Plant K^+ Channel α-Subunits Assemble Indiscriminately

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ABSTRACT In plants a large diversity of inwardly rectifying K^+ channels (K_{in} channels) has been observed between tissues and species. However, only three different types of voltage-dependent plant K^+ uptake channel subfamilies have been cloned so far; they relate either to KAT1, AKT1, or AtKC1. To explore the mechanisms underlying the channel diversity, we investigated the assembly of plant inwardly rectifying α-subunits. cRNA encoding five different K^+ channel α-subunits of the three subfamilies (KAT1, KST1, AKT1, SKT1, and AtKC1) which were isolated from different tissues, species, and plant families (*Arabidopsis thaliana* and *Solanum tuberosum*) was reciprocally co-injected into *Xenopus* oocytes. We identified plant K^+ channels as multimers. Moreover, using K^+ channel mutants expressing different sensitivities to voltage, Cs^+, Ca^{2+}, and H^+, we could prove heteromers on the basis of their altered voltage and modulator susceptibility. We discovered that, in contrast to animal K^+ channel α-subunits, functional aggregates of plant K_{in} channel α-subunits assembled indiscriminately. Interestingly, AKT-type channels from *A. thaliana* and *S. tuberosum*, which as homomers were electrically silent in oocytes after co-expression, mediated K^+ currents. Our findings suggest that K^+ channel diversity in plants results from nonselective heteromerization of different α-subunits, and thus depends on the spatial segregation of individual α-subunit pools and the degree of temporal overlap and kinetics of expression.

INTRODUCTION

Inwardly rectifying K^+ channels (K_{in} channels) have been found in all plant cells studied so far. Whereas their voltage-dependent properties were similar, their kinetics, selectivity, and susceptibility toward cationic blockers varied among cells, tissues, and plant species (see Hedrich and Dietrich, 1996, for review). Besides this large variety found in vivo, up to now on the molecular level only three different subfamilies of voltage-dependent inward K_{in} channels have been identified: they relate either to *Arabidopsis* KAT1 (Anderson et al., 1992), AKT1 (Sentenac et al., 1992) or AtKC1 (Reintanz et al., in preparation, GenBank accession no. U73325). Although these subtypes contain six transmembrane domains (S1–S6) and a pore region (P), they differ strongly in the putative extramembrane regions and in their expression pattern. The two KAT-type channels cloned as full-length clones so far, KAT1 from *Arabidopsis thaliana* and KST1 from *Solanum tuberosum*, are expressed in guard cells (Müller-Röber et al., 1995; Nakamura et al., 1995). They share an overall identity of 61%. Members of the AKT subfamily harbor, in contrast to KAT1 and KST1, a conserved ankyrin-binding domain within their C-termini. The two AKT-type channels used in this study, AKT1 from *A. thaliana*, which is predominantly expressed in roots (Basset et al., 1995; Lagarde et al., 1996), and SKT1 from *S. tuberosum* cloned from a leaf library (Müller-Röber et al., GenBank accession no. X86021) share an identity of 70%. A comparison of the AKT-type and KAT-type channels, however, revealed reciprocal identities of only ~45%. Recently, AtKC1 from *A. thaliana*, a member of a new subfamily, has been cloned (Reintanz et al., unpublished data). This channel shares identities of ~42% with KAT-type channels and of ~37% with AKT-type channels.

Functionally, plant K_{in} channels of the different types behave similarly. When expressed in yeast and animal cells like *Xenopus* oocytes or Sf9 insect cells, the gene products mediate hyperpolarization-activated, noninactivating, K^+-selective currents (Schachtman et al., 1992; Bertl et al., 1994; Bertl et al., 1995; Hedrich et al., 1995; Hoshi, 1995; Müller-Röber et al., 1995; Véry et al., 1995; Gaymard et al., 1996; Ketchum and Slayman, 1996; Marten et al., 1996). An in vivo–in vitro comparison between a cloned channel and the K^+ channel in its natural environment has only been performed for KST1 so far. When expressed in *Xenopus* oocytes this channel seems to possess all of the basic properties, such as voltage-dependence, kinetics, and selectivity, of the inward rectifier in *S. tuberosum* guard cells (Müller-Röber et al., 1995). Its pH-sensitivity and susceptibility toward Cs^+ ions, however, were less pronounced (Dietrich et al., unpublished data). This difference might indicate that KST1 is posttranslationally modified in the guard cell, or alternatively interacts with other cellular components, such as other α-subunits, β-subunits, or metabolites.

