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Proteins for Transport of Water and Mineral Nutrients across the Membranes of Plant Cells

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INTRODUCTION

The uptake and transport of water and mineral ions are among the oldest subjects in plant physiology, and numerous studies have described these processes at the whole-plant level and at the organ level (e.g., with excised roots). Subsequent work was based on isolated membrane vesicles and electrophysiology to characterize transport processes at the level of the membrane. The recent cloning of the genes for a large number of transport proteins and the availability of knockout mutants make it possible to dissect transport processes in greater detail and to begin to understand the interactions between ion uptake processes that had often been observed at the organ level. This article summarizes recent progress in characterizing the genes and the proteins involved in transporting water and major mineral nutrients.

The plasma membrane of the plant cell is a selectively permeable barrier that ensures the entry of essential ions and metabolites into the cell. Together with the vacuolar membrane (tonoplast), it permits the cytoplasm to maintain intracellular homeostasis. These membranes consist primarily of phospholipid bilayers with transmembrane proteins that permit the traversing of water, ions, and metabolites and the maintenance of a cytosolic pH that is one to three units higher than that of the cell exterior or the vacuole. Pure phospholipid bilayers are permeable to gases, such as O_2 and CO_2, but they are barely permeable to water and nearly impermeable to inorganic ions and other hydrophilic solutes, such as sucrose and amino acids. Proteins are required to transport protons, inorganic ions, and organic solutes across the plasma membrane and the tonoplast at rates sufficient to meet the needs of the cell.

Membranes contain different types of transport proteins: ATPases or ATP-powered pumps, channel proteins, and cotransporters (for other reviews on this topic, see Lalonde et al., 1999; Sze et al., 1999, in this issue). Some of these proteins are well characterized, and their subcellular location is known. The cDNAs for others have been cloned, but functional, biochemical, and cytological data are lacking. ATPases utilize the energy released upon hydrolysis of ATP to move ions across the plasma membrane against chemical and electrical gradients. In plant cells, H^+-ATPases pump protons across the plasma membrane or tonoplast to acidify the extracellular matrix or the vacuole, respectively (Sze et al., 1999). Channel proteins facilitate the diffusion of water and ions down energetically favorable gradients. These proteins form channels through which ions or water molecules pass in single file at very rapid rates—up to 10^6 to 10^7 per sec per channel. The activities of channels can be regulated: they can be opened and closed. In plant cells, both water and ion channels have been studied in detail.

Cotransporters, the third class of membrane-transport proteins, can move solutes either up or down gradients at rates of 10^2 to 10^4 molecules per second. However, depending on the conditions under which they are assayed, transporters sometimes behave like channels, and vice versa, so that the distinction between them is no longer clear. Classical models assumed that ion channels and cotransporters are clearly distinct proteins. However, recent single-channel studies on animal transporters have demonstrated that cotransporters can also function as ion channels (Fairman et al., 1995; Cammack and Schwartz, 1996). Therefore, animal researchers no longer distinguish strictly between ion channels and cotransporters, and models explaining channel modes of transporters have been derived (Larsson et al., 1996; Su et al., 1996). In plant systems, analysis of the wheat HKT1 transporter has provided evidence for two modes of transport by the same protein (Rubio et al., 1995; Gassmann et al., 1996).

Among the cotransporters, we distinguish uniporters, antiporters, and symporters. Uniporters, like channels, move substances down a concentration gradient, albeit much more slowly. Antiporters, also called exchangers, and symporters can move a given substance against its own concentration gradient, the energy requirement for which comes not from ATP but rather is satisfied by an electric potential and/or chemical gradient of a secondary substance. The movement of a solute against its concentration gradient is thus coupled to the movement of the secondary substance—in plants this is often a proton—down its concentration gradient. Thus, the high proton concentration in the

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apoplast powers the inward movement of certain ions, such as nitrate, by a symporter. Proton gradients can similarly power the movement of ions into the vacuole by the proton–sodium antiporter or the proton–calcium antiporter.

The ionic compositions of the solutions on either side of both plasma membrane and tonoplast are very different. Not only can the proton concentration be up to 1000 times greater in the apoplast and vacuole than in the cytosol, but gradients of calcium concentration can vary over an even wider range (the concentration of free Ca^{2+} is only 100 nM in the cytosol). The potassium concentration, on the other hand, is much higher in the cytoplasm than in the apoplast. These concentration gradients are maintained by the combined actions of channels, cotransporters, and pumps in the plasma membrane and tonoplast. In addition, at the neutral pH of the cytoplasm, the proteins in the protein-rich cytosolic soup are negatively charged. These and other charge imbalances result in the establishment of an electric potential of −80 to −180 mV at the plasma membrane (negative inside). This potential creates a very strong electric field that provides the energy for biochemical processes at the plasma membrane, such as the uptake of ions or solutes against their concentration gradient, and the opening and closing of ion channels (so-called voltage gated channels).

