AtKUP1: An Arabidopsis Gene Encoding High-Affinity Potassium Transport Activity

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INTRODUCTION

Potassium is the most abundant cation found in plants. As much as 6% of the dry weight of a plant may be due to potassium accumulation (Raven et al., 1976). The importance of potassium in nutrition, growth, enzyme homeostasis, and osmoregulation in plants is well documented (Epstein, 1976; Clarkson and Hanson, 1980; Glass, 1988; Schroeder et al., 1994). Plant roots are capable of accumulating potassium from micromolar concentrations from the external environment. Uptake of this cation into plant roots has been long observed to comprise at least two distinct kinetic systems (Epstein et al., 1963)—high-affinity systems, with apparent affinities of ~4 to 35 μM (Epstein et al., 1963; Newman et al., 1987) showing saturation at ~300 μM external K⁺, and constitutive low-affinity components, which have an average Kₘ of ~3 to 19 mM.

Consistent with earlier studies, two distinct mechanisms of potassium uptake have been characterized thus far at the molecular level for higher plants. Patch-clamp studies with guard cells (Schroeder and Fang, 1991) and root cells (Gassmann and Schroeder, 1994; Maathuis and Sanders, 1995) have led to the suggestion that inward-rectifying K⁺ channels provide a low-affinity pathway for K⁺ uptake in plant cells. The apparent affinity of these channels for potassium ranged from 3 to 19 mM and could also support uptake of potassium at external micromolar concentrations (Schroeder and Fang, 1991; Maathuis and Sanders, 1995). In addition, these K⁺ channels have been suggested to "sense" micromolar extracellular K⁺ concentrations and could therefore contribute to high-affinity K⁺ uptake and/or affect other transport processes (Schroeder and Fang, 1991; Gassmann et al., 1993). The first plant K⁺-transporting cDNAs to be isolated by yeast complementation represent a family of membrane proteins with high sequence homology to outward-rectifying potassium channels from animals (Anderson et al., 1992; Sentenac et al., 1992) and were shown to encode inward-rectifying potassium channels (K⁺-in) that are activated by hyperpolarization of the plasma membrane (Schachtman et al., 1992; Bertl et al., 1994; Ketchum and Slayman, 1996).

More recently, a cDNA called HKT1 (for high-affinity K⁺ transporter) was isolated from wheat roots and shown to encode a high-affinity potassium uptake transporter (Schachtman and Schroeder, 1994). The deduced amino acid sequence of HKT1 shares weak homology with TRK1 and TRK2, which are high-affinity plasma membrane potassium transporters from yeast. The transport mechanism was later delineated and shown to act as a sodium-coupled potassium uptake transporter with a micromolar affinity for potassium (apparent Kᵢ of ~3 μM K⁺) (Rubio et al., 1995; Gassmann et al., 1996). The sodium-coupled uptake of potassium is in agreement with earlier findings in charophytic algae in which sodium...
coupling of plasma membrane high-affinity $K^+$ uptake was found (Smith and Walker, 1989; Walker and Sanders, 1991). The expression of HKT1 mRNA in roots has been shown to be induced by potassium starvation, which is consistent with its proposed role as a high-affinity potassium transporter (W. Gassmann, F. Rubio, and J. I. Schroeder, unpublished data).

Although HKT1 is believed to contribute to high-affinity potassium uptake in plants, the finding that HKT1 functions as a Na$^+-K^+$ symporter (Rubio et al., 1995) suggests the possibility of additional potassium transporters in plants. Radiotracer uptake studies using whole plants have shown the presence of a high-affinity Rb$^+$ uptake system that operates independently of Na$^+$ (Maathuis et al., 1996; Walker et al., 1996; G. Mondragon, W. Gassmann, and J. I. Schroeder, unpublished data). The mechanism of high-affinity potassium uptake in Arabidopsis roots was examined and indicates proton-coupled $K^+$ uptake as one of the possible mechanisms for high-affinity $K^+$ uptake (Maathuis and Sanders, 1994). Because plants encounter a wide range of soil conditions, it is likely that multiple high- and low-affinity uptake mechanisms exist for a nutrient as vital as potassium.

We hypothesized that several redundant components exist in plants that contribute to the high- and low-affinity potassium uptake systems. Cloning of the Arabidopsis HKT1 gene (AtHKT1) and low-stringency DNA gel blot analysis suggested that AtHKT1 may be a single-copy gene in Arabidopsis (N. Uozumi, E. J. Kim, F. Rubio, S. Muto, and J. I. Schroeder, unpublished data). Therefore, to test the hypothesis that additional parallel high-affinity pathways may exist in plants, we initiated a screen for cDNA sequences showing homology to several types of potassium transporter families from other organisms in the expressed sequence tag (EST) database of GenBank and here report on a new family of Arabidopsis potassium transporters that contribute to high-affinity potassium uptake as one of the possible mechanisms for high-affinity $K^+$ uptake (Maathuis and Sanders, 1994). Because plants encounter a wide range of soil conditions, it is likely that multiple high- and low-affinity uptake mechanisms exist for a nutrient as vital as potassium.

