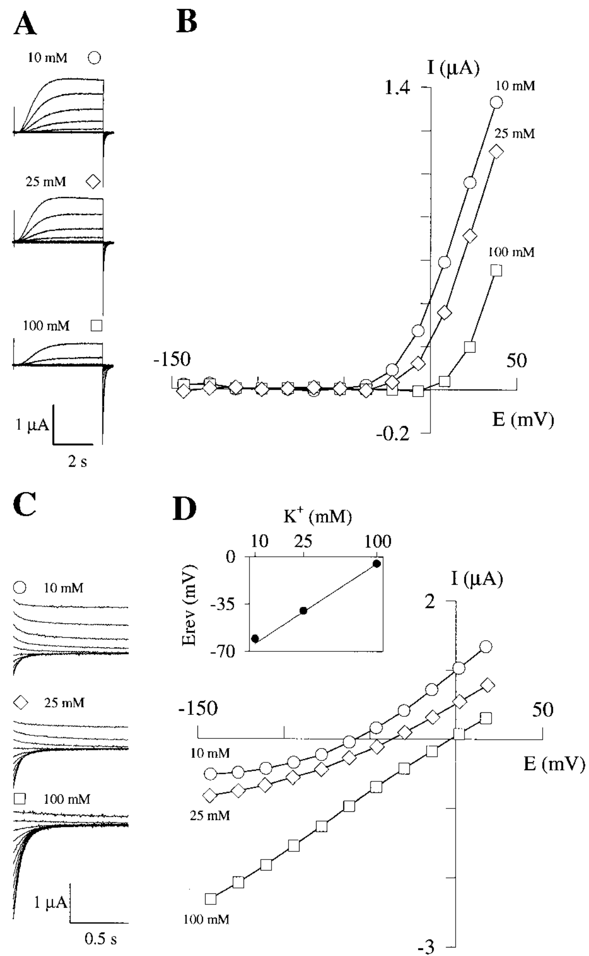


Figure 4. Functional Expression of SKOR in *Xenopus* Oocyte Assessed by Two-Electrode Voltage Clamp

Data shown were obtained from the same oocyte (injected with 30 ng of *SKOR* cRNA). The external solution contained 1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM HEPES (pH 7.4), and 10 mM KCl/90 mM NaCl (top traces in A and C, open circles in B and D), or 25 mM KCl/75 mM NaCl (middle traces in A and C, open diamonds in B and D), or 100 mM KCl (bottom traces in A and C, open squares in B and D). A linear current was mathematically subtracted after recording for leak correction.

(A and B) SKOR is an outward-rectifying channel. From a holding potential of −100 mV, the membrane potential was clamped (14 successive pulses of 4 s each) at values ranging from −140 to +40 mV with a +16 mV step. Current traces shown in (A) indicate that SKOR is a slow-activating, non-inactivating, outward-rectifying K^+ channel that displays sigmoidal activation kinetics. The steady-state current at the end of the activation step was plotted against voltage. The resulting plots (B) indicate that the potential threshold above which the current is significantly increased (activation potential) depends on the external concentration of K^+.

(C and D) SKOR is a K^+-selective channel. Reversal potential (E_{rev}) was obtained from analysis of deactivating (tail) currents elicited by a double-pulse protocol. Holding potential was −100 mV. The activating prepulse at +40 mV lasted 1 s. Tail currents were recorded during a 1 s pulse in the −140 to +20 mV range with a 16 mV step (C). The initial value of the tail current was plotted against voltage. The resulting plots (D) allowed determination of E_{rev}, which was found to depend on the external concentration of K^+. Plotting E_{rev} versus the external concentration of K^+ (inset) reveals that E_{rev} shifts by 56 mV for a 10-fold increase in external K^+ concentration, as expected for a highly selective K^+ channel. Solid line in the inset: K^+ equilibrium potential calculated with the Nernst equation.

consistent with a Ca^{2+} influx through SKOR channels. Based on this hypothesis, using an equation derived from the Goldman-Hodgkin-Katz model (Allen and Sanders, 1995), we obtained the following estimates of the permeability ratio (P_{Ca}/P_K): 0.9 ± 0.2 in 1 mM Ca^{2+} solution and of 0.04 ± 0.01 in 90 mM Ca^{2+} solution. Ca^{2+}-permeable outwardly rectifying K^+ channels have been characterized in stelar cells of barley (Wegner and De Boer, 1997) and maize (Roberts and Tester, 1997), with a similar dependency of P_{Ca}/P_K estimates on external Ca^{2+} concentrations observed for the latter.