Plant voltage-dependent K_{in} channels represent members of the large gene family of potassium channels (Jan and Jan,

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1994). On the level of the amino acid sequence they are structurally related to animal voltage-dependent outward rectifiers (K_v; Chandy and Gutman, 1994) or *eag* channels (Warmke et al., 1991) containing the *Shaker*-channel-like motives S1–S6 and P. Functionally, however, they are different. Like animal inward rectifiers (K_{ir}, Doupnik et al., 1995), upon hyperpolarization K_{in} channels catalyze K^+ uptake into the cytoplasm. In the related animal field the large functional diversity of K^+ channels at least partially seems to result from heterooligomerization of α-subunits (Christie et al., 1990; Isacoff et al., 1990; Ruppersberg et al., 1990; Covarrubias et al., 1991). Voltage-dependent outwardly rectifying channels as well as inwardly rectifying channels segregate structurally and functionally into diverse subfamilies (e.g., *Shaker*, *Shal*, *Shab*, and *Shaw*, Salkoff et al., 1992; Doupnik et al., 1995). With a few recently reported notable exceptions (Glowatzki et al., 1995; Shahidullah et al., 1995; Chen et al., 1996; Hugnot et al., 1996; Pessia et al., 1996), heteromultimers are assumed not to be formed among different subclasses, but between subunits from the same subfamily only. The assembly within subfamilies seems to require highly conserved N-terminal tetramerization domains (Li et al., 1992; Shen et al., 1993).

The structural similarity of cloned plant K^+ channels to the K_v channel family and the fact that KST1 after heterologous expression differ from its in vivo counterpart suggests that also in plants K^+ channel diversity is caused by heteromultimerization.

MATERIALS AND METHODS

Electrophysiology

Experiments were performed on RNA-injected, voltage-clamped *Xenopus* oocytes using a two-electrode voltage clamp approach as previously described by Hedrich et al. (1995). RNA concentrations were quantified spectroscopically (Spectrometer DU-64, Beckmann, Germany). Because some channel properties of plant K^+-channels differ with the expression level (Véry et al., 1994, Cao et al., 1995), oocytes with similar K^+ current amplitudes were selected for analysis only.

Solutions

External standard solution was composed of 30 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM MES/Tris, pH 5.6. Solutions for Cs^+ experiments contained additionally 200 μM–2 mM CsCl. Experiments with regard to the Ca^{2+} sensitivity were performed in 30 mM KCl, and 10 mM MES/Tris, pH 5.6. Various mol fractions of 30 mM between $CaCl_2$ and $MgCl_2$ were used to maintain the ionic strength. All solutions were adjusted to 220 mosmol/kg with sorbitol.

Biophysical analysis

Relative open probability

Relative open probabilities were deduced from a double voltage-step protocol. Time- and voltage-dependent K^+ currents were elicited in response to activation pulses (1 s) to voltages between +100 mV and −170 mV in −10-mV increments. During the second voltage step (1 s) to V_F = −70 mV K^+ currents relaxed in a time-dependent manner. The instantaneous current voltage relationship [$I_0(V)$], obtained from extrapolating the relaxation time course of the second pulse to $t = 0$ with an exponential function, is proportional to the relative open probability [$p_O(V)$] at the end of the activation pulse: $I_0(V) = N \cdot i(V_F)p_O(V) = const.\ p_O(V)$, where N denotes the number of potassium channels within the membrane and $i(V_F)$ the single channel current at −70 mV [$N \cdot i(V_F) = const.$]. To make sure not to measure a leak, in the mutant KAT1-S168R, which is not entirely closed in the voltage range tolerated by the oocyte (≈−180 mV–+100 mV), 10 mM Cs^+ were used to completely block the channels at negative potentials (cf. Becker et al., 1996). Theoretical predictions for relative open probabilities in Fig. 1 *B* were calculated according to the equation

$$p_O(V) = \frac{c_{KAT1}p_O^{KAT1}(V) + c_{KAT1\text{-}S168R}p_O^{KAT1\text{-}S168R}(V)}{c_{KAT1} + c_{KAT1\text{-}S168R}},$$

where c_{KAT1} and $c_{KAT1\text{-}S168R}$ denotes the relative amount of injected KAT1- and KAT1-S168R-RNA, respectively. The relative open probabilities $p_O^{KAT1}(V)$ and $p_O^{KAT1\text{-}S168R}(V)$ were determined experimentally (cf. Fig. 1 *A*).

