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Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*

(nucleotide sequence/amino acid homology/potassium transport/transmembrane segments/heterologous expression)

JULIE A. ANDERSON*, SHIRISH S. HUPRIKAR*, LEON V. KOCHIAN†, WILLIAM J. LUCAS‡, AND RICHARD F. GABER*§

*Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208; †U.S. Plant Soil and Nutrition Lab, U.S. Department of Agriculture-Agricultural Research Service, Cornell University, Ithaca, NY 14853; and ‡Department of Botany, University of California, Davis, CA 95616

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ABSTRACT **We report the isolation of a cDNA (KAT1) from *Arabidopsis thaliana* that encodes a probable K^+ channel. KAT1 was cloned by its ability to suppress a K^+ transport-defective phenotype in mutant *Saccharomyces cerevisiae* cells. This suppression is sensitive to known K^+ channel blockers, including tetraethylammonium and Ba^{2+} ions. The KAT1 cDNA contains an open reading frame capable of encoding a 78-kDa protein that shares structural features found in the Shaker superfamily of K^+ channels. These include a cluster of six putative membrane-spanning helices (S1–S6) at the amino terminus of the protein, a presumed voltage-sensing region containing Arg/Lys-Xaa-Xaa-Arg/Lys repeats within S4, and the highly conserved pore-forming region (known as H5 or SS1–SS2). Our results suggest that the structural motif for K^+ channels has been conserved between plants and animals.**

K^+ channels in biological membranes are essential for diverse cellular processes in all organisms studied thus far. In evolutionarily divergent organisms such as mammals and flies, the best characterized K^+ channels are voltage-sensitive channels that function in controlling the excitability of nerve and muscle cells (1). K^+ channel activity has also been detected in plant and fungal cells (2, 3), where it is believed they are required for growth, osmoregulation, cell movement, and mineral nutrition. Unlike animal cells, most plants and fungi acquire and accumulate cellular K^+ directly from the environment. Because these cells generally maintain very negative membrane potentials (−120 to −250 mV), primarily due to the functioning of the electrogenic, H^+-translocating ATPase, it has been proposed that passive, electrophoretic influx via K^+ channels could play an important role in K^+ absorption (3). However, it should be noted that a very high-affinity K^+ transport system has been identified in fungal and higher plant cells and has been proposed to mediate active K^+ influx via either a K^+-ATPase or a K^+–H^+ symporter (4, 5).

Sequence analysis of cDNAs encoding voltage-dependent K^+ channels of the Shaker family in *Drosophila* revealed structural features that are known to be conserved in insects and mammals (6–11). Each subunit of the Shaker tetramer is predicted to consist of six transmembrane helices (S1–S6) with a presumptive voltage-sensing domain in S4 (12–14) and a highly conserved domain between S5 and S6 that is proposed to line the channel pore (15–17). Expression of wild-type and mutant forms of the Shaker channel protein in *Xenopus* oocytes established that the amino terminus interacts with the ion-conducting pore to mediate channel inactivation (18, 19).

We have isolated a cDNA¶ (KAT1) from *Arabidopsis thaliana* that suppresses the mutant phenotype of a K^+ transport-deficient strain of *Saccharomyces cerevisiae*. This suppression is sensitive to known K^+ channel blockers. DNA sequence analysis of KAT1 predicts an amino acid sequence with structural similarities, including significant amino acid sequence identities, to members of the Shaker family. These include six putative transmembrane helices clustered in the amino half of the protein and the probable voltage-sensing (S4) and pore-forming (H5, SS1–SS2) domains.

MATERIALS AND METHODS

Media and Strains. YNB and LS media were prepared as described (20, 21). AA−URA is medium supplemented with all amino acids and nucleoside bases except uracil. Media with galactose or glucose as the sole carbon source are indicated as GAL and GLU. Ko and Gaber (22) have described construction of the *S. cerevisiae* strain CY162, *MATα ura3-52 trk1Δ his3Δ200 his4-15 trk2Δ1*::pCK64. Yeast transformation was performed by electroporation (23). Plasmids were selected and propagated in *Escherichia coli* strain HB101 on LB medium supplemented with ampicillin (50 μg/ml).