Increasing the external K^+ concentration (i.e., decreasing the driving force for K^+ efflux) was expected to decrease the outward SKOR current. However, an increase in the outward current was observed when the external concentration of K^+ was increased in the 0–10 mM concentration range (Figure 5A). This suggests an allosteric regulation of SKOR activity by external K^+. Such a phenomenon has been described previously for plant outward K^+ channels in guard cells (Blatt and Gradmann, 1997), and in root xylem parenchyma cells (Roberts and Tester, 1995; De Boer and Wegner, 1997), and also for some animal outward K^+ channels, such as Kv1.4 and Eag (Pardo et al., 1992; Brüggemann et al., 1993). Finally, SKOR current was decreased by several blockers of K^+ channels and by verapamil (Figure 5B), a blocker of animal L-type calcium channels and also of some outwardly rectifying K^+ channels from both animal and plants (Thomine et al., 1994; DeCoursey, 1995).

SKOR Is Involved in K^+ Translocation to the Shoots

SKOR function was assessed using a reverse genetic approach based on PCR screening of insertion mutant libraries (McKinney et al., 1995; Krysan et al., 1996). A collection of T-DNA–transformed *Arabidopsis* (Bechtold et al., 1993) was screened by PCR using primers corresponding to the T-DNA sequence (Bouchez et al., 1993) and to the *SKOR* gene, leading to the identification of a positive line and subsequently of a homozygous knockout mutant, *skor-1*. The T-DNA was inserted in the fourth exon, in the region encoding the S4 segment (Figure 1B). This localization ensures that no functional channels could be expressed. Furthermore, no transcripts likely to correspond to the *SKOR* gene were detected in Northern blot experiments (data not shown).

Disruption of the *SKOR* gene had no effect on root K^+ content, but resulted in a ca. 50% decrease in shoot K^+ content (Figure 6A). This decrease in K^+ content was not observed in *skor-1* mutant plants transformed with a genomic fragment containing the *SKOR* gene, indicating that genetic complementation was achieved (Figure 6A). Assaying K^+ in root exudate (Figure 6B) revealed that the decrease in shoot K^+ content observed in *skor-1* mutant plants is likely to result from a decrease in the rate of K^+ translocation from roots to shoots via the xylem sap flux. Inorganic cation assays in leaves indicated that the decrease in K^+ content was essentially compensated by an increase in Ca^{2+} content (Figure 6C).

SKOR mRNA Accumulation Is Modulated by Abscisic Acid

Previous physiological studies suggest that ABA plays a role in the regulation of ion translocation from roots

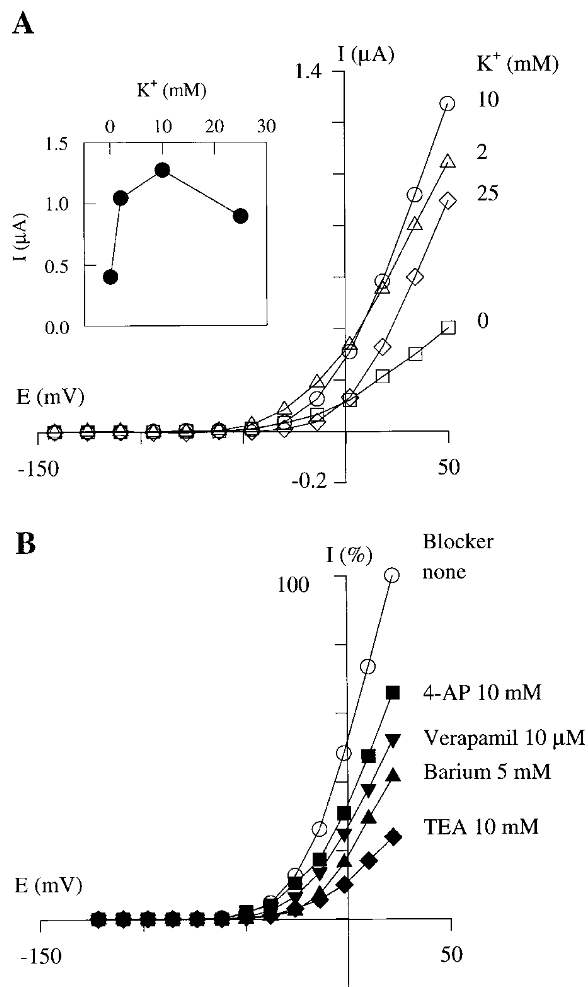
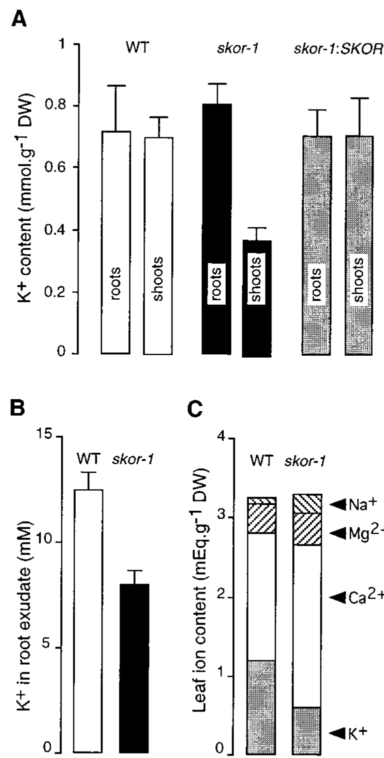


