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AtKUP1: A Dual-Affinity K^+ Transporter from Arabidopsis

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Plant roots contain both high- and low-affinity transport systems for uptake of K^+ from the soil. In this study, we characterize a K^+ transporter that functions in both high- and low-affinity uptake. Using yeast complementation analysis, we isolated a cDNA for a functional K^+ transporter from Arabidopsis (referred to as *AtKUP1* for *Arabidopsis thaliana* K^+ uptake). When expressed in a yeast mutant, AtKUP1 dramatically increased K^+ uptake capacity at both a low and high [K^+] range. Kinetic analyses showed that AtKUP1-mediated K^+ uptake displays a "biphasic" pattern similar to that observed in plant roots. The transition from the high-affinity phase (K_m of 44 μM) to the low-affinity phase (K_m of 11 mM) occurred at 100 to 200 μM external K^+. Both low- and high-affinity K^+ uptake via AtKUP1 were inhibited by 5 mM or higher concentrations of NaCl. In addition, AtKUP1-mediated K^+ uptake was inhibited by K^+ channel blockers, including tetraethylammonium, Cs^+, and Ba^{2+}. Consistent with a possible function in K^+ uptake from the soil, the *AtKUP1* gene is primarily expressed in roots. We conclude that the *AtKUP1* gene product may function as a K^+ transporter in Arabidopsis roots over a broad range of [K^+] in the soil.

INTRODUCTION

Potassium (K^+) is required for the activity of numerous enzymes in plant cells and for the maintenance of turgor, which is a prerequisite for plant growth and development (Epstein, 1972; Kochian and Lucas, 1988). As an essential macroelement, potassium normally constitutes 2% of the total dry weight of a plant, and its concentration in fresh tissues is in the range of 10 to 100 mM (Glass, 1988). Such high levels of K^+ are accumulated by roots from soil solutions that usually contain only micromolar levels of soluble K^+. As a result, plant roots have evolved uptake mechanisms that operate at low external [K^+] with high concentrative capacity (Epstein, 1973). When the rate of K^+ uptake is examined over a wide range of external [K^+], Epstein and colleagues discovered a "biphasic" pattern of K^+ uptake by barley roots (Epstein et al., 1963; Epstein, 1966, 1972). They proposed the presence of two K^+ uptake mechanisms working simultaneously at the plasma membrane (Epstein et al., 1963). The high-affinity system was thought to be mediated by a "carrier" that functions to sustain K^+ accumulation in plants when external [K^+] is in the micromolar range. The low-affinity system functions as a "passive" transporter responsible for K^+ uptake in the presence of millimolar K^+ concentrations in the soil.

The molecular basis for biphasic uptake kinetics remained elusive until recently, when two approaches in the study of plant ion transport were implemented. One is patch–clamp techniques that have been used to monitor ion fluxes across cell membranes (Neher and Sakmann, 1976). Voltage-gated K^+ channel activities are found in almost all plant cell types studied to date (Hedrich and Schroeder, 1989; Tester, 1990; Blatt, 1991), suggesting that K^+ transport is conducted at least in part by K^+ channels in the membrane. Another approach is molecular cloning. Several K^+ transporter genes have been isolated from higher plants (Anderson et al., 1992; Sentenac et al., 1992; Schachtman and Schroeder, 1994; Cao et al., 1995; Mueller-Roeber et al., 1995). Among these genes, *KAT1*, *AKT1*, and *KST1* encode closely related inward K^+ channels from Arabidopsis and potato plants (Schachtman et al., 1992; Mueller-Roeber et al., 1995; Gaymard et al., 1996).

A combination of patch–clamp and molecular analyses suggests that ion channels may act as low-affinity transporters (Kochian et al., 1993; Gassmann and Schroeder, 1994; Maathuis and Sanders, 1994, 1995; Schroeder et al., 1994). In response to the negative membrane potential generated by the H^+ pump, low-affinity K^+ uptake can be mediated by voltage-gated inward K^+ channels. High-affinity uptake has been shown to take place by coupling the movements of K^+ and other cations, such as H^+ in Arabidopsis roots (Maathuis and Sanders, 1994) and Na^+ in algae (Smith and Walker, 1989). One of the genes encoding the high-affinity K^+ transporter HKT1 from wheat has been functionally characterized in Xenopus oocytes and yeast (Schachtman and Schroeder, 1994; Rubio et al., 1995). This K^+ transporter functions as a Na^+/K^+ symporter because K^+ uptake by HKT1 expressed in oocytes and yeast is greatly facilitated by low [Na^+] (Rubio et al., 1995).

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To understand the molecular basis for biphasic K^+ uptake (Epstein, 1972), we have used yeast complementation to search for genes encoding K^+ transporters in Arabidopsis. A novel cDNA (referred to as *AtKUP1* for *Arabidopsis thaliana* K^+ uptake) was isolated and shown to encode a K^+ transporter previously unidentified in higher plants. Functional analyses using the yeast model indicate that the *AtKUP1* gene product mediates K^+ transport with dual affinity.

RESULTS

Isolation and Sequence Analysis of the *AtKUP1* cDNA

The *Saccharomyces cerevisiae TRK1* gene encodes a high-affinity K^+ transporter that is required for K^+ uptake at low external [K^+] (Ko and Gaber, 1991). A mutant strain carrying a *TRK1* deletion (CY162) is lethal when the medium contains low levels of K^+. As shown previously (Anderson et al., 1992; Sentenac et al., 1992; Schachtman and Schroeder, 1994), expression of plant genes encoding K^+ transporters rescues a *trk1Δtrk2Δ* mutant strain on low [K^+] medium. We used a similar approach and identified a number of cDNA clones that rescued lethality of a yeast *trk1Δtrk2Δ* mutant (CY162) on low K^+ medium. To isolate cDNAs for novel K^+ transporters, we first eliminated colonies containing cDNAs for known K^+ transporters by using low-stringency colony hybridization (see Methods). Partial sequence analysis revealed one cDNA clone with significant homology to the *kup* gene of *Escherichia coli* (Schleyer and Bakker, 1993) and *HAK1* of *Schwanniomyces occidentalis* (Banuelos et al., 1995). Both function in K^+ uptake. To confirm the function of the *kup*-like cDNA, we subcloned it into a different vector and introduced the plasmid into the CY162 strain. Again, the *kup*-like cDNA rescued the yeast mutant on low [K^+] medium (Figure 1).