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RESULTS

Identification of Homologs of HAK1 and KUP in Arabidopsis

The EST database of GenBank was screened for homologs of known $K^+$ transporters from other organisms, including the high-affinity Kdp transporter from E. coli (Hesse et al., 1984), the TRK1 and TRK2 genes from Saccharomyces cerevisiae (Gaber et al., 1988; Ko et al., 1990), the TOK1 gene from S. cerevisiae (Ketchum et al., 1995), the kup gene from E. coli (Schleyer and Bakker, 1993), and the HAK1 gene from Schwanniomyces occidentalis (Banuelos et al., 1995). Several ESTs of Arabidopsis related to kup and HAK1 were identified. One of these, named AtKUP1, was selected for further analysis. Complete sequencing of AtKUP1 revealed extensive homology to both the HAK1 and KUP transporters, as shown in Figure 1. AtKUP1 encodes a hydrophobic polypeptide of 712 amino acids with a predicted molecular mass of 79.1 kD, with 29 and 31% identity and 60 and 67% similarity at the amino acid level to kup and HAK1, respectively. When computer-assisted hydropathy plots were constructed from the deduced amino acid sequence, AtKUP1 was predicted to contain 12 transmembrane spans, as shown in Figure 2, which is consistent with the numbers predicted for HAK1 and KUP (Schleyer and Bakker, 1993; Banuelos et al., 1995).

Rescue of Potassium Transport-Deficient Mutants

The Arabidopsis cDNAs were first tested for their ability to rescue potassium transport deficiencies in a variety of heterologous systems. First, the cDNAs were tested for complementation of the CY162 strain of S. cerevisiae under potassium-limiting conditions. This strain has deletions in both the TRK1 and TRK2 genes, which are the two high-affinity potassium transporters identified in this species, and consequently is not able to grow in medium containing low levels of potassium (Anderson et al., 1992). Transformants of CY162 harboring the putative potassium transporter cDNA AtKUP1 in the pYE52 and pDR195 expression vectors were not able to complement the potassium-sensitive phenotype with 6.7 and 1 mM extracellular $K^+$, even after multiple attempts with more than four different constructs (data not shown).

Because AtKUP1 is homologous to the kup gene of E. coli, the AtKUP1 gene was subsequently tested for its ability to complement potassium transport deficiency in the TK2463 mutant of E. coli (Epstein and Kim, 1971). TK2463 cells containing a bacterial plasmid expressing AtKUP1 were able to grow in medium containing $\sim$2 mM potassium, as shown in Figure 3A. This is a demonstration of functional complementation of an E. coli mutant with a plant transporter. In E. coli, the kup transporter has been shown to mediate Cs$^+$ transport, although at a significantly reduced rate relative to Rb$^+$ uptake (Bossemeyer et al., 1989). Initial attempts to perform uptake experiments using E. coli cells were not successful (data not shown). Instead, we tested inhibition of AtKUP-mediated
growth of TK2463 cells on low potassium in the presence of various concentrations of Cs\(^+\). Figure 3B shows that E. coli cells expressing AtKUP1 and AtKUP2 cDNAs are capable of growing to near saturation on medium containing low potassium (\(\lesssim 2\) mM) within 9 hr. The presence of 2 mM Cs\(^+\) in this medium caused a mild inhibition of growth, whereas 10 mM Cs\(^+\) almost completely inhibited growth, indicating that Cs\(^+\) can serve as a competitor of AtKUP-mediated K\(^+\) uptake.

### Studies Using Xenopus Oocytes

Oocytes injected with AtKUP1, AtKUP2, and AtKUP3 complementary RNAs either individually or in combination were tested for novel currents. The oocytes were voltage clamped 1 to 4 days after injection and tested under a variety of pH (5.5 and 7.0), K\(^+\) (0, 0.1, 0.3, 1.0, 10, and 117 mM), and voltage (−160 to +50 mV) conditions using at least 12 different RNA preparations from six different constructs. However, in none of the cases was there a significant difference in uninjected or H\(_2\)O-injected controls. This result is consistent with findings using the fungal HAK1 gene, which also failed to show currents in the oocyte heterologous system (F. Rubio, J.I. Schroeder, and A. Rodriguez-Navarro, unpublished data).