cDNA Cloning. The *A. thaliana* cDNA library was constructed in the λYES yeast/*E. coli* shuttle vector (24) kindly provided by R. Davis (Stanford University, Stanford, CA). This library was made from mRNA extracted from leaves, shoots, stems, and flowers of plants at all stages of development (24). Expression of the cloned inserts is under control of the inducible *GAL1* promoter. The library was introduced into CY162 cells by transformation. Initial selection and subsequent screening of the transformants were carried out on AA−URA to maintain selection for the plasmids. Ura^+ transformants were selected on glucose-containing medium supplemented with 100 mM K^+ (GLU−URA 100K) and replica-plated to GAL−URA 100K to induce expression of the cloned cDNAs. After an overnight incubation, the transformants were replica-plated to GAL−URA containing 7 mM K^+ (7K) to identify cDNAs able to confer suppression of the K^+ transport-defective phenotype (Trk^-) of the recipient cells.

DNA Sequencing. Dideoxy sequencing (25) of pKAT1 was performed with Sequenase (United States Biochemical). Double-stranded template DNA was sequenced by using specific oligonucleotide primers synthesized at the Northwestern University Biotechnology Facility. DNA sequence analysis was done with The DNA Inspector Ile (Textco,

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Abbreviation: TEA, tetraethylammonium.

§To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M86990).

Lebanon, NH) and the Genetics Computer Group (GCG, Madison, WI) software.

Southern Blot Analysis. Genomic DNA extracted from *A. thaliana* (Columbia ecotype) was generously provided by K. Niyogi (Whitehead Institute, Cambridge, MA). Two micrograms of genomic DNA was digested with *Eco*RI, electrophoresed in 0.8% agarose, and transferred to nylon membrane. The KAT1 probe was prepared by random hexamer-primed [α-^{32}P]dCTP labeling (26) of the 2.2-kilobase *Xho* I insert contained in pKAT. Hybridization overnight at 65°C was followed by three washes (15 min each) at 60°C in 0.9 M NaCl/0.09 M sodium citrate, pH 7/0.1% SDS.

RESULTS AND DISCUSSION

Isolation of KAT1 cDNA. *S. cerevisiae* cells deleted for the high- and low-affinity K^+ transporter genes (*TRK1* and *TRK2*) require ≈100-fold more K^+ than wild-type cells. In previous work, genes encoding K^+ transporters from *S. cerevisiae* (21, 22), *Saccharomyces uvarum* (27), and *Schizosaccharomyces pombe* (unpublished results) were isolated and characterized by their ability to restore growth of *trk* mutants on K^+-limiting medium. We have exploited this system to isolate a probable K^+ channel from plants.

An *A. thaliana* cDNA library was screened for sequences that suppress the K^+ transport defect (Trk^- phenotype) of *trk1Δ trk2Δ* cells (CY162) and thus allow growth on K^+-limiting medium. From ≈40,000 Ura^+ transformants, a single clone was obtained that allows growth of CY162 cells on galactose-containing medium with 7 mM K^+ (GAL 7K). The cloned plasmid, pKAT1, was recovered by transformation of *E. coli* and reintroduced into CY162 by electroporation. All Ura^+ transformants containing pKAT1 were able to grow on GAL 7K.

Southern analysis using the cDNA insert contained in pKAT1 as a probe revealed the presence of homologous sequences in the *A. thaliana* genome (data not shown). The extent of this analysis was insufficient to determine whether or not multiple KAT1-related genes are contained in the *Arabidopsis* genome.

KAT1 Completely Suppresses the Trk^- Phenotype of trk1Δ trk2Δ Cells. Wild-type (*TRK1 TRK2*) *S. cerevisiae* cells are able to grow on medium supplemented with 0.2 mM KCl (0.2K) (21). To determine the level of KAT1 suppression, colonies of CY162 cells containing pKAT1 were replica-plated to GAL 0.2K. pKAT1 conferred on cells the ability to grow on GAL 0.2K but not on GLU 0.2K, consistent with the conditional expression of the cDNA (Fig. 1). Growth of CY162/pKAT1 cells on GAL 0.2K was indistinguishable from that of wild-type cells (data not shown).