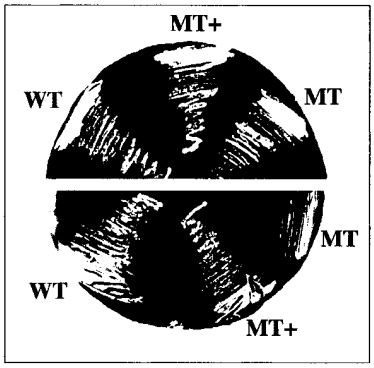

Figure 1. Complementation of the CY162 Yeast Strain by the *kup*-like cDNA.

The upper half of the circle shows growth on 50 mM KCl of wild-type (WT) yeast, mutant (MT) yeast, and mutant yeast transformed with the original 1.8-kb cDNA in the pRH98-3 vector (MT+). The lower half of the circle shows growth of the same strains at a low concentration of KCl (0.2 mM).

The *kup*-like cDNA was completely sequenced and found to be a partial cDNA clone with 3′ truncation in the coding region because no stop codon was present. The truncation also became apparent when the encoded sequence was compared with those of the *kup* and *HAK1* genes (data not shown). We used the *kup*-like sequence as a query to search the database and identified two expressed sequence tag (EST) entries in the Arabidopsis database (G10F3T7 and H4G8T7) that were nearly identical to the 5′ end of the *kup*-like cDNA sequence. One EST (G10F3T7) contains a cDNA insert with a longer 3′ sequence compared with the original *kup*-like cDNA. Both 5′ sequencing and restriction mapping suggested that the *kup*-like cDNA and EST G10F3T7 may come from the identical gene.

The EST clone (G10F3T7) was then completely sequenced and compared with the original *kup*-like cDNA sequence. This confirmed that the two cDNAs carry the same sequence over a 1671-bp common region. A full-length cDNA sequence was pieced together by combining the 5′ sequence of the *kup*-like cDNA and the 3′ sequence of EST G10F3T7 (see Methods). This full-length cDNA is referred to as *AtKUP1* (Figure 2A). After we submitted our manuscript, Quintero and Blatt (1997) reported the sequence of the *AtKT1* gene (GenBank accession number AF012656) that appears to be identical to *AtKUP1*. The *AtKUP1* cDNA codes for a protein of 712 amino acids, which is 145 amino acids longer than the original cDNA isolated by using yeast complementation. The amino acid sequence of the AtKUP1 protein is 31 and 32% identical to the *kup* and the *HAK1* gene products, respectively. Sequence alignment of the AtKUP1 and HAK1 proteins is shown in Figure 2B.

Based on the structural features, especially the hydropathy plot (data not shown), the AtKUP1 protein displays properties of a typical transporter. The first 500 amino acids are highly hydrophobic, with at least 12 regions that can serve as transmembrane domains (shown as underlined regions in Figure 2B). The C-terminal 212 amino acids are largely hydrophilic and may be located in the cytoplasm. A large portion of this hydrophilic region (from amino acids 567 to 712) is apparently not required for basic functioning because the original clone with a 145–amino acid C-terminal truncation can still complement yeast mutant CY162.

Genomic DNA Analysis and Gene Expression Pattern

Genomic DNA analysis confirmed that AtKUP1 is encoded by the Arabidopsis genome (Figure 3). Based on high-stringency DNA gel blot analysis, we determined that only one copy of the gene exists in the Arabidopsis genome. Both PstI and HindIII may cut into the genomic sequence of *AtKUP1* from the Arabidopsis Columbia ecotype, thereby

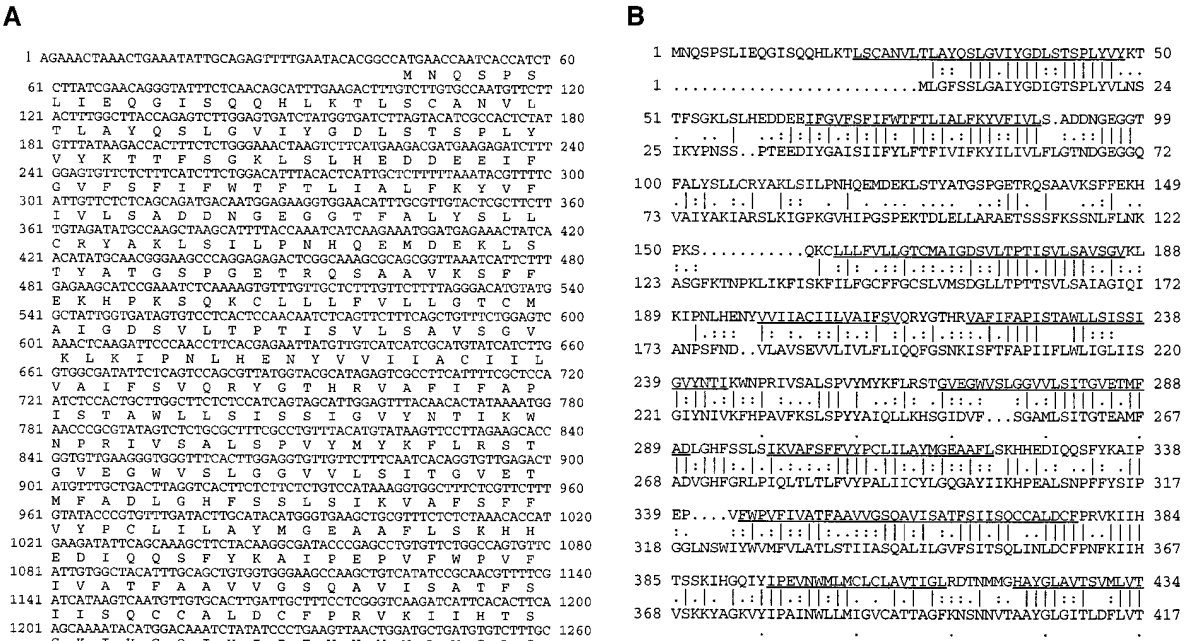

Figure 2. Sequence Analyses of the *AtKUP1* cDNA.