Many signals coming from the cell exterior (e.g., hormones) can change the electric potential across membranes by activating the H^+-ATPase or opening ion channels. Such a change may only be transient, but it sets in motion a signal transduction cascade that often leads to the activation of specific proteins and genes. Ion channels play important roles in signal transduction, but in this review, we concentrate on the proteins that are involved in nutrient acquisition and water flow.

AQUAPORINS: WATER CHANNEL PROTEINS

The Tonoplast and the Plasma Membrane Contain Water Channel Proteins Called Aquaporins

Water movement across cellular membranes is determined not only by osmotic and hydrostatic pressure gradients but also by the intrinsic permeability of the membrane. It was long thought that water molecules could simply diffuse through the lipid bilayer and that variations in water permeability arose from differences in the lipid composition of membranes. This view had to be abandoned with the discovery that proteins, now called aquaporins, specifically facilitate the passage of water through membranes. Such proteins have been found in plants, animals, and bacteria and are probably present in most cellular organisms. In plants, they are found as intrinsic proteins in the tonoplast and the plasma membrane (reviewed in Chrispeels and Maurel, 1994; Maurel, 1997; Schäffner, 1998).

Aquaporins are abundant proteins that may account for 5 to 10% of the total protein in a membrane. Indeed, when intrinsic membrane proteins are subjected to SDS-PAGE, the aquaporins, with molecular masses of 27 to 30 kD, usually form the most abundant polypeptide band. Conventional protein purification methods enable the purification of substantial quantities of aquaporins, provided that a ready supply of membranes is available (Johnson et al., 1989; Johansson et al., 1996). Aquaporins are generally highly antigenic, so that antibodies prepared to total membrane fractions sometimes recognize aquaporins specifically. Proteins other than aquaporins may also form water channels and permit the passage of water through membranes.

Aquaporins Are Tetramers of Polypeptides with Six Membrane-Spanning Domains

Aquaporins are members of the major intrinsic protein (MIP) family. All members of this family are characterized by the presence of six membrane-spanning domains and the signature sequence Asn-Pro-Ala (NPA). This NPA motif is present twice, once in a loop between the second and third membrane-spanning domains and once in a loop between the fifth and sixth membrane-spanning domains. These two loops are thought to be involved in forming the aqueous pore through which water moves. A model of aquaporin structure is presented in Figure 1.

All members of the MIP family are not aquaporins. Some members, such as the GlpF protein of *Escherichia coli*, facilitate the passage of glycerol, whereas other members transport urea. Some mammalian MIPs have a dual function,

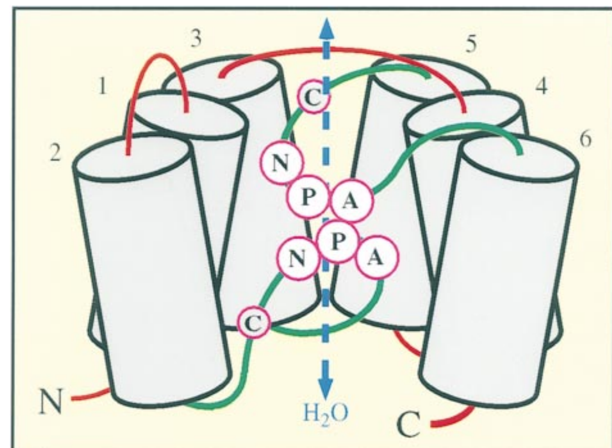

Figure 1. Model of the Structure of the Aquaporin Protomer.

The model shows six membrane-spanning domains (numbered 1 through 6) and two Asn-Pro-Ala loops folded into the membrane. The proposed path taken by water molecules is shown by the dashed double arrow.

permitting the passage of small solutes, such as glycerol, and water, but there is as yet no published evidence on dual-function aquaporins in plants. Nevertheless, Hertel and Steudle (1997) recently observed that small organic solutes (i.e., alcohols) can slip through the water channels of *Chara corallina*, and they concluded that water channels may not be as selective as generally believed. The challenge will be to identify the physiological substrates of dual-function aquaporins, because even if plant aquaporins are found to transport glycerol, this may not be their physiological substrate.

All aquaporins are not equally effective at permitting the passage of water across membranes. By surveying the Arabidopsis expressed sequence tag database, we found 23 different expressed MIPs (Weig et al., 1997). Quite a few of these have been verified as aquaporins by expressing the proteins in *Xenopus* oocytes and measuring the resulting increase in hydraulic conductivity of the oocyte plasma membrane. We do not know if these 23 members are all aquaporins or if some of them have other functions or dual functions. Amino acid sequence comparisons show that the Arabidopsis MIPs form three major groups—one group of tonoplast proteins (TIPs) and two groups of plasma membrane proteins (PIP1 and PIP2)—with a few additional aquaporins unrelated to these three groups.

The three-dimensional structure of mammalian aquaporins has been established from x-ray scattering data of aquaporin crystals. These proteins form tetramers with a central depression between the subunits. The two loops with the NPA motifs are oriented toward a central depression in the tetramer, and the loops of the four subunits probably interact.