DNA Sequence Analysis Suggests That KAT1 Encodes a K^+ Channel. The cDNA sequence in pKAT1 revealed an open reading frame of 2031 nucleotides capable of encoding a protein of 677 amino acids (78 kDa; Fig. 2). Northern blot analysis using KAT1 sequences as a probe detected a 2.2-kilobase message that was present in very low abundance (data not shown), indicating that pKAT1 contains a full-length or near full-length cDNA. Hydropathy analysis (29) of the inferred protein identified seven possible membrane-spanning domains, with six of these clustered near the amino terminus (Fig. 3), a motif found among all of the voltage-sensitive Shaker family of K^+ channels (30).

A comparison of KAT1 and Shaker-related K^+ channels revealed significant amino acid sequence identity in regions that are highly conserved among the Shaker family. These include the putative voltage-sensing domain (S4) and the region thought to line the pore of the channel (H5 or SS1–SS2). S4 domains, located between the third and fifth membrane-spanning domain, are primarily hydrophobic with arginines or lysines at every third or fourth residue (12). An S4-like region is found in KAT1 from amino acid 162 to 180 in the fourth membrane-spanning domain (Fig. 2 and Fig. 4*C*). Two notable differences between the KAT1 S4 domain and the S4 domains of other K^+ channels are at Ser^{168} and Arg^{176} (Fig. 4*A*).

The location and the amino acid sequence of the putative pore-forming domain are also conserved in KAT1 (Fig. 4 *B* and *C*). The region between S5 and S6 (Trp^{253} to Asp^{265}) shows significant sequence identity with the H5 or SS1–SS2 domain of Shaker-related channels. Eight of 13 amino acids are present in other K^+ channels, including 2 threonine residues (Thr^{259} and Thr^{260}) that have been shown to be essential for ion selectivity and are completely conserved among all members of the Shaker superfamily (16, 17). Although the overall amino acid sequence identity of KAT1 with other Shaker K^+ channels is low (<20%), the presence of six amino-terminal putative membrane-spanning domains and amino acid sequence identities in regions known to be highly conserved strongly suggests that KAT1 is a member of the Shaker superfamily. Sequence comparisons between KAT1 and other ion channels, including the Na^+ channel (12) and the Ca^{2+} channel (33), failed to identify regions of sequence conservation (data not shown).

Tetraethylammonium (TEA) and Ba^{2+} Inhibit KAT1 Function *in Vivo*. Although the ability to suppress the K^+ transport-deficient phenotype of *trk1Δ trk2Δ* cells and the struc-

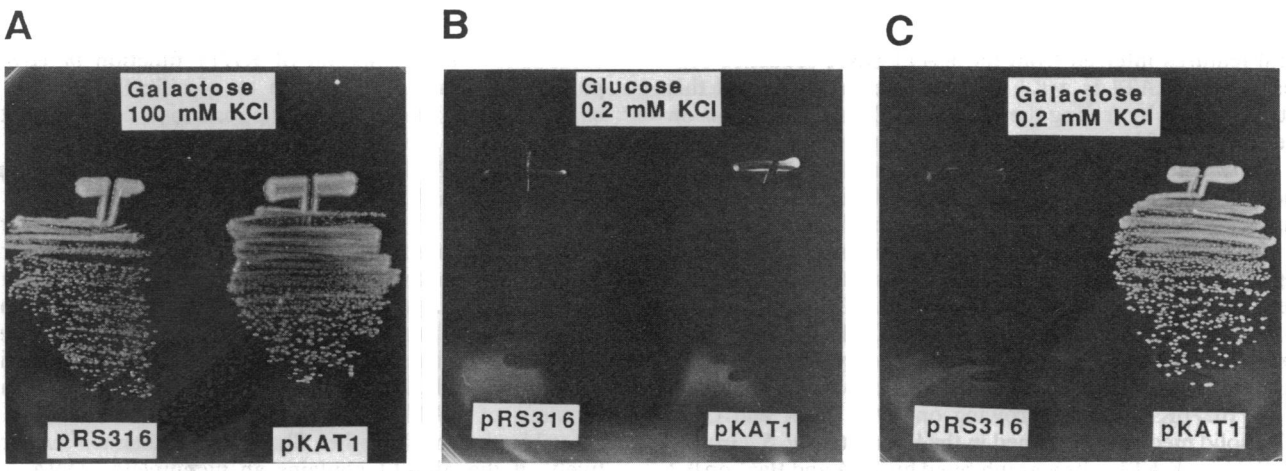

FIG. 1. Growth tests of Trk^- *S. cerevisiae* cells containing the pRS316 vector (28) or pKAT1. Cells were streaked onto AA−URA medium supplemented with 100 mM KCl (*A*) and LS−URA supplemented 0.2 mM KCl (*B* and *C*). The media contained 4% glucose or 4% galactose as indicated.