(A) The nucleotide and deduced amino acid sequences of *AtKUP1*. The asterisk indicates the stop codon. The *AtKUP1* sequence has GenBank accession number AF033118.

(B) Amino acid sequence alignment of the AtKUP1 and HAK1 proteins. The underlined regions are putative transmembrane domains, as determined on a hydropathy plot. Identical amino acids are indicated by vertical lines and similar ones by colons. Dots were introduced to optimize alignment.

generating the two bands seen on the DNA gel blot (Figure 3, lanes 4 and 6). When comparing the hybridization pattern of genomic DNA from the Columbia and Landsberg *erecta* ecotypes, a restriction fragment length polymorphism is apparent (Figure 3, lanes 3 and 6).

To determine the expression pattern of the *AtKUP1* gene, we performed RNA gel blot analyses by using total RNA isolated from various Arabidopsis tissues. As shown in Figure 4A, hybridization at 58°C revealed two closely related transcripts, with both being preferentially expressed in roots and stems. At a higher hybridization temperature (65°C), only *AtKUP1* mRNA was detected, and it was expressed primarily in root tissues. A much lower level of *AtKUP1* transcript accumulated in stems, and the mRNA level was barely

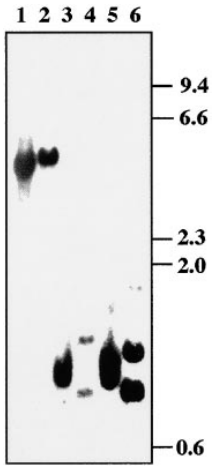

Figure 3. DNA Gel Blot Analysis of the *AtKUP1* Gene.

Arabidopsis genomic DNA was isolated from ecotypes Landsberg *erecta* (lanes 1, 3, and 5) and Columbia (lanes 2, 4, and 6) and digested with SacI (lanes 1 and 2), PstI (lanes 3 and 4), and HindIII (lanes 5 and 6), respectively. The numbers at right are in kilobases.

detectable in leaves and flowers (Figures 4B and 4C). This expression pattern suggests a possible function of the *AtKUP1* gene product in K^+ uptake by roots from the soil. The other transcript detected under lower stringency conditions may be a product of another gene highly homologous to *AtKUP1.*

The *AtKUP1* gene has been independently characterized by Kim et al. (1998). Under their growth conditions, a different RNA expression pattern was observed (Kim et al., 1998).

AtKUP1 Mediates Both Low- and High-Affinity K^+ Uptake

To test whether the AtKUP1 protein functions in K^+ uptake, we expressed the full-length *AtKUP1* cDNA in a yeast mutant strain (CY162) and performed uptake analyses by using rubidium-86 as a radioactive tracer. First, we analyzed rubidium-86 accumulation as the function of uptake time in three yeast strains, including the wild type (R757, *Trk1*$^+$*Trk2*$^+$), the mutant (CY162, *Trk1*$^-$*Trk2*$^-$), and the mutant carrying *AtKUP1* (CY162, *Trk1*$^-$*Trk2*$^-$*AtKUP1*$^+$).

Because the functional properties of K^+ transporters are often affected by external K^+ concentrations (Epstein, 1972; Schroeder et al., 1994; Maathuis and Sanders, 1996), we performed time-course analyses of K^+ uptake under three external K^+ concentrations, namely, 0.02, 0.2, and 20 mM. At 0.02 mM, K^+ uptake was slow in the mutant cells. Wild-type cells accumulated K^+ up to 13-fold faster than did the mutant. AtKUP1 increased the K^+ uptake capability of the mutant up to 5.6-fold (Figure 5A). At 0.2 mM, the AtKUP1 and wild-type yeast cells had a similar rate of K^+ uptake (three- to fourfold higher than in the mutant; Figure 5B). In the presence of 20 mM external K^+, the wild-type and the mutant cells accumulated K^+ at the same rate, whereas the AtKUP1 strain had a much higher uptake rate (up to 3.5-fold higher when compared with both wild-type and mutant cells [Figure 5C]). This result confirmed that TRK1 in wild-type yeast is responsible for high-affinity K^+ uptake at low external [K^+] (0.02 and 0.2 mM) but does not contribute to uptake under high [K^+] (20 mM) (Ko and Gaber, 1991). On the other hand, the *AtKUP1* gene product functions as a K^+ transporter in a wide range of external [K^+] (0.02 to 20 mM). In all cases, yeast cells accumulated rubidium-86 almost in a linear fashion in the first 20 min and did not reach equilibrium within 3 hr under the conditions used in this study.

According to Epstein (1972), K^+ uptake in plant roots displays a biphasic kinetics. High-affinity uptake occurs in the low [K^+] range (1 to 200 μM), whereas low-affinity uptake takes place in the high [K^+] range (1 to 50 mM). As shown in Figure 5, AtKUP1 appears to be functional in both high- and low-affinity phases. To define whether AtKUP1 functions as a low- or high-affinity transporter, we performed detailed kinetic analyses on K^+ uptake under 17 different external K^+ concentrations. To calculate the uptake rate for individual transporters, including TRK1 and AtKUP1, basal uptake in the mutant strain was subtracted from those in the wild-type and AtKUP1 strains, respectively. In Figure 6, kinetic analyses were plotted in three fragments to compare the pattern of K^+ uptake mediated by TRK1 and AtKUP1.