**Enhanced Rb\(^+\) Uptake in Transgenic Arabidopsis Suspension Cells Overexpressing AtKUP1**

All plant K\(^+\) transporter cDNAs isolated to date have been functionally characterized by expression in heterologous nonplant systems. Their functions in plants remain to be determined. Therefore, we examined the possibility of adapting a plant cell expression system for studying potassium transporter cDNAs. Suspension cultures of Arabidopsis were chosen to provide a native cell line for expression of the transgene. AtKUP1 was placed under the control of the constitutive 35S promoter of cauliflower mosaic virus within the pMON530 vector (Monsanto, St. Louis, MO) and introduced by particle bombardment into suspension cultures of Arabidopsis isolated as previously described (Axelos et al., 1992). Two days after bombardment, transformed cells were selected on media containing kanamycin. Transgenic suspension cells were then tested for Rb\(^+\) uptake after growth in medium containing 1 mM KCl, which prevents overloading of suspension cells with K\(^+\) (Bush and Jacobson, 1986) but still is sufficient to suppress the expression of inducible high-affinity uptake components (Fernando et al., 1992).

As shown in Figures 4A and 4B, uptake experiments using \(^{86}\)Rb\(^+\) clearly show enhanced uptake in cells overexpressing AtKUP1 DNA.
AtKUP1 in contrast to cells transformed with empty vector as controls. Inclusion of the translational inhibitor cycloheximide in the washing steps and during uptake was necessary to suppress induction of endogenous high-affinity uptake, which otherwise appeared within $\sim$1 hr of removing $K^+$ from the medium. The AtKUP1-induced high-affinity Rb$^+$ uptake activity in the presence of the cycloheximide, as shown in Figure 4A, illustrates that the activity assayed here was constitutively expressed, as would be expected for the 35S promoter–driven AtKUP1. Transformed cell lines were tested for expression of AtKUP1 by RNA gel blot analysis. As shown in Figure 4C, cells transformed with the 35S–AtKUP1 construct showed strong expression of AtKUP1 when grown under high potassium, in contrast to control cells where AtKUP1 message was undetectable. As shown in Figure 4A, the rate of Rb$^+$ uptake in the high-affinity range of cells overexpressing the AtKUP1 gene was found to be three to four times larger than in control cells and had an apparent $K_m$ of $\sim$22 $\mu$M for Rb$^+$, indicating that AtKUP1 is capable of mediating high-affinity potassium uptake in plant cells. Increased Rb$^+$ uptake was also detected at all Rb$^+$ concentrations tested up to 3 mM, as shown in Figure 4B. Subtraction of native uptake activities in controls from those in AtKUP1-expressing lines indicated a small increase in a linear Rb$^+$ uptake component. This suggests the possibility that AtKUP1 may contribute to both high- and low-affinity uptake, consistent with earlier findings suggesting that transporters can contribute to more than one uptake component (e.g., Gassmann et al., 1993, 1996).

**AtKUP1 Is a Member of a Multigene Family**

To test whether homologs of AtKUP1 are present in Arabidopsis, we searched the database of GenBank again, this time using the full coding sequence of AtKUP1. As shown in Table 1, the screen revealed the presence of several ESTs and genomic DNA clones with significant homology to AtKUP1. After further sequencing of some of these clones, the presence of at least three additional members in this family were identified, which we designated AtKUP2, AtKUP3, and AtKUP4, as shown in Table 1. The sequences of the various members of the AtKUP family are highly homologous: for example, the sequence of AtKUP2 is 57% identical and 83% similar at the amino acid level to AtKUP1. AtKUP2 shares 30 and 31% identity and 61 and 67% similarity to fungal HAK1 and bacterial kup, respectively, which is similar to the degree of homology of AtKUP1 to these transporters.
Differential Expression of AtKUP1 cDNAs

To examine the basis for the apparent redundancy of genes within the genome of Arabidopsis, we performed RNA gel blot analyses using RNA from various tissues and probed against the four identified AtKUP members. High-stringency RNA gel blots performed using entire cDNA inserts as probes showed transcripts of 2.9 to 3.1 kb in length, which corresponds to the expected lengths of AtKUPs. As shown in Figure 5A, AtKUP mRNAs seem to be expressed in most tissue types tested. However, there was a reduced level of AtKUP1 message in roots when compared with AtKUP2, AtKUP3, and AtKUP4 levels under the imposed conditions. The AtKUP1 gene was independently characterized (Fu and Luan, 1998). Under other growth conditions, a different RNA expression pattern was observed for AtKUP1 (Fu and Luan, 1998), as communicated by the editors after both submissions. To test whether the expression of any of the AtKUPs was modulated by potassium levels, total RNA was collected from roots of plants grown in medium containing high (2 mM) and low (40 μM) levels of external potassium. The expression of at least two members of the AtKUP family, AtKUP2 and AtKUP3, were affected by potassium levels in the growth medium, as shown in Figure 5B. Plants grown in high potassium showed a slightly increased level of expression of AtKUP2. The opposite, however, was observed with AtKUP3: the level of expression was much higher in plants grown in low potassium, indicating a strong induction of AtKUP3 by potassium starvation.