Figure 5. Effect of External K^+ Concentration and of Channel Blockers on SKOR Current

Membrane currents were recorded from oocytes injected with 30 ng of *SKOR* cRNA. A linear current was mathematically subtracted after recording for leak correction.

(A) Effect of external K^+ concentration on SKOR steady-state current. The external solution contained 1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM HEPES (pH 7.4), and 2 mM KCl/98 mM NaCl (triangles), or 10 mM KCl/90 mM NaCl (open circles), or 25 mM KCl/75 mM NaCl (diamonds), or 100 mM NaCl (squares). From a holding potential of −100 mV, the membrane potential was clamped at values from −142 to +50 mV with a +16 mV step during thirteen 4 s-long successive pulses. While the driving force for outward K^+ current was decreased by increasing external K^+ concentration from 0 to 10 mM, the current increased. This is shown by the inset where the value of the steady-state current at +50 mV is plotted against the external K^+ concentration.

(B) K^+ channel blockers inhibit SKOR. NaCl was introduced in the external solution (1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM HEPES [pH 7.4], 10 mM KCl) so that the sum of the concentration of this salt and that of the blocker (indicated on the plot) was 90 mM. Blockers: 4-aminopyridine (4-AP, squares), verapamil (down-pointing triangles), Ba^{2+} (up-pointing triangles), tetraethylammonium (TEA, diamonds). From a holding potential of −100 mV, the membrane potential was clamped to values from −122 to +22 mV with a +12 mV step during thirteen 4 s-long successive pulses. Data are shown as the percent of the current value obtained in the absence of any blocker at +22 mV.



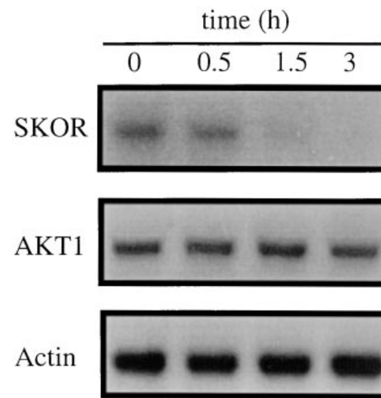


Figure 7. Northern Blot Analysis Indicates that *SKOR* mRNA Abundance Is Decreased upon Addition of Abscisic Acid to the External Solution

Ten micromolar abscissic acid was added to the culture solution (Murashige and Skoog medium supplemented with 1% sucrose and 2.5 mM MES-KOH [pH 5.7]) of *Arabidopsis thaliana* plantlets hydroponically grown for 14 days. Roots were excised 0.5 hr, 1.5 hr, or 3 hr later for RNA extraction (lanes 0.5, 1.5, and 3, respectively). Control (lane 0): roots were excised just before the addition of abscisic acid. The blot (10 μg total RNA per lane) was successively hybridized with ^{32}P-labeled DNA probes corresponding to *SKOR*, *AKT1*, and actin genes: SKOR, AKT1, and actin autoradiogram respectively.

Figure 6. Disruption of the *SKOR* Gene Results in a Decrease in K^+ Translocation to Shoots

Wild-type (WT), *skor-1*, and transgenic *skor-1* seedlings transformed with a construct containing the *SKOR* gene were grown in attapulgite-peat compost for 3–4 weeks. Ions were extracted with 0.1 N HCl and assayed by flame spectrophotometry.