Figure 6A shows the uptake rate in the low [K^+] range (0 to 40 μM). Both TRK1 and AtKUP1 increased the uptake rate

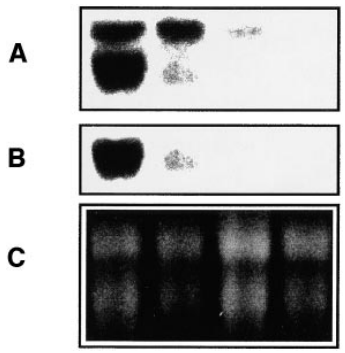

Figure 4. RNA Gel Blot Analysis of *AtKUP1* mRNA in Arabidopsis Tissues.

Total RNA was isolated from roots (first lane), stems (second lane), leaves (third lane), and flowers (fourth lane) of Arabidopsis plants.
(A) Detection of two transcript species at a hybridization temperature of 58°C.
(B) *AtKUP1* mRNA levels detected under high-stringency hybridization conditions (65°C).
(C) Ethidium bromide staining of rRNA bands after transfer to a Zeta probe membrane to show the relative amounts of total RNA analyzed.

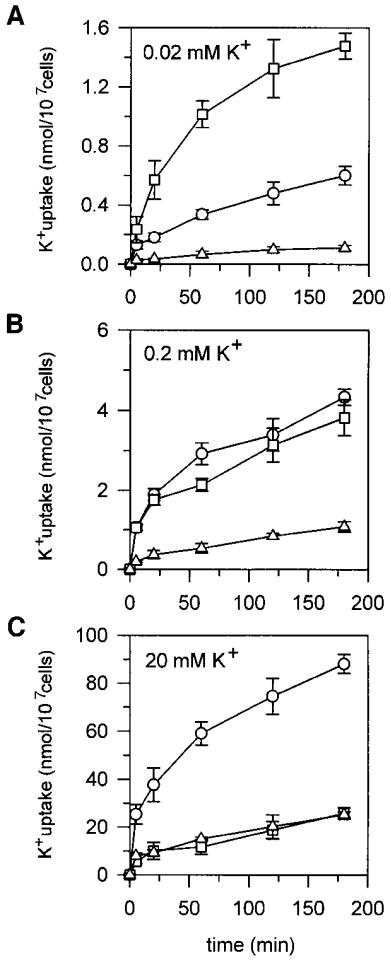

Figure 5. Time Courses of K^+ Uptake in Three Yeast Strains.

(A) K^+ uptake at 0.02 mM external [K^+].
(B) K^+ uptake at 0.2 mM external [K^+].
(C) K^+ uptake at 20 mM external [K^+].
In each case, mutant (open triangles), wild-type (open squares), and AtKUP1 (open circles) yeast cells were used.

in a linear fashion when external [K^+] increased from 1 to 20 μM. At 40 μM, K^+ uptake by TRK1 changed the slope slightly. The uptake rate of AtKUP1 changed more dramatically and almost reached saturation. As the external [K^+] continued to increase (Figure 6B), a more striking difference was observed in the kinetic pattern of K^+ uptake by AtKUP1 and TRK1. TRK1-mediated uptake followed a typical monophasic pattern and gradually decreased its slope. AtKUP1 uptake displayed a distinct pattern, with a sharp increase in uptake rate between 100 and 200 μM external [K^+] (Figure 6B). This is typical of the biphasic pattern observed on K^+ uptake by barley roots (see Figure 8 in Epstein, 1973).

TRK1 is responsible for only high-affinity K^+ uptake under low external [K^+] (Figure 5; see also Ko and Gaber, 1991). Indeed, the uptake rate in wild-type and mutant cells was the same when external [K^+] increased to 1 mM and more. The low-affinity phase of both wild-type and mutant cells reached saturation at 10 mM external [K^+] (data not shown). In contrast, uptake in AtKUP1 cells initiated a low-affinity phase that is absent in the wild-type and the mutant cells. This AtKUP1-mediated low-affinity transport was saturated at a high external [K^+] (80 to 100 mM) (Figure 6C). According

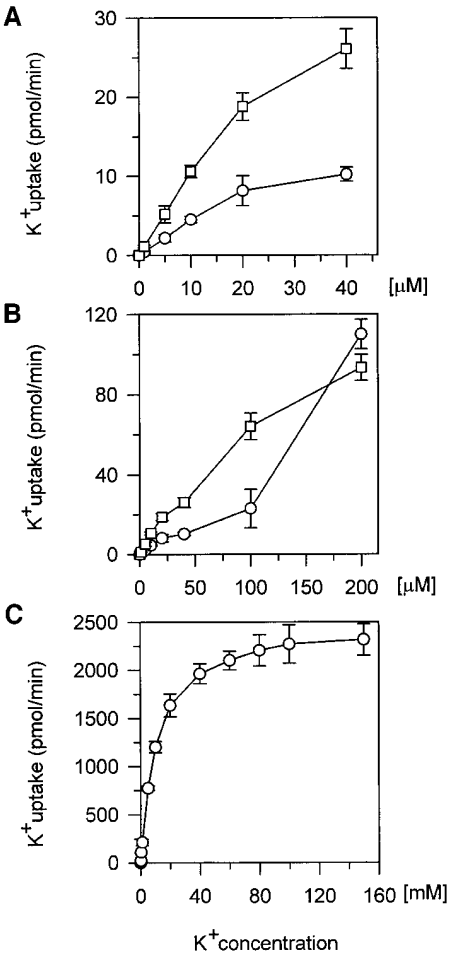

Figure 6. Kinetic Analyses of K^+ Uptake by TRK1 and AtKUP1 over a Wide Range of External [K^+].