Ion block

To obtain the instantaneous current-voltage characteristic of the open channel [$I_T(V)$] the membrane voltage after approaching steady-state acti-

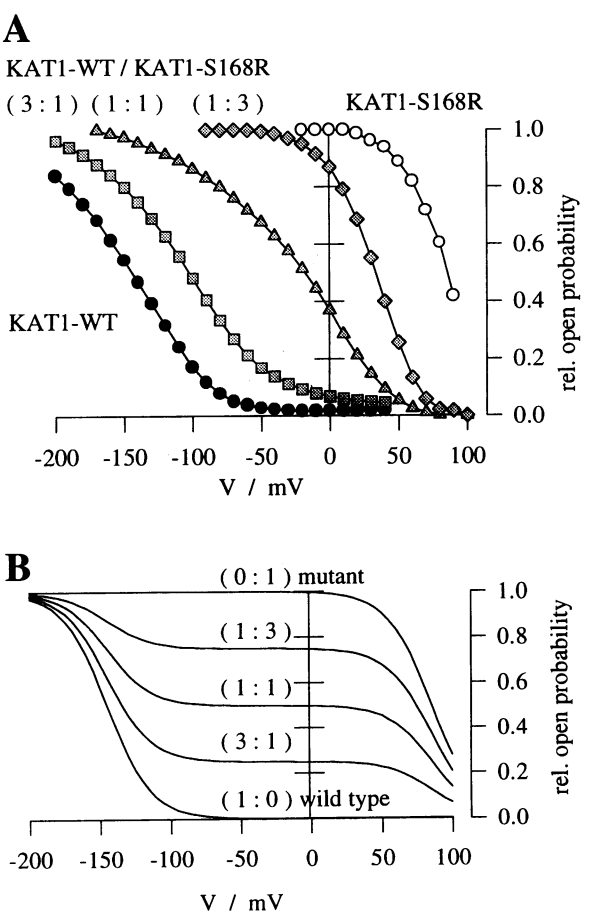

FIGURE 1 Co-expression of wild-type KAT1 and mutant KAT1-S168R in *Xenopus* oocytes. (*A*) Voltage-dependent relative open-probabilities of the K^+ channel homomers and heteromers recorded in representative oocytes injected with different fractions of KAT1-WT/KAT1-S168R cRNA: wild type (*closed circles*), KAT1-S168R (*open circles*), and 3:1 (*squares*), 1:1 (*triangles*), and 1:3 (*rhombi*) mixtures. (*B*) Theoretical predictions for relative open-probabilities for the experiment shown in (*A*) under the assumption that plant K_{in} channels are monomers.

vation at $V = -150$ mV was stepped subsequently to various values between +20 mV and −180 mV. $I_T(V)$ relationships were deduced from extrapolating the tail current onset to $t = 0$. Current-voltage relationships of tail-currents in the presence of an antagonist were fitted according to the Woodhull model (Woodhull, 1973):

$$I_T(V) = I_T^0(V)\frac{1}{1 + e^{-((zF/RT)\delta(V - V_{Block\,1/2}))}}$$

where $I_T^0(V)$ denotes the current in the absence of the blocking ion, z its valence and δ the fraction of the transmembrane voltage sensed by the ion. F, R, and T have their usual meaning. The half-blocking voltage $V_{Block\,1/2}$ could be obtained from $I_T(V_{Block\,1/2}) = \frac{1}{2} I_T^0(V_{Block\,1/2})$.

Molecular biology

The mutant KAT1-T256E was generated according to Becker et al. (1996). KAT1S168R*pYES2–2 is based on the wild-type KAT1*pYES2–2 received from Rick Gaber. The mutation was introduced by using standard polymerase chain reaction-mediated cassette mutagenesis. The segment between *Eco*R1 and *Xcm*1 of the coding sequence area was amplified using two oligonucleotides, with one of them containing the S168R mutation. The amplified segment was digested with *Eco*R1 and *Xcm*1 and ligated into KAT1*pYES2–2 digested with *Eco*R1 and partially with *Xcm*1. The PCR-amplified segment was sequenced by the University of Iowa DNA core (ABI sequencer). Finally, the reconstituted mutant cDNA was cloned into the pGEMHE expression cassette (Liman et al., 1992). To produce the mutant KST1-H271R, a silent mutation creating a novel *Bgl*II site was first introduced into the KST1 coding region upstream of the H271 codon. This modification was achieved by exchanging the *Sph*I fragment of the *KST1* cDNA in the plasmid pKST1#8–1 (Müller-Röber et al., 1995) with a corresponding PCR fragment amplified with primers P1 (5′-CCATTC-CAATCATTGATCCTCG-3′) and P2 (5′-CTCAGCATGCAGATCTC-CATAACCG-3′). In the following step, the *Bgl*II/*Sty*I fragment of the modified *KST1*-sequence was replaced by a fragment generated via PCR using reverse primer P3 (5′-TTGCTTCGGAGGGAAGTATTCAGCTTC-3′) and forward primer P4 (5′-CGGTTATGGAGATCTGCGAGCT-GAGAACTC-3′).