(A) Root and shoot K^+ contents. Root systems were separated from the aerial parts by excision below the rosette (mean values for ten plants; vertical bars: standard errors).

(B) K^+ concentration in root exudate. Shoots were excised under the rosette, and the root exudates were collected and assayed for K^+ (30 plant samples).

(C) Leaf contents of the major inorganic cations. One leaf from the third pair of leaves (the largest ones at this stage) was collected from each plant (30 plant samples, all standard errors being lower than 11% of the corresponding mean value).

to shoots (De Boer and Wegner, 1997; Roberts, 1998). The effect of ABA on *SKOR* expression in roots was therefore investigated in Northern blot experiments. Membranes were also hybridized with probes derived from actin (Nairn et al., 1988) and *AKT1*. The autoradiograms shown in Figure 7 revealed that *SKOR* transcript abundance decreased rapidly after ABA addition to the external solution: ca. 75% and 100% decrease after 1.5 hr and 3 hr, respectively (from quantitative analysis of the corresponding blots with a Storm scanning system, Molecular Dynamics; data not shown). In contrast, *AKT1* and actin transcript levels were hardly modified by the plant hormone treatment.

Discussion

Presently, SKOR is the only K^+ channel cloned in the plant kingdom to be both an outward rectifier (Figure 4) and a member of the Shaker superfamily (Figure 2). Within the Shaker superfamily of K^+ channels, SKOR may represent a link between members cloned in animal cells (outwardly rectifying) and members cloned in plant cells (inwardly rectifying). Comparative studies of the structure–function relationship of SKOR and KAT1 (both functional when expressed in *Xenopus* oocytes) could therefore provide clues toward understanding the molecular mechanisms underlying rectification of voltage-gated K^+ channels. Furthermore, as the electrophysiological properties of SKOR are reminiscent of those of as-yet-uncloned K_{out} channels characterized in guard cells (Thiel and Wolf, 1997), the use of *SKOR*-derived probes might help in the molecular identification of these channels.

The *SKOR* gene is expressed in the root pericycle and stelar parenchyma cells (Figure 3), and its disruption results in strongly decreased K^+ translocation toward the shoots (Figure 6). Together with the outward rectification, these data provide evidence that SKOR is involved in K^+ secretion into the root stelar apoplasm, toward the xylemic sap and the shoots. Taken as a whole, the results shed light on previous reports and discussions in the literature regarding two points: the site and the energetic coupling of ion secretion into the xylem sap. It was explicitly or implicitly proposed that ion secretion occurs across plasmalemma of the parenchyma cells bordering the xylem vessels (Clarkson, 1993). The expression data shown in Figure 3 provide evidence that the pericycle cells, together with the xylem parenchyma cells, are involved in this function. The energetic coupling of K^+ secretion into the xylem sap has been widely discussed. In the recent literature, characterization of K_{out} channels expressed in protoplasts obtained from stelar cells and analysis of K^+ translocation

into the xylem sap have led to the conclusion that this transport is channel-mediated (Wegner and Rashke, 1994; Roberts and Tester, 1995; Roberts and Tester, 1997; Wegner and De Boer, 1997; review by Maathuis et al., 1997). In these reports, it was assumed that the potential difference between the stelar cell cytoplasm and the xylem sap is positive to E_K. As there is no direct evidence to support this assumption, the possibility that the driving force would be against passive efflux of K^+ toward the xylem sap has been considered (review: Kochian and Lucas, 1988; Clarkson, 1993). The controversy mainly arises as reliable measurements of both the membrane potential and the apoplastic K^+ concentration of stelar cells are difficult to obtain. In this context, the phenotype of the *skor-1* knockout mutant (Figure 6) clearly indicates that passive (since channel-mediated) transport of K^+ does occur in stelar cells, contributing to at least 50% of K^+ translocation toward the shoots in our experimental conditions.

Patch-clamp recordings on protoplasts obtained from stelar cells (barley and maize roots) have identified two types of channels able to drive outward K^+ current (Wegner and Raschke, 1994; Roberts and Tester, 1995; Roberts and Tester, 1997; Wegner and De Boer, 1997; Roberts, 1998) named KORC (for *K*$^+$-selective *o*utwardly *r*ectifying *c*hannels) and NORC (for *n*onselective *o*utwardly *r*ectifying *c*hannels). Functional features of the SKOR channel are very similar to those reported for channels of the KORC type. Specifically, these similar features are: (i) sigmoidal activation (Figure 4A); (ii) activation potential following E_K (Figure 4B); (iii) increased magnitude of the outward current with increasing external K^+ concentration (in the 0 to 10 mM range, Figure 5A) suggesting allosteric regulation; (iv) sensitivity to K^+ channel blockers (Figure 5B); (v) Ca^{2+}-dependent deviation of E_{rev} with respect to E_K; (vi) sensitivity to the stress phytohormone ABA (Figure 7).