(A) The uptake rate was plotted as a function of external [K^+] from 0 to 40 μM.
(B) The uptake rate in the range of 0 to 200 μM.
(C) Uptake rate in the range of 0 to 150 mM.
TRK1- and AtKUP1-mediated uptake are shown as open squares and open circles, respectively. The uptake rate is presented as picomoles per minute for each sample of 10^7 yeast cells. In the high [K^+] range, only the kinetics for AtKUP1-mediated K^+ uptake are shown **(C)**. Five independent experiments were performed with three duplicates for each treatment. The data points are shown as mean ±SE.

to the kinetic analysis, TRK1, the high-affinity transporter in the wild-type yeast, displays one-phase uptake under low external [K^+] (200 μM and lower). Uptake by AtKUP1 displays a biphasic pattern with two K_m values: 44 μM for the high-affinity phase and 11 mM for the low-affinity phase.

AtKUP1-Mediated K^+ Uptake Is Inhibited by K^+ Channel Blockers

As a step toward understanding the mechanism of AtKUP1-mediated K^+ transport, we performed K^+ uptake assays in the presence of several K^+ channel inhibitors. Because K^+ uptake by AtKUP1 displays biphasic kinetics, we performed inhibitor studies at two external K^+ concentrations, 20 μM and 20 mM, representing high- and low-affinity [K^+], respectively. Three commonly used K^+ uptake inhibitors were chosen in this study. Tetraethylammonium (TEA) is considered as a specific blocker of voltage-gated K^+ channels. Ba^{2+} and Cs^+ inhibit K^+ uptake through most K^+ channels and some other transporters (Hedrich and Schroeder, 1989; Tester, 1990; Hille, 1992). At low [K^+] (20 μM), the mutant strain accumulated K^+ at a low rate and was not sensitive to the three inhibitors. $BaCl_2$ and CsCl but not TEA inhibited uptake by the wild-type yeast cells. K^+ uptake by AtKUP1 yeast cells is not only sensitive to Ba^{2+} and Cs^+ but is also inhibited by TEA (Figure 7A). At high [K^+] (20 mM), K^+ uptake in the wild-type and mutant cells was moderately inhibited by both Ba^{2+} and Cs^+. Unlike mutant cells, however, wild-type cells were also sensitive to TEA, implicating some difference in low-affinity uptake in the two strains (Figure 7B). Uptake rate in AtKUP1 cells was dramatically reduced by all three blockers, including Ba^{2+}, Cs^+, and TEA (Figure 7B).

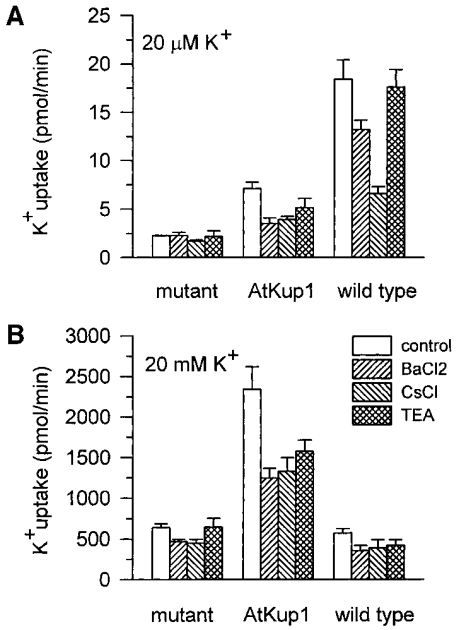

Figure 7. Inhibitor Analysis of K^+ Uptake in Three Yeast Strains.

(A) Analyses at external [K^+] of 20 μM.
(B) Analyses at external [K^+] of 20 mM.
The concentration for each inhibitor was 10 mM.

Na^+ Inhibition of AtKUP1-Mediated K^+ Uptake

Earlier studies demonstrated that K^+ uptake by plant roots is inhibited by millimolar concentrations of NaCl (reviewed in Epstein, 1973). This has been considered as one of the mechanisms for high salt-induced inhibition of plant growth (Epstein, 1972; Wu et al., 1996). Recent studies have shown that high-affinity K^+ uptake by HKT1 is stimulated by low [NaCl] (0.1 to 1 mM) and inhibited by high [NaCl] (100 to 500 mM) (Rubio et al., 1995). NaCl sensitivity thus becomes an important feature of K^+ transport in higher plants.

To determine whether K^+ uptake by AtKUP1 is affected by NaCl, we performed uptake assays in the presence of various concentrations of NaCl. At the low external [K^+] (20 μM), K^+ uptake by both wild-type and AtKUP1 cells was inhibited by high concentrations of NaCl (5 mM and higher). Interestingly, the maximal inhibition of the wild type was only 50% compared with control level and was achieved by 10 mM NaCl. In the case of AtKUP1, K^+ uptake was almost completely blocked by high concentrations of NaCl. The K^+ uptake rate was only 11% of the control level when 50 mM NaCl was present in the uptake solution (Figure 8A). The low-affinity phase of K^+ uptake by AtKUP1 was also very sensitive to NaCl. As shown in Figure 8B, NaCl at 5 mM or higher concentrations dramatically reduced the K^+ uptake by AtKUP1, whereas uptake by wild-type yeast cells was not affected. In all cases, low NaCl concentrations (0.1, 0.5, and 1 mM) did not have a significant effect on K^+ uptake compared with the uptake rate in the absence of NaCl (Figure 8).

DISCUSSION

Potassium uptake by plant roots displays a biphasic kinetics (Epstein, 1972). Recently, molecular and patch–clamp studies suggested that the two phases of K^+ uptake are generated by the presence of both high- and low-affinity transporters in higher plants. The voltage-gated K^+ channels may serve as low-affinity transporters (Kochian et al., 1993; Gassmann and Schroeder, 1994; Maathuis and Sanders, 1994, 1995; Schroeder et al., 1994). On the other hand, HKT1, a Na^+/K^+ symporter, may serve as a high-affinity transporter (Schachtman and Schroeder, 1994; Rubio et al., 1995). In this study, we have characterized a novel K^+ transporter with "dual affinity" in K^+ uptake.