Vectors used for the production of cRNA were based on plasmid pGEMHE (Liman et al., 1992). Construction of the *KST1* plasmid was described in Müller-Röber et al. (1995) and of the *KAT1* plasmid in Hedrich et al. (1995). For SKT1 the complete *SKT1* cDNA (GenBank accession no. X86021) was cloned as an *Asp*718/*Eco*R1 fragment into the *Sma*I site of pGEMHE. The *AKT1* cDNA was kindly provided by Dr. H. Sentenac. The AKT1 coding region was excised, subcloned, and inserted into the pGEMHE expression cassette. The AtKC1 coding region was excised from the *AtKC1* cDNA (GenBank accession no. U73325) and inserted into the pGEMHE expression cassette. cRNA was produced as described by Hedrich et al. (1995).

RESULTS

Plant K^+ channel α-subunits form multimers

The outward-rectifying *Shaker*-related K^+ (K_v channels), (MacKinnon, 1991; Liman et al., 1992) and the inward-rectifying IRK-related potassium channels (K_{ir} channels), (Glowatzki et al., 1995; Yang et al., 1995) show a tetrameric structure. To demonstrate whether or not the plant K_{ir} channels also form multimers, wild-type KAT1 and KAT1-S168R were co-expressed in *Xenopus* oocytes. The latter, modified in the putative voltage sensor S4, half-activates ($V_{1/2}$) at ~+85 mV compared to −140 mV for KAT1 (Fig. 1 *A*). When co-injected into oocytes, K^+ channels with intermediate gating behavior were created. With an increasing relative amount of mutant cRNA the voltage-dependent relative open probability shifted toward $V_{1/2}$ of KAT1-S168R (Fig. 1 *A*). This behavior could not be described by the sum of the relative open probabilities of the α-subunit homomers (see Materials and methods), indicating the formation of heteromeric channels rather than assembly within independent homomeric pools (Fig. 1 *B*). In contrast, co-expression of a mutant of the plant inward rectifier KAT1, KAT1-T256E, which differs from wild-type KAT1 in its susceptibility to blocking ions (Becker et al., 1996), and the structurally related outward rectifier *reag* cloned from rat brain (Ludwig et al., 1994) elicited inward and outward K^+ currents (Fig. 2 *A*). For this combination steady-state current-voltage characteristics as well as voltage-dependent relative open probabilities could be resolved and demonstrated as arising from two homomeric entities (Fig. 2, *B* and *C*), indicating differential assembly of KAT1 and *reag*. In line with the hypothesis that not even the animal K^+

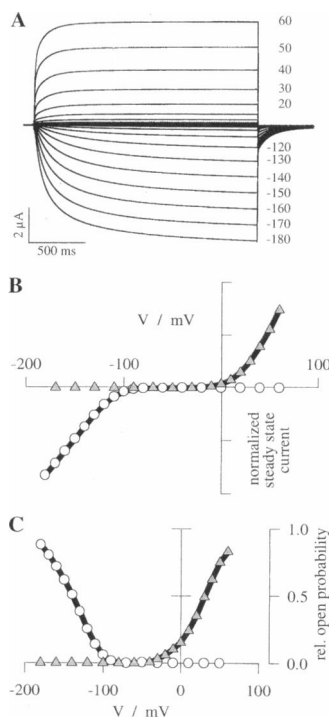

FIGURE 2 Co-expression of plant KAT1-T256E and *reag* (5:1 mixture) in *Xenopus* oocytes. The fraction of 5:1 was used to obtain inward and outward currents of similar amplitudes. Different fractions did not change the result qualitatively. (*A*) Representative inward and outward K^+ currents. From a holding potential of −30 mV currents were evoked by activating voltage-pulses to voltages indicated in millivolts. (*B*) Steady-state current-voltage characteristic (*solid lines*) and (*C*) voltage-dependent relative open probability (*solid lines*) for the oocyte shown in (*A*) superimposed with the respective relationships of homomeric KAT1-T256E (*open circles*) and *reag* (*gray triangles*). Relative open probabilities and steady-state current-voltage relationships of the homomers were determined in oocytes into which similar amounts of either KAT1-T256E- or *reag*-RNA, respectively, were injected. Steady-state current voltage characteristics of the single transporters were normalized to maximum currents measured in the mixture.

channels with the highest degree of homology to the plant inward rectifiers assemble with the "green" channels, permeation properties of K^+ inward currents were not altered (cf. next paragraphs). Inward currents could, e.g., be entirely suppressed by 1 mM Cs^+ (data not shown) as has been shown for KAT1-T256E homomers [Becker et al., 1996; cf. Fig. 2, *B* and *C* (*gray triangles*)].