Membranes that have abundant aquaporins have a low activation energy for water movement, $\sim$16 kJ mol^{-1}, which is similar to the movement of bulk water. Water molecules are thought to pass through the aqueous pore in a single file, and an osmotic difference of 100 mM can permit the passage of $\sim 10^6$ water molecules per sec per pore. Within the channels, water molecules experience surroundings similar to those in bulk water.

In spite of the tetrameric structure of aquaporins, the aqueous channel does not form from among the four subunits, but rather each 27-kD polypeptide is a water-conducting unit. For those mammalian aquaporins that transport small solutes, it is possible that the passage of the solute molecules occurs through the center of the tetramer and results from a change in the way the NPA loops interact with each other (Echevarría et al., 1996). Recent evidence shows that two amino acid mutations in the sixth transmembrane domain of an insect aquaporin converts it from a water channel to a glycerol channel (Lagrée et al., 1999).

The Activity of Some Aquaporins Is Regulated by Phosphorylation

Are aquaporins always "open," or is their activity somehow regulated? Two groups have compared the hydraulic permeability of isolated tonoplast and plasma membrane vesicles and found the tonoplast vesicles to be 10 to 50 times more permeable to water than the plasma membrane vesicles (Maurel et al., 1997b; Niemietz and Tyerman, 1997). Furthermore, the measured activation energy for water movement across the two types of membranes indicated that water movement through the tonoplast vesicles probably involved aquaporins (low energy of activation), whereas water movement across the plasma membrane probably occurred by diffusion through the lipid bilayer (high energy of activation). This rather surprising result indicates that the particular plasma membrane vesicles used (from tobacco cells and wheat roots) had either few aquaporins or aquaporins that were largely inactive.

Recent results (Maurel et al., 1997b; Johansson et al., 1998) indicate that aquaporin activity may be regulated by phosphorylation. Aquaporins have conserved phosphorylation motifs in which certain serine residues are phosphorylated in vivo (Johnson and Chrispeels, 1992; Johansson et al., 1996). Phosphorylation increases the activity of tonoplast aquaporin α-TIP from bean and plasma membrane aquaporin PM28A from spinach. This conclusion is based not only on the expression of wild-type and mutant (serine to alanine) aquaporins in *Xenopus* oocytes but also on the determination of oocyte swelling rates in the presence of inhibitors of protein kinases and protein phosphatases. Interestingly, PM28A is poorly phosphorylated under water deficit, leading to the suggestion that plant cells can at least partially close their aquaporins upon a loss of turgor. Johansson et al. (1998) proposed a model in which loss of turgor caused by water deficit results in the dephosphorylation of PM28A so as to decrease aquaporin activity and conserve water.

Plant Aquaporin Genes Are Differentially Expressed

With so many aquaporins and/or MIPs, it is not surprising that the corresponding genes are differentially regulated. Through promoter–β glucuronidase (GUS) fusion analyses and in situ hybridization with gene-specific probes, the transcription of a number of aquaporin genes has been investigated. These studies show dramatic differences in aquaporin gene expression among various tissues and cell types, but whether differences in transcription correlate with aquaporin levels is unclear. Nevertheless, these gene expression or, more correctly, mRNA abundance studies have led to the following conclusions: (1) many aquaporins are highly expressed in vascular tissues; (2) some tonoplast aquaporins are highly expressed in meristems (where vacuolar biogenesis takes place); and (3) aquaporins are highly expressed in tissues that can experience high water or metabolite flux (see Barrieu et al., 1998, and references therein).

The differential expression of aquaporin genes has been postulated to account for three aspects of water balance in

plants. First, the high expression of tonoplast aquaporins in meristematic cells is necessary to sustain vacuole biogenesis and to prepare the cells for the rapid increase in vacuolar volume that accompanies cell enlargement (for a review of vacuole biogenesis, see Marty, 1999, in this issue). Second, in tissues or cells that experience high metabolite fluxes, rapid osmotic equilibration of the cytosol with water from the vacuole may be a prerequisite. Third, in tissues that experience transcellular water flow, aquaporins would greatly reduce the resistance to this flow. Furthermore, because the permeability of the tonoplast is much greater than that of the plasma membrane (Maurel et al., 1997b; Niemietz and Tyerman, 1997), the regulation of this flow may well occur at the plasma membrane.

Functional Investigations of Aquaporins in the Plant

Much of the evidence for the function of plant aquaporins has been gathered in heterologous systems. What do we know about plant aquaporins in vivo? Maggio and Joly (1995) found that mercuric chloride rapidly reduces pressure-induced root water flux in decapitated tomato plants and that this inhibition is largely reversed by subsequent treatment with β-mercaptoethanol. Similarly, many aquaporins are also reversibly inhibited by mercuric chloride, which led the authors to conclude that aquaporins largely account for the hydraulic conductivity of the root system. Tazawa et al. (1996) made similar observations with *Chara* cells. Barone et al. (1997) recently showed that mercuric chloride induces conformational changes in plant MIPs, and this observation may provide an explanation for the observed inhibition of water transport. However, the possibility that mercuric chloride and β-mercaptoethanol exert their effects through other membrane proteins or cellular processes cannot be excluded.

