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Cloning and Expression in Yeast of a Plant Potassium Ion Transport System

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A membrane polypeptide involved in K^+ transport in a higher plant was cloned by complementation of a yeast mutant defective in K^+ uptake with a complementary DNA library from *Arabidopsis thaliana*. A 2.65-kilobase complementary DNA conferred ability to grow on media with K^+ concentration in the micromolar range and to absorb K^+ (or $^{86}Rb^+$) at rates similar to those in wild-type yeast. The predicted amino acid sequence (838 amino acids) has three domains: a channel-forming region homologous to animal K^+ channels, a cyclic nucleotide-binding site, and an ankyrin-like region.

In contrast to animal cells, plant cells are generally exposed to low K^+ concentrations, often in the micromolar range. Growth in such conditions is made possible by high affinity K^+ transport systems in the plasma membrane (*1*). Biochemical efforts to purify the transporters are difficult, because of the low abundance of these transport proteins, and screening cDNA libraries with heterologous DNA probes has been generally unsuccessful in plants (*2*).

A mutant (*3*) of *Saccharomyces cerevisiae*, unable to grow on low K^+ medium and belonging to the same complementation group as the *TRK1* (*4*) K^+ transport system, was here complemented with a cDNA library made from *Arabidopsis thaliana* seedlings. An *Arabidopsis* clone (AKT1) was able to complement the yeast mutant and effect K^+ transport (Fig. 1). In the low (micromolar) K^+ concentration range, the K^+ (or $^{86}Rb^+$) uptake rates were similar in the wild-type and complemented yeast strains; both were much higher than in the mutant strain. When the K^+ concentration was increased to the millimolar range, the uptake rate reached a saturation plateau in the wild-type strain but in the complemented strain continued to increase with increasing K^+ concentration. The kinetics of the K^+ transport in the complemented strain were complex (Fig. 1B) and did not fit classical (Michaelian) saturation kinetics. The kinetics of K^+ uptake in plant roots are also quite complex (*1*) and may nevertheless result from the activity of a single transport system (*5*).

The capacity of the protein encoded by AKT1 to accumulate K^+ was verified by transferring complemented yeast into a K^+-free medium. After an initial loss of K^+ (*3*), which increased the external K^+ concentration to 10 μM, a net influx developed, decreasing the external K^+ concentration to 0.65 μM (*6*). Under these conditions, the cytosolic K^+ concentration was estimated to be 0.17 M (*7*). Thus, yeast transformed with AKT1 maintained a high K^+ accumulation ratio (K_{int}/K_{ext} approximately 2.6×10^5), which corresponded to an equilibrium potential difference (E_K) of about −320 mV. No estimate of the actual membrane potential difference is available, and it was not possible to determine whether the protein encoded by AKT1 mediated passive or active K^+ transport (*8*).

A Southern (DNA) blot of Eco RI–digested genomic DNA from *Arabidopsis* probed with the 2.65-kb AKT1 cDNA showed a single band. Northern (RNA) blot of total RNA from *Arabidopsis* indicated that a single 2.8-kb transcript hybridized with the AKT1 cDNA. The difference in length between the transcript and the cDNA may result from the loss of the polyadenylate tail or from cloning a cDNA incomplete in the 5′ upstream region (Fig. 2).

The AKT1 cDNA encodes a predicted peptide of 838 amino acids (MW 95.4 kD) (Fig. 2). No homology was found with the yeast *TRK1* gene product (*4*) or with K^+-transporting ATPases from bacteria and

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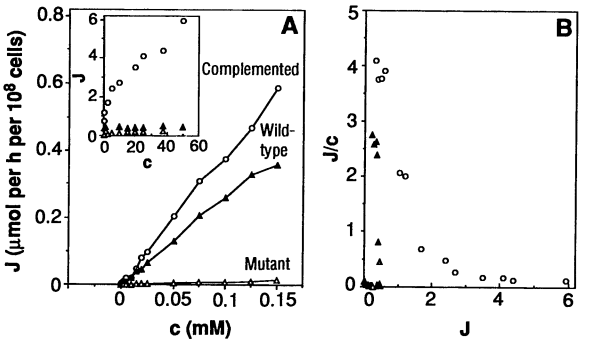