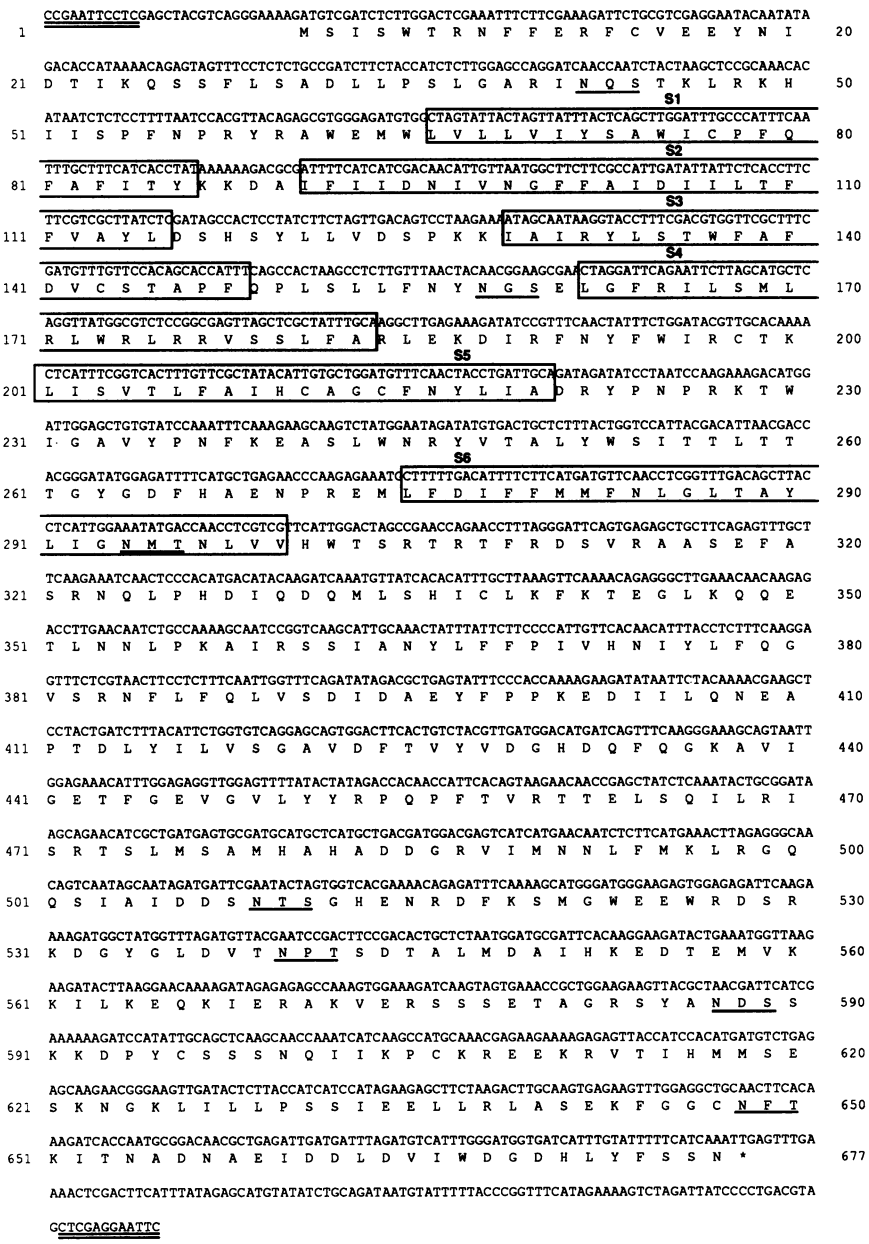

FIG. 2. Nucleotide and predicted amino acid sequence of KAT1. The potential membrane-spanning regions S1–S6, suggested by hydropathy analysis, are boxed. Amino acids are numbered and the termination codon is highlighted with an asterisk. Sites of potential N-linked glycosylation, NX(S/T), are underlined. Sequences encoded by the *Xho* I–*Eco*RI linker of the vector are doubly underlined.