Using a cell pressure probe, Hertel and Steudle (1997) measured the hydraulic conductivity, solute permeability, and reflection coefficients of *C. corallina* internodes in the temperature range of 10 to 35°C. They found that water does indeed use water channels to cross the membrane and that the changes in the reflection coefficient with temperature are a sensitive measure for the open/closed state of the channels. When the channels are open, the energy of activation is ~10 to 15 kJ mol^{-1}, but when they are closed, the energy of activation increases to 40 to 60 kJ mol^{-1}.

Transgenic plants demonstrate that water channels are important both at the cellular and at the whole-plant level. Kaldenhoff et al. (1995, 1998) expressed an Arabidopsis *PIP1b* construct in the antisense orientation. The antisense plants had reduced steady-state levels of *PIP1a* and *PIP1b* mRNAs, and there was no detectable cross-reactivity with antibodies to PIP1a protein. The protoplasts of the transgenic plants, furthermore, swelled and burst more slowly when exposed to hypotonic conditions than did those of the control plants. Correspondingly, the osmotic water permeability coefficient of the cells was reduced threefold in the antisense plants. Interestingly, the root mass of the antisense plants was five times greater than that of the control plants, although both lines had similar shoot masses and developed similarly. The authors suggested that the reduced water permeability of the plasma membranes caused the plants to overproduce roots as a compensatory mechanism to maintain water flow in the xylem. These findings argue that water flux is generally regulated by the plasma membrane rather than by the tonoplast.

UPTAKE OF NITRATE, PHOSPHATE, AND SULFATE

Energetics, Mechanisms, and Regulation of Anion Uptake

Nitrogen, phosphorus, and sulfur are macronutrients that are required for plant growth and reproduction. These elements are present in the soil solution as organic and inorganic forms, including the anionic species nitrate, phosphate, and sulfate. The uptake of these anions presents a challenge to plants because their concentration in the soil can vary by two to three orders of magnitude and because their uptake often occurs against concentration and electrical gradients. To meet these challenges, plants have evolved active transport systems that are regulated in response to changes in environmental and internal conditions.

The physiological analysis of ion uptake by plant roots has revealed several distinct classes of regulated transport activities. The original work of Epstein classified these activities into two mechanisms (reviewed in Epstein, 1966, 1972). Mechanism I operates at concentrations below 200 μM and behaves as a saturable carrier. At higher concentrations, mechanism II becomes apparent and displays either linear or multiple saturation kinetics. These mechanisms have also been called high- and low-affinity uptake systems, respectively. Figure 2 shows a graphical representation of these types of kinetics.

For anion uptake, both high- and low-affinity systems have a characteristic feature: they are electrogenic. When plant cells are exposed to nitrate, sulfate, or phosphate, an initial depolarization of the membrane is induced, rendering the inside of the cell more positive (for examples, see Ullrich and Novacky, 1990; Glass et al., 1992; Meharg and Blatt, 1995). Depolarization is followed by repolarization of the membrane, which has been attributed to enhanced H^+-ATPase activity in the plasma membrane. The amplitude of the initial depolarization is inversely proportional to the pH of the bath solution and is associated with an alkalization of the extracellular environment. These data indicate that uptake of anions requires cotransport of two or more protons for each anion, although hydroxide antiport cannot be ruled out.

Another characteristic feature of anion uptake is that it is regulated (reviewed in Clarkson and Luttge, 1991; von Wiren

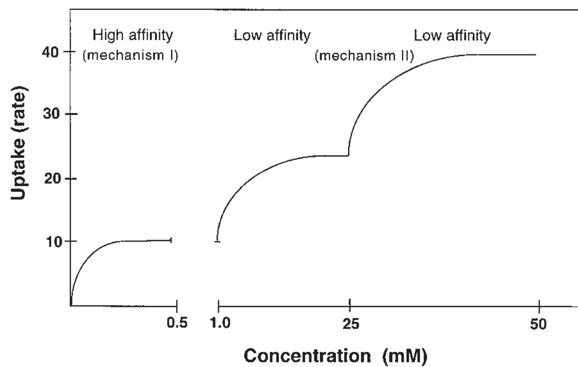

Figure 2. Generalized Diagram of the Rate of Absorption of a Cation as a Function of Its Concentration.

The three curves represent postulated uptake systems with different affinities for the cation. The work of E. Epstein and collaborators showed that many plant nutrients have absorption isotherms of this nature.

et al., 1997; Crawford and Glass, 1998; Schachtman et al., 1998). In the case of phosphate and sulfate, high-affinity transporter activities are derepressed when the plants are deprived of the nutrient. In the case of nitrate, high-affinity transporters are induced by nitrate and are feedback inhibited by reduced and organic forms of nitrogen. These responses allow plants to adjust anion uptake to environmental conditions and internal metabolism.