Complementation of yeast *trk1Δtrk2Δ* strain has been used successfully in the isolation of several plant genes en-

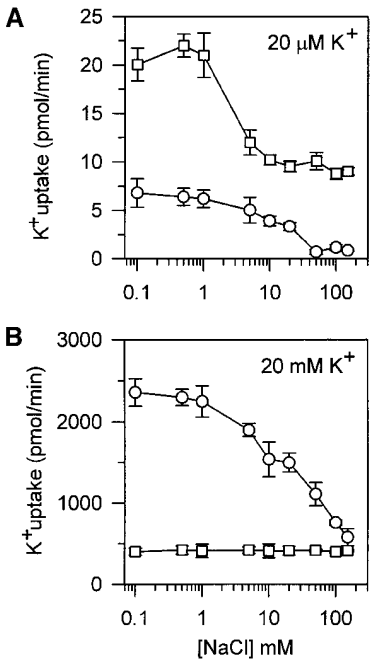

Figure 8. Inhibition of AtKUP1-Mediated K^+ Uptake by NaCl.

(A) NaCl effect at 20 μM external [K^+].
(B) NaCl effect at 20 mM external [K^+].
NaCl concentrations included in the uptake assay are detailed in Methods. Uptake in wild-type and AtKUP1 cells is presented as open squares and open circles, respectively.

coding K^+ transporters (Anderson et al., 1992; Sentenac et al., 1992; Schachtman and Schroeder, 1994). Using the same approach, we isolated the *AtKUP1* cDNA from Arabidopsis. Its sequence is highly homologous to those of two K^+ transporters found in the GenBank database—*kup* from *E. coli* (Schleyer and Bakker, 1993) and *HAK1* from *Schwanniomyces occidentalis* (Banuelos et al., 1995). Sequence comparison and hydropathy analysis indicate that all three K^+ transporters contain at least 12 putative transmembrane domains. It may be of evolutionary significance that the ancient Kup transporter in a prokaryotic system is conserved in *S. occidentalis* and Arabidopsis, a soil yeast and a higher plant, respectively, but not in the budding yeast *S. cerevisiae.* A different K^+ transporter in budding yeast, TRK1, also contains 12 transmembrane domains (Ko and Gaber, 1991) and may be the K^+ uptake system functionally equivalent to the Kup-like systems in organisms discussed above.

Perhaps the most striking feature of AtKUP1-mediated K^+ uptake is its biphasic kinetics (Figure 6), which are similar to those of plant roots (Epstein, 1972) but distinct from any of the other known K^+ transporters identified from higher plants. In the case of barley roots, the high-affinity phase of K^+ uptake is saturated by 100 to 200 μM external [K^+]. The low-affinity phase starts at 0.5 to 1 mM K^+ and is saturated by 50 mM external [K^+] (Epstein, 1972). When expressed in the *trk1*Δtrk2Δ mutant strain, AtKUP1 confers on yeast cells a higher capability of K^+ uptake at both low and high external [K^+]. In the low [K^+] range, AtKUP1-mediated K^+ uptake reaches a plateau at 40 to 100 μM K^+. Strikingly, between 100 and 200 μM [K^+], the uptake rate sharply increases and initiates the low-affinity phase that reaches saturation at high external [K^+] (80 to 100 mM). The uptake rate as a function of external [K^+] displays a typical biphasic pattern with a high-affinity K_m of ~44 μM and a low-affinity K_m of ~11.0 mM. In a study with the high-affinity transporter HKT1, the K_m value obtained in the yeast model was 29 μM (Schachtman and Schroeder, 1994). A low-affinity transporter, KAT1, had a K_m of 620 μM, using the same model system (Kochian et al., 1993).

Comparing the kinetics of K^+ uptake by AtKUP1 with that by barley roots, a parallel is apparent, except that the transition between the high- and low-affinity phases occurs earlier in AtKUP1-mediated K^+ uptake (at 100 to 200 μM as opposed to 500 to 1000 μM in barley roots). This is not surprising considering the different systems (yeast cells versus plant roots) used in these studies. Moreover, AtKUP1 may be only one of several K^+ transporters that contribute to K^+ uptake in plant roots. The uptake kinetics in plant roots represent an integrated pattern of all of these individual transporters that contribute to the two phases of K^+ uptake. Despite the apparent similarity between AtKUP1-mediated K^+ uptake and uptake by barley roots, we must interpret the kinetic data obtained from a heterologous system with caution. Although this study and a number of previous studies have obtained different uptake kinetics with different transporter genes studied in the *trk1Δtrk2Δ* mutant strain, we cannot completely exclude the the possibility that an exogenous transporter may interact with endogenous transporters and change the uptake property of the mutant strain. As a result, the kinetics obtained for AtKUP1 expressed in yeast may not truly represent its endogenous function in Arabidopsis plants. On the other hand, an independent study (Kim et al., 1998) indicates that AtKUP1 can mediate high-affinity K^+ uptake and an additional small low-affinity K^+ uptake in transgenic plant cells, which is consistent with the kinetic analyses in this study.

It has been generally accepted that low-affinity K^+ uptake is achieved mainly by voltage-gated channels (Schroeder et al., 1994; Maathuis and Sanders, 1996). In this study, the low-affinity phase of K^+ uptake by AtKUP1 is strongly inhibited by TEA, Ba^{2+}, and Cs^+. Although this result suggests that AtKUP1 may function like a voltage-gated K^+ channel, the structure of the AtKUP1 protein does not appear to support this notion. Another possibility is that these inhibitors (including TEA) may block K^+ influx through other K^+ transporters in addition to voltage-gated K^+ channels (Hille, 1992). In any case, it may be informative to compare the behavior of AtKUP1 and TRK systems in yeast cells.