Fig. 1. Heterologous expression of an *A. thaliana* K^+ channel in *S. cerivisiae*. (**A**) $^{86}Rb^+$ influx (J), in media of various K^+ concentrations (c). (**B**) Eadie-Hofstee transformation of the data shown in (A). A double mutant defective in K^+ uptake and deleted for the *URA3* gene was obtained from a cross between the PC1 strain (*3*) and a *URA3Δ* strain. This double mutant was complemented by the spheroplast transformation method with an *Arabidopsis* cDNA library and the shuttle vector pFL61 (*30*) bearing the yeast *URA3* marker. Expression of the cDNA was controlled by the constitutive promotor of the phosphoglycerate kinase gene. The transformants were grown on a solid medium containing 20 μM K^+. Twelve independent clones with growth characteristics similar to the wild-type strain were obtained from 6 × 10^5 transformants. The corresponding plasmids were subcloned in *E. coli* and reintroduced into the mutant yeast strain, which they again stably complemented. K^+ transport was studied (*31*) with the mutant strain complemented by one of the plasmids (pHS41).

Fig. 2. Deduced amino acid (*20*) sequence encoded by AKT1 cDNA in plasmid pHS41. The nucleotide sequence of the 2649-bp cDNA revealed a single 2517-bp open reading frame (nucleotides 58 through 2574). The cloned cDNA did not have a polyadenylate tail. The putative transmembrane segments (S1 through S6) and the H5 region in the predicted peptide are underlined. The EMBL accession number of the nucleotide and amino acid sequences is X62907.

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  1  MRGGALLCGQ  VQDEIEQLSR  ESSHFSLSTG  ILPSLGARSN  RRVKLRRFVV
 51  SPYDHKYRIW  EAFLVVLVVY  TAWVSPFEFG  ELRKPRPPLS  LTDNIVNAFF
                          S1
101  AIDIIMTFFV  GYLDKSTYLI  VDDRKQIAFK  YLRSWFLLDL  VSTIPSEAAM
                S2                                   S3
151  RISSQSYGLF  NMLRLWRLRR  VGALFARLEK  DRNFNYFWVR  CAKLVCVTLF
                                    S4
201  AVHCAACFYY  LIAARNSNPA  KTWIGANVAN  FLEESLWMRY  VTSMYWSITT
           S5
251  LTTVGYGDLH  PVNTKEMIFD  IFYMLFNLGL  TAYLIGNMTN  LVVHGTSRTR
           S5                                   S6
301  NFRDTIQAAS  NFAHRNHLPP  RLQDQMLAHL  CLKYRTDSEG  LQQQETLDAL
351  PKAIRSSISH  FLFYSLMDKV  YLFRGVSNDL  LFQLVSEMKA  EYFPPKEDVI
401  LQNEAPTDFY  ILVNGTADLV  DVDTGTESIV  REVKAGDIIG  EIGVLCYRPQ
451  LFTVRTKRLC  QLLRMNRTTF  LNIIQANVGD  GTIIMNNLLQ  HLKEMNDPVM
501  TNVLLEIENM  LARGKMDLPL  NLCFAAIRED  DLLLHQLLKR  GLDPNESDNN
551  GRTPLHIAAS  KGTLNCVLLL  LEYHADPNCR  DAEGSVPLWE  AMVEGHEKVV
601  KVLLEHGSTI  DAGDVGHFAC  TAAEQGNLKL  LKEIVLHGGD  VTRPRRTGTS
651  ALHTAVCEEN  IEMVKYLLEQ  GADVNKQDMH  GWTPRDLAEQ  QGHEDIKALF
701  REKLHERRVH  IETSSSVPIL  KTGIRFLGRF  TSEPNIRPAS  REVSFRIRET
751  RARRKTNNFD  NSLFGILANQ  SVPKNGLATV  DEGRTGNPVR  VTISCAEKDD
801  IAGKLVLLLE  FQGVARIGFQ  QVWYCCYQSY  EQRQQCRD
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A