tural features inferred from the KAT1 DNA sequence suggested that KAT1 is a K^+ channel, we further tested this interpretation by testing the effect of TEA and Ba^{2+} ions on the function of KAT1. TEA and Ba^{2+} are specific inhibitors of many voltage-gated K^+ channels (1) and appear to block channel conductance by interacting with sites normally occupied by K^+ ions (31, 32). This has been further supported by recent experiments in which mutations residing in the region thought to constitute the lining of the channel pore were shown to affect the binding of TEA (15, 16).

If KAT1 is a K^+ channel, growth of CY162/pKAT1 cells on K^+-limiting medium might be inhibited by TEA and Ba^{2+}. TEA and Ba^{2+} were applied to filter discs placed onto lawns of CY162/pKAT1 cells growing on GAL 100K and GAL 0.2K solid media as described in the legend to Fig. 5. Growth of CY162/pKAT1 cells was inhibited by TEA and Ba^{2+} on 0.2 mM K^+ but not on 100 mM K^+. In contrast, similar tests using CY162 cells containing *TRK1* carried on a centromeric plasmid (pRG295-1; ref. 21) showed no inhibition by these compounds. The inhibition of KAT1 function *in vivo* by pharmacological agents known to specifically bind to K^+ channels in animals further supports our conclusion that KAT1 encodes a Shaker-related K^+ channel.

KAT1 May Be Incapable of Inactivating Due to a Short Amino-Terminal End. The length of the amino-terminal region upstream of the S1 domain in KAT1 is significantly shorter than is observed in other Shaker-related K^+ channels (Fig. 2). The inactivation of K^+ channels following entry into the closed state has been shown to be dependent on the amino terminus of the protein. Mutants of Shaker with deletions in the amino terminus are unable to undergo channel inactivation (18, 19). If KAT1 is a full-length cDNA, the short amino terminus of KAT1 suggests that its inactivation may not resemble that of the other Shaker-type channels. Alternatively, if the pKAT1 contains an incomplete cDNA, it is possible that suppression of the Trk^- phenotype of CY162 cells by pKAT1 could be due to an inability of the truncated channel to undergo inactivation.

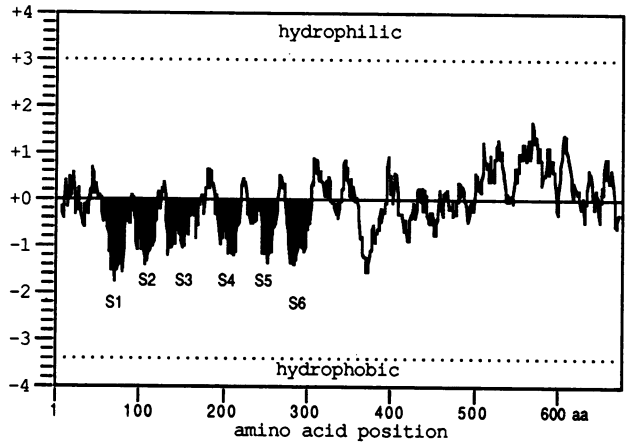

FIG. 3. Hydropathy plot of the predicted amino acid sequence of KAT1. The profile was generated by the method of Hopp and Woods (29) with a window of 12 amino acids. Negative values indicate hydrophobic regions and positive values indicate hydrophilic regions. Hydrophobic regions corresponding to the putative membrane-spanning domains of the Shaker-type channels are shaded and designated S1–S6.

Implications. Shaker-related K^+ channels in animal cells are primarily involved in electrical signaling and not with appreciable changes in total cellular K^+ content. Although a similar function has been ascribed to K^+ channels involved in the action potential of giant algal cells (for example, see ref. 3), plant K^+ channels also appear to be involved in osmotic adjustment, turgor-mediated growth, cell movement, and nutrient absorption (3). Along these lines, the complete suppression of the Trk^- phenotype of *trk1Δ trk2Δ* cells by KAT1 suggests that K^+ channels in plants play a fundamentally different role than their animal counterparts. K^+ channels in plants could provide a principal mechanism for K^+ uptake. Consistent with this, the electrical potential across plant plasma membranes is reportedly large enough to allow for K^+ uptake via a passive mechanism such as a channel (3).