DISCUSSION

AtKUP1 Is a Potassium Transporter from Arabidopsis

We report here on the characterization of a member of a novel gene family of putative potassium transporters in plants. The AtKUP gene family shares considerable sequence homology with the genes encoding Kup (Schleyer and Bakker, 1993) and HAK1 (Banellos et al., 1995), which are potassium transporters from E. coli and S. occidentalis, respectively. After submission of our manuscript, GenBank entries and a report appeared describing sequences of the AtKUP gene family by Quintero and Blatt (1997). In their study, AtKT2 (GenBank accession number AF012657; essentially identical

Figure 3. AtKUP1 and AtKUP2 Complement Potassium Transport Deficiency in E. coli TK2463 Cells.

(A) Complementation of TK2463 cells by AtKUP1. The E. coli TK2463 mutant is defective in three K⁺ uptake transporters (Trk, Kup, and Kdp) (Epstein and Kim, 1971) and was transformed with plasmids containing the AtKUP1 gene or with an empty vector. Transformants were streaked either on medium containing 134 mM K⁺ or on medium containing ~2 mM K⁺.

(B) Growth of control and AtKUP1 (black bars)-expressing and AtKUP2 (hatched bars)-expressing TK2463 cells in liquid culture containing ~2 mM K⁺ and various concentrations of Cs⁺. Saturated overnight cultures grown in KML medium were washed once and added to low-potassium medium to a starting density of OD₆₀₀ = 0.05. Optical density was measured 9 hr later. Open bars represent vector-transformed controls.
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AtKUP2 was found to complement growth of potassium transport–deficient CY162 yeast cells under conditions of limiting potassium. AtKT1 (GenBank accession number AF012656; essentially identical to AtKUP1) was not able to complement CY162 cells (Quintero and Blatt, 1997), as was found with our AtKUP1 constructs.

In E. coli, Kup is a component of the constitutive potassium uptake system, with an apparent $K_m$ of $\sim$0.25 to 0.4 mM (Dosch et al., 1991; Schleyer and Bakker, 1993). However, because the $V_{max}$ of Kup is an order of magnitude lower than that of the Trk systems within E. coli (Rhoads et al., 1976; Bossemeyer et al., 1989; Dosch et al., 1991), kup is thought to play a relatively minor role in contributing to K$^+$ uptake, and as a consequence, little is known of its transport mechanism. However, the ability of kup to mediate cesium transport in addition to K$^+$ uptake and the finding that

Figure 4. Enhanced $^{86}$Rb$^+$ Uptake in Transgenic Arabidopsis Suspension Cells Expressing AtKUP1.

(A) Uptake rates of $^{86}$Rb$^+$ (in nanomoles per minute per gram fresh weight [FW] of cells) plotted as a function of micromolar Rb$^+$ concentrations in cells transformed with the 35S–AtKUP1 construct (closed triangles) or vector (open triangles). Error bars denote standard error.

(B) $^{86}$Rb$^+$ uptake rates at higher concentrations of Rb$^+$ expressed as a function of external Rb$^+$ concentrations in cells transformed with the 35S–AtKUP1 construct (closed triangles) or vector (open triangles).

(C) RNA gel blot analysis of cells transformed with vector or 35S–AtKUP1 grown on Murashige and Skoog medium. Approximately 40 $\mu$g of total RNA was probed with radiolabeled AtKUP1 and the 18S rRNA, as indicated.
only mutations within the kup gene affected specific, saturable cesium transport in Escherichia coli (Bossemeyer et al., 1989) provided strong evidence that kup encodes a transport system that is independent of the other genetically identified components in this organism.

A homolog of the kup gene, HAK1, was identified from the yeast *S. occidentalis* by complementation of the *trk1 trk2* mutations in *S. cerevisiae* (Banuelos et al., 1995). Unlike kup, the HAK1 cDNA was found to encode a high-affinity potassium uptake mechanism, and HAK1-expressing cells were found to be capable of depleting the medium K⁺ to <0.03 μM (Banuelos et al., 1995). Furthermore, in contrast to the relatively minor role that kup is believed to play in K⁺ uptake in *E. coli*, HAK1 is thought to comprise the major high-affinity K⁺ uptake pathway in *S. occidentalis* (Banuelos et al., 1995).