The physiological studies described above have laid the groundwork for the molecular and genetic analysis of anion uptake (reviewed in Tanner and Caspari, 1996; von Wiren et al., 1997; Crawford and Glass, 1998; Forde and Clarkson, 1998; Schachtman et al., 1998). A major challenge has been to identify the components of each uptake system and determine the contribution that each makes to nutrient uptake and plant growth. Many anion transporter genes have now been cloned, and all have predicted membrane topologies found in cotransporters with 12 membrane-spanning domains. Many of the cloned genes also show regulated expression and produce functional products in heterologous systems such as yeast or *Xenopus* oocytes. In one case, plants with mutations in the transporter gene have been identified and used for functional analysis. The initial conclusions from this work are that multiple, regulated genes are involved in the uptake of a particular anion and that plant transporters share sequence and functional similarity with equivalent proteins in fungi and yeast (see below).

The Identification of Regulated, High-Affinity Phosphate Transporters

In most soils, phosphate is not mobile and is quickly depleted from the rhizosphere. Often, plants must take up soluble, inorganic phosphate from concentrations of <10 μM while maintaining cytosolic concentrations of 5 to 10 mM (Lee and Ratcliffe, 1993). Plant roots have a high-affinity uptake system that is specifically derepressed by phosphate deprivation (reviewed in Clarkson and Luttge, 1991; Schachtman et al., 1998). The cloning and analysis of plant phosphate transporter genes were made possible by the availability of fungal and yeast transporter mutants and clones. The plant genes show extensive sequence similarity among themselves, and they show regulation in response to phosphate starvation (reviewed in Schachtman et al., 1998).

Eukaryotic high-affinity phosphate transporter genes were initially isolated from *Saccharomyces cerevisiae* (*PHO84*; Bun-ya et al., 1991), *Neurospora crassa* (*PHO-5*; Versaw, 1995), and the arbuscular mycorrhizal fungus *Glomus versiforme* (*GvPT*; Harrison and van Buuren, 1995). cDNA clones with related sequences were then identified in the Arabidopsis expressed sequence tag databases. Subsequently, phosphate transporter genes were isolated from Arabidopsis (Muchhal et al., 1996; Lu et al., 1997; Smith et al., 1997a), tomato (Daram et al., 1998; C. Liu et al., 1998), potato (Leggewie et al., 1997), and *Medicago truncatula* (H. Liu et al., 1998). The plant genes typically show 75 to 85% identity among themselves and 25 to 35% identity with the fungal transporters at the amino acid level. In one case, two genes (*APT1* and *APT2*) are 99% identical and are genetically linked (Smith et al., 1997a). All of the plant genes respond to phosphate deprivation with a dramatic increase in transcript levels, and most of the corresponding proteins are localized in the roots. For the tomato genes, expression is primarily restricted to the root epidermis (C. Liu et al., 1998). Interestingly, both *M. truncatula* genes are downregulated when roots are colonized by mycorrhizal fungi (H. Liu et al., 1998). Lastly, the functional analysis of the plant phosphate transporters showed that most complement the yeast *pho84* high-affinity phosphate transporter mutant (Bun-ya et al., 1991).

The findings summarized above indicate that the cloned plant genes encode high-affinity phosphate transporters. One would therefore expect that these transporters would have a K_m between 1 and 10 μM for phosphate uptake. However, the measured K_m values for the plant transporters expressed in yeast are all above 30 μM: 130 μM for StPT2 and 280 μM for StPT1 from potato, 192 μM for MtPT1 from *M. truncatula*, and 31 μM for LePT1 from tomato (Leggewie et al., 1997; Daram et al., 1998; H. Liu et al., 1998). To explain this discrepancy, a recent report suggests that the high K_m values observed in yeast are due to the foreign environment in which these plant proteins are expressed (Mitsukawa et al., 1997). This work found enhanced phosphate uptake activity in tobacco tissue culture cells that overexpress the *APT2* transporter (also named *AtPT1* and *PHT1*) even though no *APT2* complementation of the yeast *pho84* mutant was detected. The phosphate uptake activity in tobacco attributed to the *APT2* gene had a K_m of 3 μM and was inhibited by protonophores, indicating that APT2 is

a high-affinity proton/phosphate symporter. To explain the difference in activity or K_m values between yeast and plants, it has been suggested that the plant phosphate transporters may be modified in plants. Alternatively, they may interact with other plant proteins (as has been postulated in yeast [Bun-ya et al., 1996]) to generate a transport complex with altered kinetic properties (Mitsukawa et al., 1997; Smith et al., 1997a).

The fungal genes described above are all H^+–phosphate symporters; however, fungi also possess Na^+–phosphate symporters. A genome database search shows that a homolog to the Na^+–phosphate symporter *PHO-4* exists in the Arabidopsis genome (GenBank accession number X97484), indicating that Na^+ coupling may also exist in higher plants (see below for coupling to K^+).