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E. coli CAP   24-110  YPSKSTLIHQGEKAETLYYIVKGSVAVLIKDEEGKEMILSYLNQGDFIGELGLFEEGQERSAWVRAKTACEVAEISYKKFRQLIQV
AKT1          393-477 FPPKEDVILQNEAPTDFYILVNGT-ADLVDVDTGTESIVREVKAGDIIGEIGVLCYRPQLFT-VRTKRLCQLLRMNRTTFLNIIQA
cAMP-gated    475-558 FSPGDYICRKGDIGKEMYIIKEGK-LAVVADDGVTQYAL--LSAGSCFGEISILNIKGS--K-MGNRRTANIRSLGYSDLFCLSKD
channel
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B

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Anky    x G x T P L H x A A x x G H x x x V x x L L x x G A x x N x x x x
Cons    x G x T P L H x A A x x x x x x x V x x L L x x G A x x x x x D x

517-549  D L P L N L C F A A I R E D D L L L H Q L L K R G L D P N E S D N
550-582  N G R T P L H I A A S K G T L N C V L L L E Y H A D P N C R D A
583-614  E G S V P L W E A M V E G H E K V V K V L L E H G S T I D A G D -
615-646  V G H F A - C T A A E Q G N L K L L K E I V L H G G D V T R P R R
647-679  T G T S A L H T A V C E E N I E M V K Y L L E Q G A D V N K Q D M
680-712  H G W T P R D L A E Q Q G H E D I K A L F R E K L H E R R V H I E
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Fig. 3. Analysis of the AKT1 polypeptide sequence. (**A**) Homologies with putative cyclic nucleotide–binding sites. The displayed region was identified as a putative cyclic nucleotide–binding site by comparison with *E. coli* CAP (*24*) and cyclic nucleotide–gated channels (*10, 11*). (**B**) Comparison of aligned ankyrin-like repeats of AKT1 with the erythrocyte ankyrin consensus (*26*) (Anky), and with the consensus (Cons) for similar repeats in nine other proteins (*28*). An "x" in the Anky and Cons sequences marks a position where no consensus exists. Amino acid gaps (marked by hyphens) were introduced for maximal alignments (*13*). Identical residues are indicated by colons or by boxes, and conservative substitutions by single points. Numbers: location of the first and last amino acids of each repeat. Amino acid groups for conservative changes: [K, R]; [D, E]; [N, Q]; [A, G]; [S, T]; [I, M, L, V]; [F, W, Y].

mammals (*9*). However, homologies with cAMP-gated channels from rat or bovine olfactory epithelium (*10, 11*) extend from amino acids 36 to 522 [22.3 or 22.5% identity within that region]. Homologies with the cGMP-gated channel from bovine rods (*12*) extend from amino acids 49 to 507 (22.7% identity).

Homologies were found (*13*) with K^+ channels of the Shaker family found in insects and mammals (*14*). The Shaker channels are thought to consist of four subunits (*15*) arranged about a central pore; each subunit consists of six transmembrane segments called S1 to S6 (*16*). Site-directed mutagenesis and electrophysiological measurements suggest that S4 acts as a voltage sensor (*17*). The sequence of S4 is characterized by repetition of basic amino acids at every third or fourth position (*14*). In the protein encoded by AKT1, a region homologous to S4 is present between amino acids 160 and 185 that has six basic residues conserved out of the seven present in the corresponding region of the Shaker K^+ channel ShB (*14, 17*).

Among K^+ channels, the highest degree of sequence identity is found in the putative pore-forming sequence (*18*), called H5 (*14*), located between S5 and S6 and thought to span the membrane as a β hairpin. Amino acids 242 to 266 of the protein encoded by AKT1 show homology with the H5 region (*14*) of voltage- or cyclic nucleotide–gated K^+ channels. The ends of the H5 segment are parts of the external vestibule of the channel (*19*), and the amino acid motif T-T-V (*20*) within H5 (corresponding to amino acids 252 to 254 in the protein encoded by AKT1) are involved in the formation of the internal vestibule (*18*). In the Shaker K^+ channel ShB, amino acids 433 (external vestibule), 441, and 442 control the cation selectivity (*21*). In the protein encoded by AKT1, the F at position 433 is conservatively replaced

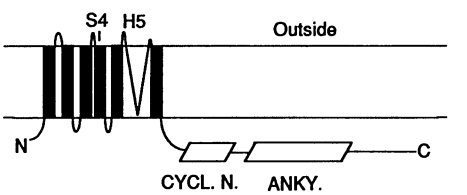

Fig. 4. Proposed topography of the AKT1 polypeptide. The averaged hydropathicity index values (*32*) were calculated with a window size of 11 amino acids. We assumed that the ankyrin-related domain (ANKY.), the putative cyclic nucleotide–binding site (CYCL. N.), and the middle portion of H5 (*18*) were intracellular, and that the ends of H5 were extracellular (*19*). The six transmembrane segments, including S4 (*17*), are shaded. N, NH_2-terminus; C, COOH-terminus.

by a Y at 245, and the Ts at 441 and 442 are conserved (252 and 253).