The Ca^{2+}-dependent deviation of E_{rev} with respect to E_K suggests that Ca^{2+} influx could occur through these channels. As discussed by Roberts and Tester (1997), such an influx could be involved in the reabsorption of Ca^{2+} from the transpiration stream of the stelar apoplast or in signal transduction. The observation that the decrease in K^+ shoot content due to *SKOR* gene disruption is accompanied by an increase in the Ca^{2+} content (Figure 6) would rather support the former hypothesis. However, Ca^{2+} permeation through SKOR channels could perhaps be involved in the feedback control of the cell membrane channel activity, since the cytosolic concentration of Ca^{2+} in xylem parenchyma cells seems to influence the K^+-selective and nonselective outwardly rectifying conductances of the membrane (Wegner and De Boer, 1997).

ABA is involved in the control of diverse physiological processes that occur in plant tissues notably upon water stress. Increased ABA synthesis has been reported in root systems under drought conditions (Zhang and Tardieu, 1996). Diverse (although contradictory) responses of root and especially of stelar tissues to exogenously applied ABA, which result in a modification of the volume and ionic composition of the upward-flowing xylem sap, are recognized. These events, together with the well-documented stomatal response triggered by ABA in shoots (MacRobbie, 1997), could participate in an integrated, whole-plant response to water stress. In a recent paper, the inhibition of K_{out} channels in stelar cells was unambiguously demonstrated in ABA-pretreated plants (Roberts, 1998). It is known that root growth under reduced soil water content requires considerable osmotic adjustment in apical growing segments to maintain a high turgor. This is usually achieved (Pritchard et al., 1991), and the largest part of solute accumulation in growing elements is due to an increase in K^+ content (Sharp et al., 1990). Decreased K^+ release into the xylem sap, in response to increased apoplasmic ABA concentrations, could be involved in this response. The cloning of the *SKOR* gene and the finding that its expression is sensitive to ABA are therefore significant results in this field of plant physiology.

Experimental Procedures

Plant Culture

Plants were either (i) grown in attapulgite-peat compost in a greenhouse, or (ii) hydroponically grown on sterile solutions (25°C, with a photoperiod of 16 hr light, 100 $\mu E.s^{-1}.m^{-2}$), or (iii) grown on agar plates in petri dishes (21°C/18°C 16 hr/8 hr day/night, 150 $\mu E.s^{-1}.m^{-2}$), depending on the subsequent experiment: (i) K^+ assays in shoots and roots and in root exudate, (ii) effect of the plant hormone ABA on K^+ channel expression, or (iii) *GUS* reporter gene expression analysis, respectively. Germination was synchronized by placing seeds, after sowing, for 2 days at 4°C in the dark. The compost was a mix (50–50, v-v) of peat compost (Humin substrat N2 Neuhaus, Klasmann-Deilmann, Germany) and of attapulgite clay minerals (Oil Dri US special, Lobbe, Germany), which facilitated the recovery of entire root systems. For hydroponic cultures, seeds were surface-sterilized and transferred into Petri dishes in a liquid medium containing Murashige and Skoog salts (M5524, Sigma) and vitamins (M3900, Sigma), 1% sucrose, and 2.5 mM MES-KOH (pH 5.7). The dishes were kept in the dark at 4°C for 2 days, and then transferred into the growth chamber, and incubated with constant shaking (ca. 80 rpm) for 3 days. The seedlings were then transferred onto nylon grids (6 cm × 6 cm, 0.5 mm mesh, ca. 20 seedlings per grid), layered onto culture solution (60 ml; same composition as above) in Magenta boxes (base: 7.6 cm × 7.6 cm; height: 10.2 cm), and incubated with constant shaking. The culture medium was changed after 7 days, and the plants were allowed to develop for 7 days more before ABA was introduced to the solution. For growth on agar plates, seeds were surface-sterilized and placed on the same medium as above but supplemented with 0.8% agar.