Identification of Sulfate Transporters with Different Affinities for Sulfate and Different Expression Patterns

Sulfate uptake is characterized by a starvation-induced, high-affinity system with a K_m between 10 and 20 μM along with a nonsaturable, low-affinity system (Legget and Epstein, 1956; reviewed in Clarkson and Luttge, 1991; Hawkesford et al., 1993). High-affinity sulfate transporter genes were originally identified in *N. crassa* (Ketter et al., 1991) and yeast (Smith et al., 1995b). A yeast mutant was used to clone three sulfate transporter cDNA clones from the tropical legume *Stylosanthes hamata* (Smith et al., 1995a). The SHST1 and SHST2 proteins are 95% identical and, when expressed in yeast, show an acid-enhanced uptake of sulfate with a K_m of 10 μM. Expression of the *SHST1* and *SHST2* genes is root specific and derepressed by sulfate deprivation. The third gene, *SHST3*, belongs to a different class because it is ~50% identical to the first two genes (at the amino acid level), displays a higher K_m (100 μM) for sulfate uptake in yeast, and expresses much less mRNA in plants than do the *SHST1* and *SHST2* genes. The *SHST3* gene has another interesting property: its mRNA levels are higher in shoots than in roots of plants supplied with adequate sulfur but may decrease (in the leaves) under sulfate limiting conditions.

Additional sulfate transporter genes have more recently been identified in Arabidopsis (*AST56* and *AST68*; Takahashi et al., 1996, 1997) and barley (*HVST1*; Smith et al., 1997b). The HVST1 protein is most similar in sequence and activity to the SHST1 and SHST2 proteins of *S. hamata.* HVST1 expressed in yeast shows a K_m of 7 μM for sulfate uptake, and its mRNA is found exclusively in roots, increasing in abundance in response to sulfate deprivation. The products from the two Arabidopsis genes, however, are more similar to SHST3 (60% peptide sequence identity) than to the SHST1 and SHST2 proteins (50% identity). *AST56* and *AST68* expression patterns are also not root specific. *AST56* is constitutively expressed, whereas *AST68* is induced with sulfate starvation.

These findings indicate that the SHST1/SHST2/HVST1 class of sulfate transporters is part of the starvation-induced, high-affinity uptake system in plant roots. The second class (composed of SHST3, AST56, and AST68) is thought to contain low-affinity transporters that either load sulfate into the vascular tissue in roots and unload sulfate into leaf cells (for AST68; Takahashi et al., 1997) or transport sulfate internally between cellular or subcellular compartments (for SHST3; Smith et al., 1995a). No transporters have been assigned to a low-affinity sulfate uptake system in the root.

Identification of High- and Low-Affinity Nitrate Transporters

Inorganic nitrogen nutrition differs from that of phosphate and sulfate in that not one but two inorganic forms, ammonium and nitrate, are readily used by plants (in addition, N_2 gas is used by nitrogen fixers). For many plants, the presence of both ammonium and nitrate is optimal for growth, but nitrate is taken up in the largest quantity. High-affinity nitrate uptake is characterized by K_m values for nitrate between 7 and 80 μM and saturation below 200 μM (reviewed in Glass and Siddiqi, 1995; Crawford and Glass, 1998). High-affinity uptake activity also displays both positive and negative regulation. In plants that have not been exposed to nitrate, high-affinity uptake activity is detected at a basal level. When a root is exposed to nitrate, high-affinity uptake activity increases, and a new activity with a distinct K_m appears. If nitrate accumulates in the root or if the root is exposed to ammonium or certain amino acids, uptake is downregulated. Plants also possess another uptake activity that is detectable at nitrate concentrations above 0.2 to 0.5 mM and that displays linear (nonsaturable) kinetics. This low-affinity activity does not appear to be induced by nitrate in plants such as barley. From these findings, a model was proposed that plants have three nitrate uptake systems: a constitutive high-affinity uptake system (cHATS), an inducible high-affinity system (iHATS), and a constitutive low-affinity system (cLATS) (reviewed in Glass and Siddiqi, 1995; Crawford and Glass, 1998).

Efforts to identify the components of these three uptake systems led to the cloning and characterization of several nitrate transporter genes (reviewed in Crawford and Glass, 1998). Two gene families, named *NRT1* and *NRT2*, have been identified so far. The first gene identified in the *NRT2* family was *crnA* of *Aspergillus nidulans* (Unkles et al., 1991). A mutation in this gene reduces nitrate uptake two- to fourfold in conidiospores and young mycelia but not in older mycelia (Brownlee and Arst, 1983). Subsequently, a pair of *crnA*-related algal genes (*Nrt2;1* and *Nrt2;2*, originally called *nar-3* and *nar-4*) was isolated from Chlamydomonas (Quesada et al., 1994). Mutations in these genes impair high-affinity nitrate uptake. Some time later, *NRT2* genes were identified in higher plants (reviewed in Crawford and Glass, 1998). The expression of these genes is root specific,

nitrate inducible, and ammonium/glutamine repressible. Recently, an *NRT2* gene (*YNT1*) was isolated from the yeast *Hansenula polymorpha*, and a mutant defective in nitrate uptake was generated by disrupting the *YNT1* gene (Perez et al., 1997). A barley *NRT2* cDNA, under the control of a suitable yeast promoter, has been shown to partially restore high-affinity nitrate uptake in the *ynt1* mutant (B. Forde and J. Siverio, personal communication). These data indicate that the *NRT2* genes are components of the iHATS in plants.