Strategy to investigate assembly of plant K^+ channels

To test whether α-subunits from different plant species and tissues form heteromers we used the following strategy (Fig. 3): α-subunits "A" and "B" were co-expressed in *Xenopus* oocytes. Whereas "A" exhibited a pronounced phenotype, homomeric "B" did not carry K^+ currents. A change in the phenotype "A" thus provides evidence for the heteromerization of the two different α-subunits.

K^+ channel α-subunits from guard cells of different species co-aggregate

Initially we probed for the assembly of the guard cell K^+ channels KAT1 from *A. thaliana* (Anderson et al., 1992; Nakamura et al., 1995) and KST1 from *S. tuberosum* (Müller-Röber et al., 1995). Because both channels differ in their kinetics but are similar in their selectivity and in their sensitivity to blocking ions, to discriminate between selective and nonselective assembly more easily we used mutants with distinct properties: besides the increased Cs^+ sensitivity, KAT1-T256E, in contrast to the wild-type KAT1, is characterized by a voltage-dependent Ca^{2+} block (Becker et al., 1996), whereas KST1-H271R is altered in its pH-dependence. At pH 5.6 the latter activates more negative than -180 mV. At higher pH values the activation threshold shifts more positive (Hoth et al., 1997). Therefore, under our experimental conditions (an external pH of 5.6 and voltages between -170 mV and $+20$ mV) the homomeric KST1-H271R channel was electrically silent. In contrast to the Ca^{2+}-sensitive KAT1 mutant KAT1-T256E, KST1-H271R like the KST1 wild type was insensitive to extracellular Ca^{2+} (data not shown). When co-injected at a ratio of 1:3, kinetics of K^+ currents mediated by the hybrid channels were slowed more than threefold compared to KAT1-T256E (Fig. 4 *A*). Moreover, K^+ currents exhibited an intermediate susceptibility toward Ca^{2+} ions. Whereas

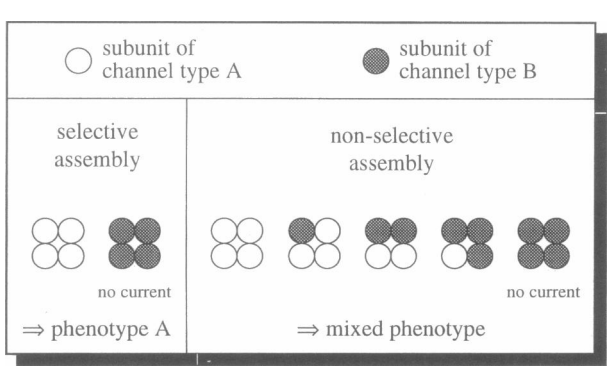

FIGURE 3 Cartoon of K^+ channel phenotypes following selective and nonselective assembly from a current-mediating ("A") and an electrically silent ("B") subunit. Because plant K^+ channels might be formed by four subunits, like the *Shaker* and IRK K^+ channels (MacKinnon, 1991; Liman et al., 1992; Glowatzki et al., 1995; Yang et al., 1995), in the presence of subunits from two different channel types, subunits could either assemble indiscriminately or form homomultimers.