We have provided evidence that strongly suggests that AtKUP1 functions as a potassium transporter or is a component of it. However, it is difficult at this time to determine whether AtKUP1 alone is sufficient for expression of the high-affinity potassium uptake system or whether additional subunits are necessary for AtKUP1 function. Although the inability to obtain functional expression in yeast or in oocytes of *Xenopus* could be interpreted as a need for additional subunits for AtKUP1 function, the evidence from its nonplant homologs suggests that AtKUP1 does not require a larger complex to function. Complementation of the *E. coli* strain TK2463 disrupted in the three major potassium uptake pathways (Figure 3) as well as functional complementation of the yeast mutant with the fungal HAK1 (Banuelos et al., 1995) suggests that AtKUP1 by itself can function as a K⁺ uptake transporter.

**E. coli Expression System Provides a Powerful Tool for the Study of Plant Ion Transport**

The isolation and characterization of plant transporters have been greatly aided by the presence of heterologous expression systems, allowing quantitative functional studies of

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**Table 1. AtKUP GenBank Accession Numbers**

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*GenBank accession numbers are in parentheses.

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**Figure 5. Differential Expression of AtKUP mRNAs.**

(A) Approximately 30 μg of total RNA isolated from flowers, leaves, stems, and roots was loaded on each lane and hybridized with 32P-radiolabeled AtKUP1, AtKUP2, AtKUP3, AtKUP4, and the 18S rRNA, as indicated.

(B) Twenty micrograms of total RNA was isolated from roots grown in medium containing high (2 mM) or low (40 μM) K⁺ and probed with 32P-radiolabeled AtKUP2, AtKUP3, and the 18S rRNA.
these genes in the absence of other plant proteins (Frommer and Ninemann, 1994). A number of potassium transporter cDNAs have been isolated by using complementation of yeast transport mutants (Anderson et al., 1992; Sentenac et al., 1992; Schachtman and Schroeder, 1994). Furthermore, in a number of cases, functional expression in oocytes has led to the elucidation of the underlying transport mechanisms. We have now demonstrated the feasibility of complementation of an E. coli mutant by a plant transporter, which can provide the same advantages as in yeast, with some additional features. First, as a prokaryotic system, E. coli possesses only one membrane system; therefore, it is possible to functionally express organellar transporters or those that may not otherwise be targeted to the plasma membrane in yeast cells. Second, the genetic system that exists for E. coli provides distinct advantages over the use of S. cerevisiae, because many of the analyses can be performed more extensively in a much shorter period of time. For example, a single study of the high-affinity potassium uptake system encoded by the Kdp locus (Buurman et al., 1995) identified mutations in 13 positions within the KdpA gene, providing evidence for two sequentially occupied sites for cation binding. The abundance of transport mutants in E. coli should prove to be advantageous for investigations of transporters of a wide variety of substrates, if other transporters are also expressed functionally in the E. coli system, as has been demonstrated for AtKUP1 and AtKUP2 here. Initial attempts to perform Rb\textsuperscript{+} uptake experiments using E. coli cells were not successful. Therefore, we used growth assays to establish that Cs\textsuperscript{+} can act as a competitor of AtKUP1 and AtKUP2, inhibiting their ability to support growth of the TK2463 cells in low potassium.

**AtKUP1-Mediated High-Affinity Rb\textsuperscript{+} Uptake in Plant Cells**

Despite the obvious advantages provided by these heterologous expression systems, studies with native plant systems are needed to predict physiological components encoded by each gene. In addition, it is clear that many transporters are not expressed functionally in certain heterologous systems. For example, a plasma membrane H\textsuperscript{+}-ATPase cDNA from Arabidopsis (Harper et al., 1989) was not able to complement a deletion in the yeast H\textsuperscript{+}-ATPase, because an insufficient amount of the pump was targeted to the plasma membrane (Villalba et al., 1992). The use of Arabidopsis suspension cells in our study provides a rapid means to address their functions in plant cells. Expression of AtKUP1 in Arabidopsis suspension culture cells produced a constitutive high-affinity Rb\textsuperscript{+} uptake component that is reminiscent of the classic high-affinity Rb\textsuperscript{+} uptake measured in various plant tissues (Epstein et al., 1963; Glass, 1976; Kochian and Lucas, 1982). A recent study of an Arabidopsis phosphate transporter (Mitsukawa et al., 1997) used transgenic tobacco cultured cells to analyze the function of the phosphate transporter, which could not successfully complement yeast mutants in phosphate transport (Mitsukawa et al., 1997; Smith et al., 1997). Both studies show that the use of plant cells for the study of transport function provides a powerful tool for the functional analysis of novel transporter genes.