Molecular Techniques

Standard molecular biology techniques were performed according to Ausubel et al. (1993). Total RNA was extracted according to Lobréaux et al. (1992). Genomic DNA was isolated as described by Dellaporta et al. (1983). Sequencing was performed using the T7 sequencing kit (Pharmacia). DNA amplification experiments were performed with *Thermus brockianus* thermostable polymerase (ExtraPol I, Eurobio) following the manufacturer's recommendations.

Generation of the Full-Length *SKOR* cDNA

The EST clone (partial cDNA, accession number Z 33794) was provided by Dr. Desprez (Laboratoire de Biologie Cellulaire, INRA Versailles, France). The DNA insert (1.8 kb) was sequenced. The full-length sequence of the cDNA was obtained by 5′-RACE. Four independent clones were sequenced. They all shared the same sequence. The full-length cDNA (2.6 kb) was reconstituted by PCR on an *Arabidopsis* cDNA library (Minet et al., 1992). It was sequenced to ensure identity with the 5′-RACE–amplified fragments, with the EST, and with the genomic clone.

Genomic Clone

The cDNA was used to screen an *Arabidopsis* genomic library prepared in λFix (Voytas et al., 1990). From about 5.5×10^5 plaque-forming units, six independent clones were selected and purified.

Bacteriophage genomic DNA was prepared by PEG precipitation according to Sambrook et al. (1989). Four clones showed a 5.7 kb SacI fragment that hydridized with the cDNA probe. This fragment was cloned in pBS-SK (Stratagene) and sequenced.

Expression in *Xenopus* Oocytes and Electrophysiology

SKOR coding sequence was cloned between the noncoding 5′ and 3′ flanking regions of *Xenopus* β-globin gene in a transcription vector (pBSTA). In vitro transcription (T7 RNA polymerase) produced *SKOR* cRNA, which was injected into *Xenopus* oocytes (30 nl of cRNA solution containing ca. 1 μg cRNA.μl^{-1} per oocyte). Control oocytes were injected with 30 nl of deionized water. Whole-cell currents were recorded using the two-electrode voltage-clamp technique, 3 to 7 days after the oocyte injection as previously described (Véry et al., 1995).

Transgenic Plants

The 5.7 kb SacI genomic fragment corresponding to the *SKOR* gene (1.8 kb promoter region) was introduced in pBS-SK, the BamHI polylinker site situated upstream of the promoter region. A NcoI site was introduced just upstream of the initiator ATG (detailed information available upon request). This construct was digested by BamHI and NcoI and the resulting fragment was cloned in pBI320.X (Dr. R. Derose; this plasmid carries a unique NcoI site at the first inititiation codon of the promoterless *GUS*-3′ nopaline synthase gene), leading to a translational fusion between the *SKOR* promoter region and *GUS* coding sequence. This construct was introduced into the SacI site of pMOG 402 binary vector (Dr. H. Hoekema). The resulting plasmid was introduced into *Agrobacterium tumefasciens* MP90 (Höfgen and Willmitzer, 1988). *Arabidopsis thaliana* was transformed with agrobacteria using the infiltration method according to Bechtold et al. (1993). GUS histochemical staining was performed according to Lagarde et al. (1996). Cross sections of GUS-stained material were prepared on hydroxyethyl methacrylate (Technovit 7100, Heraus-Kulzer GmbH, Wehrheim, Germany) embedded tissues with an LKB microtome and were counterstained by ponceau 2R-toluidine blue staining (detailed information available upon request).

Isolation of a T-DNA-Tagged Plant Disrupted in the *SKOR* Gene

A library of *Arabidopsis* T-DNA insertion mutants (constructed by Laboratoire de Génétique et Amélioration des Plantes, INRA Versailles, France; about 22,000 plants) was screened by PCR using primers corresponding to the right and left borders of the T-DNA and to the *SKOR* gene. One positive line was detected, the corresponding plant was selected, and the position of the disrupting T-DNA in the *SKOR* gene was determined by sequencing PCR-amplified fragments. To ensure that physiological features observed in plants carrying the disrupting T-DNA are genetically linked to *SKOR* disruption, plants homozygous for the disruption were backcrossed with the Wassilewskija ecotype, and further studies were performed on F2 progeny homozygous for the disruption. To show complementation of *skor-1*, homozygous mutants were transformed with the 5.7 kb SacI genomic fragment corresponding to the *SKOR* gene (detailed information available upon request). K^+ accumulation experiments were performed on progeny of five independent T2 plants.

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EMBL Accession Numbers

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