The first member of the *NRT1* family was identified in a chlorate-resistant mutant (*chl1*) of Arabidopsis that had reduced nitrate uptake above 1 mM nitrate (i.e., in the low-affinity range) (Doddema and Telkamp, 1979; Tsay et al., 1993). The *CHL1* gene was cloned from a T-DNA insertion mutant and shown to encode a protein with electrogenic nitrate uptake activity in *Xenopus* oocytes (Tsay et al., 1993). Initial experiments showed that CHL1 has a K_m of ~8 mM for nitrate in *Xenopus* oocytes (Huang et al., 1996). Figure 3 is representative of the data showing the depolarization response to nitrate in *Xenopus* oocytes expressing CHL1. In plants, the *CHL1* gene is nitrate induced, has higher mRNA levels at acidic pH, and is expressed predominantly in the outer layers of the root (i.e., endodermis to epidermis) after nitrate induction (Tsay et al., 1993; Huang et al., 1996). Together with physiological data showing that *chl1* mutants are defective in low-affinity nitrate uptake (Doddema and Telkamp, 1979), these findings indicated that CHL1 is a component of the low-affinity nitrate uptake system.

The assignment of CHL1 to the low-affinity system best fits the physiological data in Arabidopsis; however, it ran counter to previous descriptions of the low-affinity system in other plants, that is, cLATS shows no sign of nitrate induction. A solution to this contradiction became apparent when it was discovered that the contribution of CHL1 to low-affinity nitrate uptake is variable and depends on the composition of the nutrient solution and that more than one differentially regulated *NRT1* gene exists in plants (Huang et al., 1996; Touraine and Glass, 1997). If Arabidopsis plants are grown with nitrate but no ammonium, short-term, low-affinity nitrate uptake rates for wild-type and *chl1* mutant plants are similar (12 μmol g^{-1} hr^{-1} at 5 mM nitrate); however, if plants are grown on ammonium nitrate, uptake rates are much lower for *chl1* mutants (2 μmol g^{-1} hr^{-1} at 5 mM nitrate) than for the wild type (7.5 μmol g^{-1} hr^{-1}) (Touraine and Glass, 1997). The implication from these results is that the contribution that CHL1 makes to low-affinity uptake depends on the relative expression or activity levels of two transporters. A candidate gene that could encode this second transporter has been identified in Arabidopsis and is called *NRT3* (Huang et al., 1996; see also Genbank accession number AF073361). Interestingly, this protein is a member of the NRT1 family, being 36% identical to CHL1.

The same situation may also apply in tomato, in which two *NRT1* genes that are 65% identical to *CHL1* have been cloned (Lauter et al., 1996). The first is expressed predominantly in root hairs and is nitrate inducible. The second is

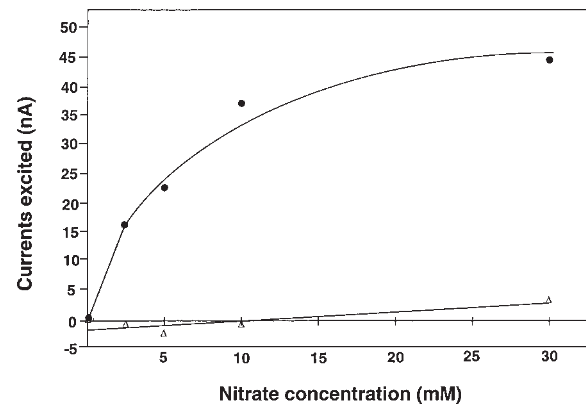

Figure 3. Kinetics of Nitrate Uptake by *Xenopus* Oocytes Expressing CHL1.

Transporters can be assayed by injecting the corresponding mRNA into oocytes and assaying transport at the plasma membrane 2 to 3 days later. Transport can be measured with radioactive tracers or by measuring currents in the case of ions. Here, current (nA) elicited in a single oocyte injected with *CHL1* mRNA (filled circles) or with water (open triangles) was plotted as a function of nitrate concentration (taken from Huang et al., 1996). The K_m value calculated by using a Lineweaver-Burk plot for this experiment is 7.4 mM, indicating that CHL1 has low-affinity transport activity.

more ubiquitously expressed in roots and is not nitrate regulated. Given their high sequence identity to *CHL1* and their expression in roots, it is possible that both genes encode nitrate transporters that are components of the low-affinity system.

CHL1 Is a Dual-Affinity Nitrate Transporter

The results described above appeared to fit into a simple model: members of the *NRT1* gene family, which includes *CHL1*, encode the transporters of the low-affinity system (which has both constitutive and inducible components); and *NRT2* genes make up the inducible high-affinity system. Our understanding of these systems took an interesting turn, however, when a high-affinity nitrate uptake mutant was discovered in Arabidopsis that had a transposon insertion in the *CHL1* gene (R. Wang et al., 1998). Further analysis demonstrated that CHL1 is a major component of the high-affinity uptake system when plants are grown on ammonium nitrate but that it plays a minor role when ammonium is absent (Touraine and Glass, 1997; R. Wang et al., 1998; Liu et al., 1999). Thus, as is the case in the LATS, the contribution of CHL1 depends on the composition of the nutrient solution.