Suppression of the K^+ requirement of *trk1Δ trk2Δ* cells by a probable K^+ channel demonstrates that K^+ uptake in yeast could be mediated by passive transport. It is possible that TRK1 and TRK2, structurally dissimilar to known K^+ channels, also confer passive K^+ uptake and are members of a distinct family of K^+ channels or channel regulators. Because the K^+ concentrations used for the screening experiments conducted in this study were well above the K_m of the putative high-affinity K^+ transport system studied previously in fungal and higher plant cells (4, 5), it is still not clear whether an active K^+ transport system is operating in addition to the K^+ channel described here.

The probable K^+ channel described here appears to mediate K^+ influx and thus might be categorized as an inward rectifying channel, although this will require verification via experiments aimed at characterizing the transport properties of the system. However, the Shaker-type K^+ channels in animals are associated with outward K^+ currents (1). Therefore, although the *Arabidopsis* K^+ channel described here may share significant structural features with the Shaker superfamily of K^+ channels, there appear to be significant physiological and functional differences.

The isolation of KAT1 indicates that *S. cerevisiae* can be used as a powerful and convenient method of isolating K^+ channel cDNAs from higher eukaryotes.

We have cloned a cDNA encoding a probable plant K^+ channel by its ability to suppress the K^+ transport defect in mutant *S. cerevisiae* cells. Several lines of evidence support our conclusion that KAT1 is a K^+ channel: (*i*) KAT1 suppresses the Trk^- phenotype of *S. cerevisiae* cells deleted for their endogenous K^+ transporters; (*ii*) the inferred protein sequence includes a cluster of six putative membrane-