An earlier study of potassium uptake in carrot suspension cells (Bush and Jacobson, 1986) showed that cells grown on Murashige and Skoog medium (Murashige and Skoog, 1962), which contains nearly 20 mM K\textsuperscript{+}, release potassium into the medium when uptake is analyzed under low-potassium concentrations. However, when potassium concentrations are reduced to micromolar levels in the medium, the induction of endogenous high-affinity Rb\textsuperscript{+} uptake results in large uptake rates in control cells. We have circumvented both of these problems by exposing cells to medium containing intermediate (1 mM) levels of K\textsuperscript{+} 1 to 2 days before uptake is to be performed and by washing and performing uptake in the presence of the translational inhibitor cycloheximide. Under these conditions, the uptake rate of the AtKUP1-overexpressing cells was three- to fourfold higher than that found with control cells in the micromolar Rb\textsuperscript{+} concentrations in the uptake buffer. When tested at higher Rb\textsuperscript{+} concentrations (Figure 4B), a small, low-affinity uptake component was also apparent, but it is difficult to establish its significance, because the AtKUP1-mediated uptake activity accounts for only a fraction of total uptake in the millimolar range. In an independent study, AtKUP1 with a short C-terminal truncation was isolated by complementation of the potassium transport deficiency of CY162 and was found to support low-affinity uptake in yeast (Fu and Luan, 1998).

We cannot rule out the possibility that high-affinity Rb\textsuperscript{+} uptake observed in Figure 4A was the result of overexpression of a low-affinity transporter, because low-level expression of a high-affinity transporter results in a low-affinity uptake component. However, it is unlikely that overexpression of a low-affinity transporter would result in its Rb\textsuperscript{+} uptake accounting for such a large portion of total high-affinity uptake without great enhancement of low-affinity uptake. Together with the complementation of a K\textsuperscript{+} uptake-deficient E. coli strain, we deduce that AtKUP1 encodes a high-affinity potassium uptake transporter that may also provide a small low-affinity Rb\textsuperscript{+} uptake component.

**Multiple Potassium Uptake Transporters Suggest a Complex Molecular Basis of Plant K\textsuperscript{+} Nutrition**

A cDNA from wheat called HKT1, which is capable of mediating high-affinity K\textsuperscript{+} uptake, has been previously characterized (Schachtman and Schroeder, 1994). A homolog of HKT1 has recently been isolated from Arabidopsis (N. Uozumi, E.J. Kim, F. Rubio, S. Muto, and J.I. Schroeder, manuscript in preparation) and rice (Golldack et al., 1997). AtKUP1 and the structurally unrelated HKT1 transporter are both capable of mediating high-affinity potassium uptake. Their presence
may explain why no single high-affinity K⁺ transport mechanism could be detected in roots in numerous detailed studies by using several complementary techniques (Newman et al., 1987; Kochian et al., 1989). For example, external acidification does not stimulate high-affinity Rb⁺ influx in intact corn or wheat roots (Kochian et al., 1989; W. Gassmann, F. Rubio, and J.I. Schroeder, unpublished data). K⁺ transporter complexity may help to reconcile some of the apparent disagreements in the findings of numerous investigations concerning the mechanism of high-affinity potassium uptake in intact plants. In retrospect, however, it is not surprising to witness the growing complexity of potassium uptake mechanisms in plants when considering that even the simple prokaryote E. coli possesses at least three major pathways consisting of 11 operons for potassium uptake (Epstein et al., 1993).

Because AtKUP1 is not expressed in Xenopus oocytes, the K⁺ transport mechanism of AtKUP1 remains to be elucidated. It is likely that plants may possess multiple and redundant mechanisms for both high- and low-affinity potassium uptake, as has been previously discussed (Epstein and Rains, 1965; Rubio et al., 1996). The apparent complexity of potassium uptake in plants may be important to ensure uptake under many different soil environments.

The expression of the AtKUP mRNAs showed that most of these were expressed in all of the tissue types tested. The slightly elevated expression of AtKUP2 mRNA in the presence of high potassium in the growth medium is somewhat unexpected. AtKUP2 therefore may encode K⁺ transporters of different affinity and/or localization, similar to CHL1, the nitrate-inducible low-affinity nitrate transport systems in Arabidopsis (Crawford, 1995). In barley, a basal level of high-affinity uptake has been reported, and induction of potassium uptake is on the order of threefold (Fernando et al., 1992). A recent study with Arabidopsis has also demonstrated the presence of both inducible and constitutive high-affinity K⁺ uptake components (Ding and Zhu, 1997). The strongly expressed level of AtKUP3 mRNA in roots of plants grown on low-potassium growth medium suggests that AtKUP3 encodes an inducible high-affinity component in Arabidopsis. HKT1 transcript levels have also been shown to be induced in potassium-starved wheat, rice, and barley (Goldtack et al., 1997; W. Gassmann, F. Rubio, and J.I. Schroeder, unpublished data). Therefore, more than one high-affinity transport component may be induced under starvation conditions.