Based on the study by Bertl et al. (1995), wild-type yeast cells (with TRK1 and TRK2) carry a voltage-dependent inward K^+ current that is completely abolished in *trk1Δtrk2Δ* mutant

cells, suggesting that one or both of the TRK systems are somehow responsible for this inward channel activity. In our study, only K^+ uptake in wild-type yeast cells, but not that in mutant cells, was inhibited by TEA in the low-affinity phase, which is consistent with the notion that TRK systems may constitute a functional channel. Both AtKUP1 and TRK systems belong to the K^+ transporter family with 12 transmembrane domains. If TRK systems function as voltage-dependent K^+ channels, AtKUP1 may also carry the structural elements of a functional K^+ channel. In fact, one highly conserved region in the AtKUP1 protein (amino acids 34 to 49) is homologous to the signature motif in the K^+ channel pore (lYGD in AtKUP1 as opposed to GYGD in most K^+ channels). More work is required to determine whether AtKUP1 (and TRK systems) can indeed function as a voltage-activated K^+ channel, especially in the low-affinity phase.

Studies by Sanders and colleagues suggest that H^+/K^+ symport may constitute a major energization mechanism for high-affinity uptake in Arabidopsis roots (Maathuis and Sanders, 1994, 1995, 1996). On the other hand, studies with a wheat K^+ transporter, HKT1, indicate that Na^+/K^+ symport may take place for high-affinity K^+ uptake in higher plants (Schachtman and Schroeder, 1994; Rubio et al., 1995). Our studies show that AtKUP1, unlike HKT1, is not affected by low [NaCl]. Higher [NaCl] ($>$5 mM) dramatically inhibits both low- and high-affinity uptake by AtKUP1. Similar effective NaCl concentrations have been observed for inhibition of AtKUP1-mediated K^+ uptake and K^+ uptake by plant roots (Epstein, 1973; Wu et al., 1996). The close parallels between K^+ uptake by AtKUP1 and by plant roots suggest that AtKUP1 functions as a major K^+ transporter in plant roots, although more work is needed to test this possibility. Under conditions used in this study, it is difficult to determine whether AtKUP1 serves as a H^+/K^+ symporter, as shown in Arabidopsis roots by Maathuis and Sanders (1994). Improved approaches, such as electrophysiological techniques, may be helpful in resolving this issue.

In summary, we have identified a novel K^+ transporter that may function in both high- and low-affinity phases of K^+ uptake in plant roots. It will be particularly interesting to dissect the structural basis for dual-affinity ion transport by a single protein. We speculate that conformational changes in the transporter protein might be responsible for switching between the high- and the low-affinity mode. Changes in external [K^+] or membrane potential might trigger such a conformational switch.

METHODS

Yeast Complementation

The yeast strains CY162 (*ura3-52 trk1Δ his3Δ200 his4-15 trk2Δ1::pCK64*) and R757 (*ura3-52*) were kindly provided by R. Gaber (Northwestern University, Evanston, IL). For the complementation experiment, an *Arabidopsis thaliana* cDNA library constructed in the λYES yeast/*Escherichia coli* shuttle vector (Elledge et al., 1990) was kindly provided by R. Davis (Stanford University, Palo Alto, CA). The cDNAs were prepared from mRNA purified from several tissues of Arabidopsis plants at various developmental stages, and expression of the cDNA inserts was controlled by an inducible *GAL1* promoter (Elledge et al., 1990).

For transformation of CY162 cells, we used an electroporation procedure previously described by Becker and Guarente (1991). Briefly, a single colony was picked and cultured at 30°C overnight in 5 mL of rich medium containing YPD (Gibco BRL) and 100 mM KCl. The overnight culture was transferred to 500 mL of the same medium in a 2-liter flask and grown for another 4 to 5 hr with vigorous shaking until OD_{600} reached 1.0. Cells were collected by centrifugation at 2000*g* for 5 min at 4°C and washed in cold double-distilled water three times. Cells were then washed once with 1 M cold sorbitol and resuspended in a final volume of 0.5 mL of sorbitol before being used for electroporation. An aliquot of 40 μL of yeast suspension and 0.2 μg of library DNA was mixed in an Eppendorf tube and transferred to a 0.2-cm sterile electroporation cuvette (Bio-Rad). A Bio-Rad gene pulser was used to apply an electric pulse (at 1.5 kV, 25 μF, and 200 Ω) to the cuvette. Immediately following the pulse, 1 mL of sorbitol was added to the cuvette and mixed thoroughly by inverting several times. Cells were plated onto uracil-deficient synthetic medium containing 2% glucose, 100 mM KCl, and 1 M sorbitol.

The plates were incubated at 30°C for 3 days. The Ura^+ colonies were pooled in the uracil-deficient liquid medium containing 4% galactose and 20 mM KCl and cultured for 4 hr at 30°C to induce expression of cloned cDNAs. The cultured cells were plated onto uracil-deficient medium containing 4% galactose and 2 mM KCl. After incubating at 30°C for 5 or 6 days, positive colonies were replicated onto the same plates and used in further analyses. Approximately 2×10^6 Ura^+ transformants were screened, and 36 positive colonies were identified.

Isolation of the *kup*-like cDNA

To isolate cDNAs for novel K^+ transporters, a colony hybridization procedure similar to that for bacterial colonies (Sambrook et al., 1989) was used to identify the positive colonies containing cDNAs for known K^+ transporters. Briefly, yeast colonies were lifted onto a Hybond nylon membrane (Amersham Life Science, Buckinghamshire, UK). The membranes with yeast colonies were treated with zymolyase solution (20 mM EDTA, 1 M sorbitol, and 1 mg/mL zymolyase 100,000) for 2 hr at 37°C in a covered glass tray. The membranes were then treated with the denaturing and neutralizing solutions (Sambrook et al., 1989). After baking for 2 hr at 80°C, the membranes were prehybridized in a solution described in Sambrook et al. (1989) at 42°C for 2 hr. The mixed cDNAs (*KAT1* and *AKT1* at 1:1 ratio) were labeled by using a random priming kit from Promega (Madison, WI) and added to the prehybridization solution. Hybridization was continued for 12 hr at 42°C before washing and autoradiography. Among the initial 36 positive clones, 27 hybridized with the probe containing ^{32}P-labeled *AKT1* and *KAT1* cDNA (data not shown). Plasmid DNA was recovered from the nine hybridization ("negative") colonies and used to transform *E. coli* by electroporation (Gunn and Nickoloff, 1995). Plasmid DNA was then amplified and used for sequence analysis with a Sequenase 2.0 kit (U.S. Biochemicals). Sequence alignment and homology searches were performed using the programs of

the National Center for Biotechnology Information available on the Internet.