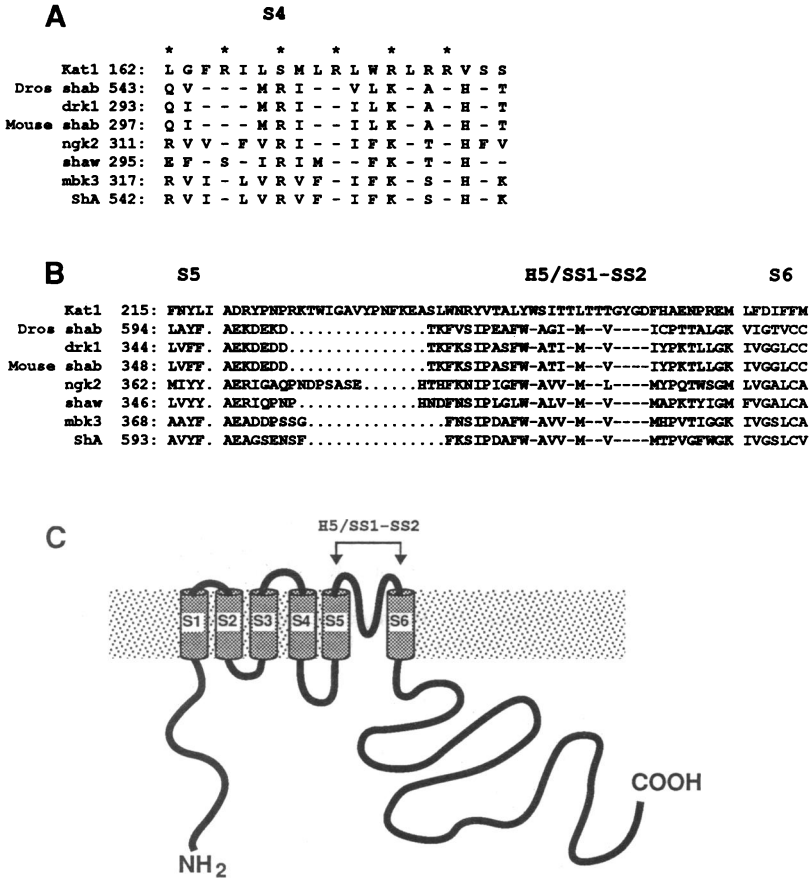

FIG. 4. (*A*) Comparison of the S4 region of KAT1 with the S4 regions of other voltage-gated K^+ channels. Amino acid sequences of the S4 regions are compared for KAT1 and K^+ channels from *Drosophila* [Dros shab (9), shaw (9), and ShA (6)], mouse [Mouse shab (31), ngk2 (32), and mbk3 (11)], and rat [drk1 (10)]. The numbers apply to the first residue in each of the aligned sequences. Identities with KAT1 are indicated by dashed lines. The positions of Arg/Lys residues associated with the overall pattern of Arg/Lys-Xaa-Xaa observed in members of this channel family are indicated by asterisks. (*B*) Comparison of the H5/SS1–SS2 region of KAT1 with the analogous regions of other voltage-gated K^+ channels. Amino acid sequences of the H5/SS1–SS2 regions are compared for KAT1 and the same K^+ channels as in *A*. Gaps in the protein sequences were introduced to allow optimal sequence alignment and are indicated with dots. Identities between all sequences are designated with dashed lines. (*C*) Proposed model of KAT1 indicating the locations of regions presented in *A* and *B*. Hydrophobic regions analogous to those thought to be membrane-spanning domains in Shaker-type channels are indicated as S1–S6. The region analogous to the putative pore-forming domain of Shaker channels is designated H5/SS1–SS2.

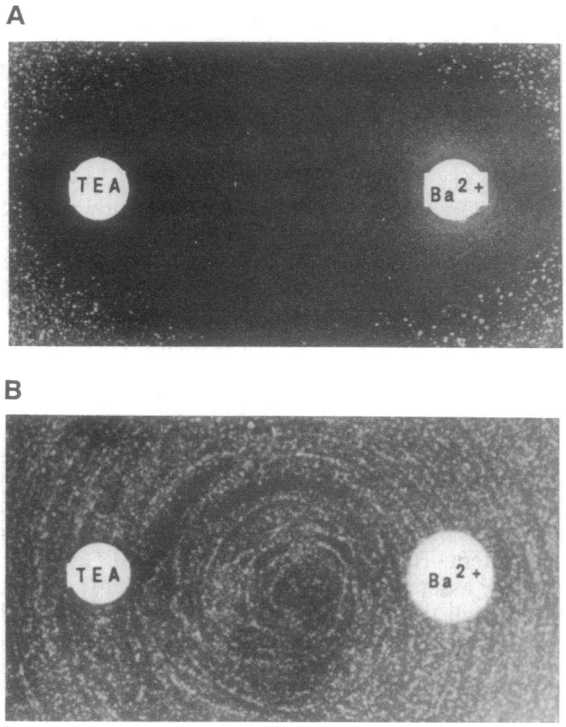

FIG. 5. TEA and Ba^{2+} inhibition of KAT1 *in vivo*. Approximately 10^5 CY162/pKAT1 cells were plated onto GAL−URA 0.2K (*A*) and GAL−URA 100K (*B*) solid media. Twenty microliters of 1 M TEA and 20 μl of 1 M $BaCl_2$ were applied to sterile filter disks placed on the media. A halo of inhibited cell growth can be seen around the filters on the 0.2 mM K^+-containing plate. $BaCl_2$ precipitated out of the medium in the region surrounding the Ba^{2+} filter disk.

spanning domains and conserved amino acid sequences corresponding to the presumptive voltage-sensing (S4) and pore-forming (SS1–SS2 or H5) regions; and (*iii*) K^+ channel-specific blockers (TEA and Ba^{2+}) inhibit KAT1 *in vivo*. Although the primary amino acid sequences of KAT1 and Shaker-related K^+ channels have diverged significantly, the structures defining K^+ channels remain conserved between plants and animals.

Note Added in Proof. Since the submission of this work we have discovered significant sequence identity between KAT1 and the Drosophila K^+ channel encoded by ether á go-go (*eag*; ref. 34). A comparison between all of the Drosophila K^+ channel sequences and KAT1 revealed that KAT1 and Eag are more closely related to each other than either one is to the other members of the Shaker superfamily. We have also become aware of another putative K^+ channel cDNA, designated AKT1 (35), isolated from *A. thaliana* by suppression of a K^+ transport defect in *S. cerevisiae*. The amino acid sequences of these two molecules share extensive identity but are clearly not allelic, demonstrating the existence of multigene K^+ channel families in higher plants.