The region between amino acids 393 and 477 is homologous with mammalian ion channels (*10–12*) and protein kinases (*22*) that are regulated by cGMP or cAMP, and with the catabolite gene activator protein (CAP) of *Escherichia coli* (*23*) (Fig. 3A), which includes a cAMP binding site (*24*). Similarly, the corresponding regions of cyclic nucleotide–gated channels are thought to bind cAMP (*10, 11*). The significance of homologies to these regions in the protein encoded by AKT1 is not clear because there is as yet no conclusive evidence for the presence of cyclic nucleotides in higher plants (*25*).

The six imperfect repeating sequences of 32 or 33 amino acids between positions 517 and 712 of the protein encoded by AKT1 (Fig. 3B) show homology to a 33-residue motif repeated 22 times in tandem in erythrocyte ankyrin (*26*), a protein that attaches integral membrane proteins to cytoskeleton components. In brain, ankyrin links a voltage-dependent Na^+ channel to spectrin, and thus may restrict the channel to specific locations in the neuronal membrane (*27*). Similar repeats have been observed in a variety of proteins and are thought to tether the subunits of regulatory proteins (*28, 29*). Thus, the presence of ankyrin-like repeats in the protein encoded by AKT1 suggests that this transport system may interact with the cytoskeleton or with regulatory proteins.

The sequence homologies between the protein encoded by AKT1 and cyclic nucleotide–gated channels encompass the six putative transmembrane segments present in the channels between the NH_2-terminus and the cyclic nucleotide–binding region. These homologies with this region of the channels suggest that the protein encoded by AKT1 also has six transmembrane segments (Fig. 4), although only four (S1, S2, S5, and S6) may be inferred from the hydropathicity plot. Whatever the exact topography, the presence in a plant transport system of both the highly conserved S4 and H5 regions, typical of Shaker channels, and of a cyclic nucleotide–binding site supports the hypothesis of an ancient common origin of voltage-gated and cyclic nucleotide–gated channels (*14*).

It is unlikely that the protein encoded by AKT1 is an accessory polypeptide interacting with yeast transport proteins, because the protein encoded by AKT1 was not homologous to the yeast *TRK1* gene product (*4*), and expression of AKT1 in the mutant yeast strain was sufficient to form a functional K^+ uptake system. The homologous Shaker polypeptides form functional channels when expressed in *Xenopus* oocytes (*15*).

Note added in proof: Functional expression of another putative K^+ transport system cDNA (*KAT1*) from *Arabidopsis thaliana* in a yeast *trk1*Δ *trk2*Δ mutant has just been reported (*29a*). The predicted amino acid sequences of *AKT1* and *KAT1* share extensive identity but are not allelic.

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DNA Hydrolyzing Autoantibodies

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A DNA-nicking activity was detected in the sera of patients with various autoimmune pathologies and was shown to be a property of autoantibodies. The DNA hydrolyzing activity, which was purified by affinity and high-performance liquid chromatography, corresponded in size to immunoglobulin M (IgM) and IgG and had a positive response to antibodies to human IgG. The DNA hydrolyzing autoantibodies were stable to acid shock and yielded a DNA degradation pattern that was different from that of deoxyribonuclease (DNase) I and blood DNase.

Patients with autoimmune diseases produce autoantibodies to nucleoprotein complexes (*1*), to DNA, and to enzymes that participate in nucleic acid metabolism (*2*). In autoimmune diseases, there can be spontaneous induction of anti-idiotypic antibodies (Abs), which are Abs elicited by a primary antigen. These anti-idiotypic Abs may have characteristics of the primary antigen, including catalytic activity. In some cases, the sera of patients with scleroderma, systemic lupus erythematosus (SLE), or rheumatoid arthritis have an

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