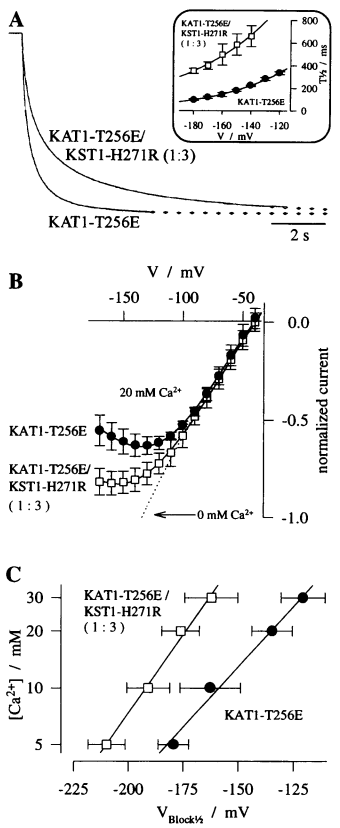

FIGURE 4 Co-expression of the Ca^{2+}-sensitive KAT1 mutant KAT1-T256E and the Ca^{2+}-insensitive KST1 mutant KST1-H271R. At pH 5.6 KST1-H271R activates more negatively than -180 mV (Hoth et al., 1997). Each data point represents the mean of five measurements $\pm$ standard error of the mean. (*A*) Co-expression slows activation kinetics of KAT1-T256E. Representative K^+ currents mediated by KAT1-T256E and KAT1-T256E/KST1-H271R mixtures (1:3). Currents were evoked by activation pulses to -150 mV starting from a holding potential of -30 mV. Current amplitudes were normalized to the steady-state value of -2.2 μA for KAT1-T256E and -2.4 μA for the KAT1-T256E/KST1-H271R mixture (1:3). *Inset*: Comparison of the voltage-dependence of the half-activation time ($T_{1/2}$). Solid lines represent best fits with an exponential function. (*B*) Co-expression reduces the Ca^{2+} sensitivity of KAT1-T256E. Tail currents recorded in 20 mM Ca^{2+} for KAT1-T256E (*closed circles*) and KAT1-T256E/KST1-H271R (1:3, *open squares*) after an activating pre-pulse to -150 mV are superimposed. The dotted line represents tail currents through KAT1-T256E and KAT1-T256E/KST1-H271R in the absence of Ca^{2+} ions, and solid lines represent best fits according to the Woodhull model (Woodhull, 1973). (*C*) Concentration- and voltage-dependence of the Ca^{2+} block. Half-blocking voltages ($V_{Block1/2}$) were determined in different Ca^{2+} concentrations (5, 10, 20, and 30 mM). Note the logarithmic scale of the concentration axis.

for KAT1-T256E 7 mM Ca^{2+} were sufficient to inhibit the K^+ current at −170 mV by ~50%, a related block of the hybrid channels required more than three times the Ca^{2+} concentration (Fig. 4, *B* and *C*).

Guard cell and root K^+ channels from different species form heteromers

Because the two guard cell channels from *A. thaliana* and *S. tuberosum*, KAT1 and KST1, belong to the same K^+ channel subfamily, we could not exclude the possibility that the assembly was tissue- or subtype-dependent. Therefore, we co-injected the guard cell KAT1-related mutants with AKT1 (Sentenac et al., 1992) and SKT1 (GenBank accession no. X86021), members of the AKT subtype, as well as with AtKC1 (Reintanz et al., GenBank accession no. U73325), a member of a new subfamily. AKT1 and AtKC1 from *A. thaliana* are predominantly expressed in roots (Basset et al., 1995, Lagarde et al., 1996; Reintanz et al., unpublished data) whereas SKT1 was cloned from a leaf library of *S. tuberosum* but also showed expression in roots (Müller-Röber et al., unpublished data). When injected alone into *Xenopus* oocytes these channels were electrically silent. After co-injection of SKT1 with the KAT1 mutant T256E, however, K^+ currents were present that were less Ca^{2+} sensitive (Fig. 5 *A*) and had slower kinetics than KAT1-T256E homomers (data not shown, cf. Fig. 4 *A*). Co-expression of SKT1 and KST1 wild type, both cloned from an epidermal fragment library, resulted in channels with increased Cs^+ sensitivity (Fig. 5 *B*). When co-injecting the two silent subunits, KST1-H271R and SKT1, together they created K^+ currents of intermediate pH dependence compared to the mutant and KST1 wild type (data not shown). This might indicate that the presence of SKT1 α-subunits rescues KST1-H271R from the inability to activate at moderate voltages under acidic conditions. Similar results were obtained by co-expressing AKT1 with KAT1-T256E and KST1, respectively. K^+ currents resulting from hybrid AKT1/KAT1-T256E channels were less susceptible to Ca^{2+} (Fig. 5 *C*). At −170 mV the K_i was 25 mM compared to 7 mM for homomeric KAT1-T256E multimers. K^+ currents obtained from AKT1/KST1 mixtures had an increased Cs^+-sensitivity (Fig. 5 *D*). The K_i at −150 mV was 40 μM compared to 500 μM for the KST1 wild type.

The heterooligomerization between AtKC1 and KST1 on one side, and AtKC1 and KAT1-S168R on the other, has already been apparent during steady-state activation of the multimers. These subunit mixtures shifted the voltage dependence ($V_{1/2}$) to more negative potentials than KAT1-S168R and KST1, respectively (Fig. 5, *E* and *F*). An unusual S4 segment of AtKC1 may bring about a $V_{1/2}$ more negative than −200 mV, which is not tolerated by *Xenopus* oocytes. When heterooligomerized with other channel subunits, however, its activation threshold is shifted positive into the voltage range tolerated by *Xenopus* oocytes. Like SKT1 and AKT1, the presence of AtKC1 significantly