The apparent expression of the AtKUP mRNAs in all tissues tested is interesting. It is presently not clear why non-root cells need multiple potassium transporters. AtKUP-mediated potassium uptake may be favorable under certain conditions over K⁺ in channel-mediated uptake. Moreover, it is possible that some members of the AtKUP family encode potassium transporters of different cellular or organellar localization and/or affinity. Additional experiments need to be performed to determine the contribution of individual AtKUP members in potassium nutrition in plants. The abundance of multiple high-affinity K⁺ uptake transporters in plants points to the need to disrupt genes of individual components to dissect their relative roles and interactions during K⁺ nutrition and uptake into different plant cell and tissue types.

METHODS

Strains

Escherichia coli TK2463 (F⁻ thi lacZam82 rhaΔ (trkA) trkD1 Δ(kdp-FAB) ß endA) was generously provided by W. Epstein (University of Chicago) (Epstein et al., 1993). Saccharomyces cerevisiae CY162 (MATa ura3-52 trk1Δ his3Δ1200 his4-15 trk2Δ1Δ: pCK64) was a kind gift from R. Gaber (Northwestern University, Chicago, IL) (Anderson et al., 1992).

Gene Identification

Arabidopsis thaliana cDNA clones homologous to either HAK1 or kup were identified from the expressed sequence tag (EST) and nonredundant (NR) databases by using BLAST (Altschul et al., 1990). Sequences homologous to the AtKUP1 coding region were also selected using the same method. The GenBank accession numbers (within parentheses) for the selected Arabidopsis ESTs are as follows: N96203 (G10F3), T20469 (B1F3), W43757 (H4G8), T04361 (39B1), W43598 (H2E6), AA042476 (H10G7), W43749 (H5F9), and W43758 (H4G9). The GenBank accession numbers (within parentheses) for bacterial artificial chromosomes are as follows: B10149 (F20B15), B10459 (F19B9), and AC002336 (T02P04) (see Table 1).

Complementation Tests with S. cerevisiae

cDNA clones were tested for their ability to complement the potassium transport deficiency of CY162. The AtKUP1 cDNA was inserted into the yeast expression vector pYES2 (Invitrogen, La Jolla, CA) or pSE420 (Invitrogen). Cells were transformed by standard methods and selected on synthetic minimal medium (10 g of tryptone, 2 g of yeast extract, and 10 g of KCl per liter) (Epstein and Kim, 1971). Transformants were then tested for their ability to grow in medium containing low (~2 mM) potassium (10 g of tryptone, 2 g of yeast
extract, and 100 mmol of mannitol per liter), pH 7.0, for 2 days. All E. coli cultures were grown at 37°C.

Expression in Xenopus laevis Oocytes

Complementary RNA was generated from the AtKUP1, AtKUP2, and AtKUP3 cDNAs either in pBluescript SK− or between the 5’ and 3’ untranslated regions of the Xenopus β-globin gene of pGEM-HE (Liman et al., 1992). Capped complementary RNA was transcribed from linearized plasmids in vitro using the mMessage mMachine (Ambion, Austin, TX), according to the manufacturer’s instructions. Complementary RNA (10 to 50 ng) was injected into Xenopus oocytes prepared and handled as described elsewhere (Cao et al., 1992). Oocytes were kept in modified Barth’s solution (Cao et al., 1992) for 1 to 4 days at 18°C before recording, and ionic currents were measured using two-electrode voltage clamping. Vortage-pulse protocols, data acquisition, and data analysis were performed with an 80386-based microcomputer using the Cornerstone model TEV-200 amplifier (Dagan Corp., Minneapols, MN) and the PCLAMP or AXOTAPE software packages (Axon Instruments, Inc., Foster City, CA), as previously described (Cao et al., 1992). All experiments were performed without leak subtraction. Membrane currents were measured in a solution that contained 1.8 mM CaCl₂, 6 mM MgCl₂, and 10 mM Mes-BTP, pH 5.5 or 7.0, with concentrations of K⁺ ranging from 0 to 117 mM (K⁺ was added as a glutamate salt).