To confirm complementation of CY162 by the *kup*-like cDNA, the 1.8-kb cDNA insert was subcloned into pRH98-3 vector that carries the glyceraldehyde-3-phosphate dehydrogenase gene (*GPD*) promoter and the phosphoglycerate kinase (*PGK*) gene terminator (Yalovsky et al., 1997). The *kup*-like cDNA fragment with EcoRI-XhoI sites at the 5′ and 3′ end was cloned into the EcoRI and SalI sites between the *GPD* promoter and *PGK* terminator. The constructed plasmid was transformed into CY162 by using the electroporation procedure (Becker and Guarente, 1991) described above. Because the *GPD* promoter is constitutively active, the complementation assay was performed on glucose-containing medium, as shown in Figure 1.

DNA and RNA Gel Blots

Genomic DNA was isolated from Arabidopsis (Columbia and Landsberg *erecta* ecotypes) and cut with the restriction enzymes indicated in the legend to Figure 3. Digested DNA (5 μg in each lane) was loaded and separated on a 0.7% agarose gel and blotted onto a Zeta probe membrane (Bio-Rad). The membrane was prehybridized in a buffer containing 0.5 M Na_2HPO_4, pH 7.2, and 7% SDS for 1 hr before adding the ^{32}P-labeled *AtKUP1* cDNA probe prepared by using a random labeling kit from Promega. After 16 hr of hybridization at 65°C, the membrane was washed three times for 15 min in 0.5% SDS and 40 mM Na_2HPO_4 at 60°C. The membrane was exposed to the Kodak XAR-50 x-ray film at −80°C with an intensifying screen.

For RNA isolation, Arabidopsis (Columbia ecotype) plants were grown in a greenhouse under long-day conditions (a 16-hr-light/8 hr-dark cycle) to the flowering stage. During the growth period, plants were watered twice a week (once with tap water and once with one-quarter strength Hoagland's solution). Total RNA was isolated from roots, stems, leaves, and flowers by using a procedure described in detail in a previous report (Luan et al., 1994). The RNA samples (10 μg per lane) were separated on a 1.2% agarose–formaldehyde gel and blotted to a Zeta probe membrane. The membrane was hybridized as described for the DNA gel blot, except that two hybridization temperatures were used, as indicated in the legend to Figure 4. After 16 hr of hybridization at 58°C, the membrane was washed in 40 mM Na_2HPO_4 and 0.5% SDS twice at room temperature and three times at 42°C. After these washes, the membrane was exposed to the Kodak XAR-50 x-ray film at −80°C with an intensifying screen (as a low-stringency blot). The membrane was then striped, rehybridized at 65°C, washed at 60°C, and exposed (as the high-stringency blot).

Construction of AtKUP1 Strain and Transport Assays

After the full-length cDNA sequence of *AtKUP1* was obtained, a HindIII site in the middle of the cDNA (nucleotide 1038 from the 5′ end) was identified and used to construct a full-length *AtKUP1* cDNA. Briefly, the G10F3T7 expressed sequence tag (EST) cDNA was digested with HindIII so that the 5′ half of the cDNA was released from the insert. The EST clone was then religated and contained only the 3′ half of the cDNA. This cDNA clone was digested using HindIII and BamHI to release the 3′ half of the cDNA. Meanwhile, the *kup*-like cDNA in pRH98-3 was digested with HindIII and BamHI to release the 3′ half of the *kup*-like cDNA. The 3′ half from the EST clone was then ligated to the 5′ half of the *kup*-like cDNA in the pRH98-3 vector so that the full-length *AtKUP1* cDNA was under the control of the *GPD* promoter. This plasmid was transformed into CY162 to construct the AtKUP1 strain. Both CY162 and the wild-type yeast strain (R757) were transformed by pRH98-3 vector. Therefore, all three strains were able to grow in the uracil-deficient medium.

Using the vector-containing CY162 and R757 together with the AtKUP1 strain, we performed rubidium-86 tracer experiments according to previous procedures (Kochian et al., 1993), with modifications. The ^{86}RbCl salt with a specific activity of 9.03 mCi/mg was purchased from Du Pont–New England Nuclear (Boston, MA). Yeast cells were grown to mid-log phase in the uracil-deficient synthetic medium containing 50 mM [K^+]. Cells were harvested and incubated in K^+-free medium for 4 hr before the uptake assay was conducted. For the time-course analyses, 5×10^8 cells were added to 50 mL of the uptake solution containing 0.5 μCi of rubidium-86 per mL and 0.02, 0.2, and 20 mM KCl in culture flasks and gently shaken on a platform. A fraction of the cells was harvested at the indicated time (Figure 5). For kinetic analyses, 10^7 cells were used in each uptake sample in a 1-mL solution containing 0.5 μCi rubidium-86 and various concentrations of KCl (Figure 6). For inhibitor analyses, 10 mM of tetraethylammonium (TEA), $BaCl_2$, or CsCl was included in the uptake assays (Figure 7). For the NaCl sensitivity study, 10 concentrations of NaCl (0, 0.1, 0.5, 1, 5, 10, 20, 50, 100, and 150 mM) were tested at both 20 μM and 20 mM external [K^+] (Figure 8). The radioactivity retained by the cells was measured by a liquid scintillation counter (model LS6000IC; Beckman Instruments, Allendale, NJ). All K^+ uptake experiments were repeated three times unless otherwise stated. Each treatment was performed in three duplicates. Values of K^+ uptake were obtained based on rubidium-86 uptake and the external [K^+] (Figures 5 to 8). Data are shown as mean ±SE.

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