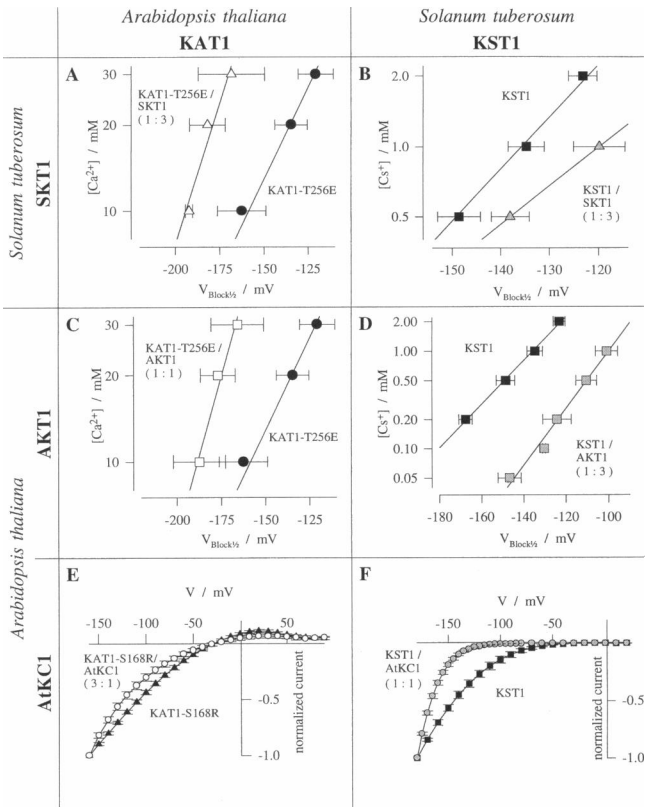

FIGURE 5 Co-expression of K^+ channel α-subunits from different cells/tissues and plant species. KAT-homologs (KAT1, KST1) and AKT-homologs (AKT1, SKT1) were from *Arabidopsis thaliana* and *Solanum tuberosum* and AtKC1 from *A. thaliana*. AKT1 and AtKC1, as well as SKT1 homomultimers, were electrically silent when expressed alone in oocytes. Each data point represents the mean of 3 to 10 measurements ± standard error of the mean. (*A* and *C*) Concentration- and voltage-dependence of the Ca^{2+} block in KAT1-T256E (*closed circles*), and in the subunit mixtures KAT1-T256E/SKT1 (1:3, *A*, *open triangles*), and KAT1-T256E/AKT1 (1:1, *C*, *open squares*). (*B* and *D*) Cs^+-sensitivity of KST1 wild type (*closed squares*), and the mixtures KST1/SKT1 (1:3, *B*, *gray triangles*), and KST1/AKT1 (1:3, *D*, *gray squares*). (*E* and *F*) Steady-state current-voltage relationships of KAT1-S168R (*E*, *closed triangles*), KAT1-S168R/AtKC1 (3:1, *F*, *open circles*), KST1 wild type (*F*, *closed squares*), and KST1/AtKC1 (1:1, *F*, *gray circles*). Here a normalized current of −1.0 corresponds to a whole-cell current of ~−1.1 μA in KAT1-S168R/AtKC1-, −1.8 μA in KAT1-S168R-, −4.5 μA in KST1-, and −1 μA in KST1/AtKC1-oocytes. Note the logarithmic scale of the concentration axes in (*A*—*D*).

increased the Cs^+-sensitivity of the K^+ currents compared to wild-type KST1 and KAT1-S168R (data not shown).

Since neither AtKC1 alone nor in combination with the electrically silent subunits SKT1 and AKT1 did carry measurable K^+ currents, a proof for or against assembly between these α-subunits could not be furnished. To our surprise, however, co-expression of the two silent channels SKT1 and AKT1 produced K^+ currents in *Xenopus* oocytes (Fig. 6). These currents show the highest Cs^+ sensitivity observed so far. The Cs^+ block was already visible at a concentration of 2 μM. At −150 mV the half-blocking concentration was 15 μM, a K_i ~70-fold lower than for

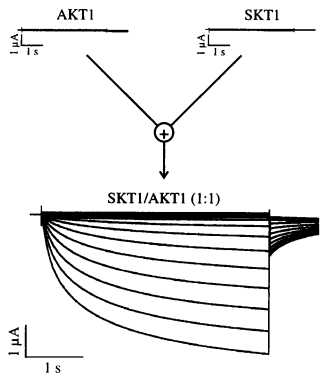

FIGURE 6 The two silent subunits, AKT1 and SKT1, form active channels upon co-expression. Representative current families from oocytes expressing AKT1, SKT1, and AKT1/SKT1 (1:1 mixture), respectively. Currents were elicited by 4-s voltage steps from +10 mV to −150 mV (10-mV steps) followed by a voltage step to −70 mV from a holding potential of −30 mV.

KAT1 and KST1 when studied at comparable current amplitudes. Thus the Cs^+ sensitivity of AKT1 and SKT1 might account for the increased Cs^+ sensitivity in KST1/SKT1 and KST1/AKT1 heteromers as well (cf. Fig. 5, *B* and *D*).