Analysis of RNA Gel Blots

To detect AtKUP mRNAs in various tissue types, Arabidopsis ecotype Columbia plants were grown in soil for ~6 or 7 weeks in a controlled environment growth chamber (Conviron, Ashville, NC) at 20°C in constant light. For K⁺ induction studies, RNA was isolated from roots of plants grown vertically on Petri dishes for 2 or 3 weeks in medium (0.8% [w/v] agarose, 1 mM MgSO₄, 7.5 mM Ca(NO₃)₂, 3% [w/v] sucrose, 5 mM H₃PO₄, 3 mM Mes-Ca(OH)₂, pH 5.7, 7 µM H₂BO₃, 1.4 µM MnSO₄, 3.8 µM NaClO₄, 0.2 µM Na₂MO₄, and 0.01 µM CoCl₂) containing low (40 µM) or high (2 mM) KCl. Total cellular RNA was prepared using the Trizol reagent (Life Technologies, Rockville, MD). Thirty-five- for tissue-specific expression studies) and twenty-microgram (for induction studies) samples of RNA were fractionated on a denaturing 1.2% formaldehyde agarose gel and transferred onto a Hybond-N nylon membrane (Amersham). Prehybridization and hybridization were performed as previously described (Kwak et al., 1997). Membranes were first washed in 0.5 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS at room temperature for 15 min and then in 0.2 × SSC and 0.1% SDS at 45°C for 15 min. Loading was normalized by hybridizing with an 18S rRNA gene of Brassica napus (Park et al., 1993).

Overexpression of the AtKUP1 Gene in Arabidopisis Suspension Cultures

The KpnI-XhoI fragment of the AtKUP1 gene was placed downstream of the cauliflower mosaic virus 35S promoter (Odell et al., 1985) within the pMON530 expression vector (Monsanto, St. Louis, MO). The resulting plasmid was introduced into suspension cells of Arabidopsis isolated as previously described (Axelos et al., 1992). Bombardments were performed using the Biolistic PDC-1000/He system (Bio-Rad), with a modification to previously described methods (Zhang et al., 1996). The manufacturer’s instructions were followed for coating the mixture of 1.0-µm gold carriers (Bio-Rad) with plasmid DNA that was prepared using Qiagen-tip 100 purification columns (Qiagen, Chatsworth, CA). An ~0.5-g sample of cell suspensions that had been subcultured 3 days before was plated in the center of Petri dishes containing Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with 85 vitamins, 3% (w/v) sucrose, 300 mM mannitol, 5 mM Mes-KOH, pH 5.7, and 0.25% (w/v) Phytagel (Sigma) for 2 hr before bombardment. The plates were placed 8 cm beneath the stopping plate of the gun. A helium pressure of 1100 psi was used to accelerate the particles. After bombardment, the cultures were maintained on the same plates at 20 to 24°C under constant light for 48 hr and transferred either to Petri dishes containing the same medium as described above but without mannitol and with 30 µg/ml (final concentration) of kanamycin or to 6 ml of liquid medium. Three to four weeks later, kanamycin-resistant cells were identified and transferred to liquid medium.

Measurement of ⁸⁶Rb⁺ Uptake in Transgenic Suspension Culture Cells

Transgenic suspension cultures were grown in liquid Murashige and Skoog medium and subcultured every 5 days. Approximately 24 hr before uptake studies were to be performed, suspension cells were washed and resuspended in modified growth medium (1 mM MgSO₄, 7.5 mM Ca(NO₃)₂, 3% [w/v] sucrose, 5 mM H₃PO₄, 3 mM Mes-Ca(OH)₂, pH 5.7, 7 µM H₂BO₃, 1.4 µM MnSO₄, 3.8 µM NaClO₄, 0.2 µM Na₂MO₄, and 0.01 µM CoCl₂) containing 1 mM KCl. Immediately before initiation of uptake, cells were washed three times in uptake solution (2.5 mM Mes-1,3-bis(tris(hydroxymethyl)methylamino) propane, pH 6.0, 0.3 M mannitol, 1.0 mM MgSO₄, 0.1 mM CaCl₂, and 100 µM cycloheximide). Uptake was then initiated by the addition of ⁸⁶Rb⁺ diluted to a specific activity of 0.005 to 0.05 Ci per nmol with cold RbCl. Uptake was performed in 60 × 15 mm Petri dish plates on an orbital shaker platform (~100 rpm). At specified time points, a 0.9-ml fraction was removed using a wide-bore pipette and collected on filter disks (HA type, 0.8-µm pore size; Millipore). Cells were then washed with 10 ml of wash buffer (100 mM CaCl₂ and 10 mM cold RbCl) for 10 sec. Individual samples were weighed, and radioactivity in the cells was measured by liquid scintillation spectrometry (Beckman Instruments, Fullerton, CA).

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