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1. Hille, B. (1991) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
2. Gustin, M. C., Martinac, B., Saimi, Y., Culbertson, M. R. & Kung, C. (1986) *Science* **233**, 1195–1197.
3. Schroeder, J. I. & Hedrich, R. (1989) *Trends Biochem. Sci.* **14**, 187–192.
4. Rodriguez-Navarro, A., Blatt, M. R. & Slayman, C. L. (1986) *J. Gen. Physiol.* **87**, 649–674.
5. Kochian, L. V., Shaff, J. E. & Lucas, W. J. (1989) *Plant Physiol.* **91**, 1202–1211.
6. Tempel, B. L., Papazian, D. M., Schwarz, T. L., Jan, Y. N. & Jan, L. Y. (1987) *Science* **237**, 770–775.
7. Schwartz, T. L., Tempel, B. L., Papazian, D. M., Jan, Y. N. & Jan, Y. J. (1988) *Nature (London)* **331**, 137–142.
8. Tempel, B. L., Jan, Y. N. & Jan, L. Y. (1988) *Nature (London)* **332**, 837–839.
9. Butler, A., Wei, A., Baker, K. & Salkoff, L. (1989) *Science* **243**, 943–947.
10. Frech, G. C., VanDongen, A. M. J., Schuster, G., Brown, A. M. & Joho, R. H. (1989) *Nature (London)* **340**, 642–645.
11. Chandy, K. G., Williams, C. B., Spencer, R. H., Aguilar, B. A., Ghansani, S., Tempel, B. L. & Gutman, G. A. (1990) *Science* **247**, 973–975.
12. Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. & Numa, S. (1984) *Nature (London)* **312**, 121–127.
13. Greenblatt, R. E., Blatt, Y. & Montal, M. (1985) *FEBS Lett.* **193**, 125–134.
14. Guy, H. R. & Seetharanulu, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 508–512.
15. MacKinnon, R. & Yellen, G. (1990) *Science* **250**, 276–278.
16. Yellen, G., Jurman, M. E., Abramson, T. & MacKinnon, R. (1991) *Science* **251**, 939–941.
17. Yool, A. J. & Schwartz, T. L. (1991) *Nature (London)* **349**, 700–704.
18. Hoshi, T., Zagotta, W. N. & Aldrich, R. W. (1990) *Science* **250**, 533–538.
19. Zagotta, W. N., Hoshi, T. & Aldrich, R. W. (1990) *Science* **250**, 567–571.
20. Sherman, F., Fink, G. R. & Hicks, J. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Gaber, R. F., Styles, C. A. & Fink, G. R. (1988) *Mol. Cell. Biol.* **8**, 2848–2859.
22. Ko, C. H. & Gaber, R. F. (1991) *Mol. Cell. Biol.* **11**, 4266–4273.
23. Becker, D. M. & Guarente, L. (1991) *Methods Enzymol.* **194**, 182–187.
24. Elledge, S. J., Mulligan, J. T., Ranier, S. W., Spottswood, M. & Davis, R. W. (1990) *Proc. Natl. Acad. Sci. USA* **88**, 1731–1735.
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6.
27. Anderson, J. A., Best, L. A. & Gaber, R. F. (1991) *Gene* **99**, 39–46.
28. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.
29. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3824–3828.
30. Jan, L. Y. & Jan, Y. N. (1989) *Cell* **56**, 13–24.
31. Hille, B. (1967) *J. Gen. Physiol.* **50**, 1287–1302.
32. Armstrong, C. M. (1971) *J. Gen. Physiol.* **58**, 413–437.
33. Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. & Numa, S. (1987) *Nature (London)* **328**, 313–318.
34. Warmke, J., Drysdale, R. & Ganetzky, B. (1991) *Science* **252**, 1560–1562.
35. Sentenac, H., Bonnaud, N., Minet, M., Lacroute, F., Salmon, J.-M., Gaymard, F. & Grignon, C. (1992) *Science* **256**, in press.