DISCUSSION

By taking advantage of K^+ channel mutants affected in sensitivity toward changes in voltage, pH, Cs^+, and Ca^{2+}, we could demonstrate, from the change of two independent biophysical parameters (gating and permeation), heteromeric assembly of plant K_{in} channel α-subunits. Although heteromerization within a K^+ channel subfamily (KAT1 with KST1) was not a surprising result, assembly of plant K^+ channel subunits was different from processes known for the animal K_v and K_{ir} channel families (Covarrubias et al., 1991; Salkoff et al., 1992; Pessia et al., 1996). Whereas in animals a molecular barrier to heteropolymerization seems to exist, for plants we could show the nonselective assembly among channel subtypes originating from different tissues and even different plant families.

The most striking result was the oligomerization of KAT1 with SKT1 α-subunits and of KST1 with AKT1 or AtKC1 α-subunits. This indiscriminate assembly would be comparable to a heteromultimerization of, e.g., *Shaker*-type subunits from rat brain and *Shal*-type subunits from human heart.

Physiologically significant, however, was the heterooligomerization of α-subunits of different channel subfamilies within one and the same species (KST1 with SKT1, and KAT1 with AKT1 or AtKC1). This interesting finding might explain the above-mentioned (see Introduction) difference between the heterologously expressed channel KST1 and the channel in its natural environment. For example, the Cs^+ sensitivity of the inwardly rectifying potassium channel in *S. tuberosum* guard cells is ~10-fold more pronounced than in KST1 expressed in oocytes. Heteromerization of KST1 α-subunits with subunits of the AKT-type (not necessarily SKT1) in guard cells could be responsible for this contrariety.

Less striking but more spectacular was the result of co-injecting *SKT1*- and *AKT1*-RNA. In contrast to the SKT1 and AKT1 homomers that were inactive in *Xenopus* oocytes, their heteromers formed active K^+ channels of the highest Cs^+ sensitivity observed so far. This result becomes even more strange when taking into account that AKT1 and SKT1 homomers were functionally expressed in Sf9 insect cells and yeast (Sentenac et al., 1992; Bertl et al., 1994; Gaymard et al., 1996; Müller-Röber et al., unpublished data). Although the genuine reason for this discrepancy is still unknown, some recent experimental findings indicate modifications of heterologously expressed channels by the expression system. First evidence for expression system-dependent functional differences have been observed with KAT1. Like all investigated plant inward rectifiers in vivo (Hedrich and Dietrich, 1996), when expressed in oocytes KAT1 is characterized by a significant NH_4^+ permeability (Schachtman et al., 1992). Expressed in Sf9 cells and yeast, however, KAT1 lost its NH_4^+ permeability (Bertl et al., 1995; Marten et al., 1996). This change in the permeation properties might result either from differences in protein glycosylation and phosphorylation, or that native peptides assemble with K^+ channel α-subunits. In a similar way cytoplasmic factors or even β-subunits present in individual expression systems may enable functional expression of channels. Recently, putative plant β-subunits (Tang et al., 1995) with a high degree of homology with the animal K^+ channel β-subunits (Heinemann et al., 1994; Pongs, 1995) have been discovered. Inasmuch as the latter contain amino acid stretches similar to regions in the N-terminus of the *Shaker* channels, they might be able to interact with the α-subunit.

Even if plant K^+ channel α-subunits seem to heterooligomerize without preference, to date we cannot exclude defined stoichiometries within the aggregates. Nevertheless, the physiological aspects of the following questions can be discussed: why is the assembly within the plant K_{in} family different from that in the animal K_v or K_{ir} families? Why is the molecular barrier to heteropolymerization—if it actually exists in the K_{in} family—so weak? What could be the advantage for the plant?

In contrast to animal potassium channels, plant K_{in} channels are not involved in fast signal transduction but are predominantly required for long-term potassium uptake. The centralized processing of signals in animals demands a maximum amount of synchronization of the transducing cells. To reduce their sensitivity to disturbances, blending of multiple K^+ channel types might be the best way for each cell to fine-tune its electrical properties. Inevitably the cells accept the disadvantage to have to drive a high expenditure in protein expression. The local decentralized perception and response to environmental signals, on the other hand, makes plants less sensitive to disturbances in signal transduction. Not facing this handicap, nonselective assembly of

K^+ channel α-subunits could be advantageous for the plant to be able to adapt more easily to its environment. By temporal overlap and kinetics of α-subunit expression, plants might obtain a maximum of flexibility to alter the whole-cell K^+ current features during cell growth, development, and reproduction with a minimum of expenditure.

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