Further work showed that CHL1 contributes to both iHATS and cHATS (R. Wang et al., 1998). Its contribution to the constitutive system is pH dependent, being most significant

at acidic pH. To explain how CHL1 could be a major component of both LATS and HATS, it was proposed that CHL1 is a dual-affinity transporter with both high- and low-affinity K_m values (R. Wang et al., 1998; Liu et al., 1999). These new findings show that nitrate uptake is not mediated by a one-affinity-type/one-transporter system but is more complex and dynamic, with multiple transporters contributing to a given system (e.g., at least two *NRT1* genes for LATS) and a single transporter contributing to multiple uptake systems in an environmentally dependent manner (e.g., CHL1 contributes to cHATS, iHATS, cLATS, and iLATS when ammonium is present and/or the pH is low).

UPTAKE AND CELLULAR TRANSPORT OF POTASSIUM

Positively charged macronutrients such as potassium (K^+), ammonium (NH_4^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) are required in relatively large amounts for plant growth and development. Additional cationic micronutrients (iron, manganese, zinc, copper, and nickel) play essential roles as cofactors and activators of enzymes. In the past few years, transporters for several of these cations have been cloned from plants, including transporters for ammonium, iron, copper, and potassium (Sentenac et al., 1992; Ninnemann et al., 1994; Kampfenkel et al., 1995; Eide et al., 1996). As is the case for H^+-ATPases (Sussman, 1994), data from many laboratories show that for each cation, multiple genes and even multiple gene families appear to be responsible for transport. This is not surprising, considering that different plant tissues have different nutritional and energy requirements. Furthermore, the transport of nutrients across a variety of different membranes (e.g., plasma membrane, tonoplast, and the inner and outer plastid membranes) is required. In addition, multiple membrane proteins may be needed for cation uptake from soils to adapt to varying extracellular conditions and nutrient availability. To illustrate the underlying complexity and open questions, we focus here on recent advances in the understanding of K^+ transport mechanisms.

Physiology of K^+ Uptake in Roots

Potassium is the most abundant cation in plants and plays an important role in processes such as cell elongation, leaf movements, tropisms, metabolic homeostasis, germination, osmoregulation, stomatal movements, and sodium (Na^+) stress (reviewed in Kochian and Lucas, 1988; Maathuis et al., 1997). Because no convenient radioactive isotope of K^+ exists, transport of K^+ is measured with radioactive Rb^+. Classical studies of K^+ (Rb^+) uptake into roots showed two main transport components, described as high-affinity (mechanism I) and low-affinity (mechanism II) transport, respectively (reviewed in Epstein, 1972; see Figure 2). One of the high-affinity K^+ uptake components is induced by removing K^+ from the nutrient medium.

A comparison of K^+ uptake in K^+-starved and K^+-supplied barley and maize roots shows constitutive and inducible high-affinity K^+ uptake components (Glass and Dunlop, 1978; Kochian and Lucas, 1982). The genetic separation of constitutive from K^+ starvation-induced high-affinity K^+ uptake systems was recently demonstrated with the sodium-sensitive Arabidopsis mutant *sos1* (Wu et al., 1996). Specifically, uptake studies showed that the *sos1* mutant is defective in inducible high-affinity K^+ uptake, whereas constitutive (i.e., background) high-affinity K^+ uptake remains unaltered (Ding and Zhu, 1997). Because of the importance of K^+ uptake for plant nutrition, research in several laboratories is focused on determining the transport mechanisms and molecular bases of high- and low-affinity K^+ transport components.

Multidisciplinary studies have been pursued to determine the nature of the proteins (e.g., ATPase, cotransporter, and/or channel) that might mediate high- and low-affinity K^+ uptake in roots. In intact maize roots, the nonsaturable low-affinity component of Rb^+ uptake is inhibited by the K^+ channel blocker tetraethylammonium, suggesting the involvement of K^+ channels in low-affinity K^+ (Rb^+) uptake (Kochian and Lucas, 1982). Patch clamp studies of different root cell types (root cortex, root hair, stele, and parenchyma) in several laboratories demonstrated the existence of hyperpolarization-activated K^+ (K^+_{in}) channels, leading to the suggestion that these K^+_{in} channels may contribute to low-affinity channel–mediated K^+ uptake (reviewed in Maathuis et al., 1997). These K^+ channels are activated by plasma membrane potentials more negative than the K^+ equilibrium potential, suggesting that activated K^+_{in} channels "draw" K^+ into cells upon sufficient activation of proton pumps. Tracer flux studies suggest that multiple low-affinity K^+ (Rb^+) uptake components exist, with distinguishable K_m values for K^+ in the millimolar concentration range (Epstein, 1972). Further research is needed to determine the relative contributions of K^+_{in} channels in different root cells to physiological K^+ uptake.

A Single Mechanism for High-Affinity K^+ Transport Could Not Be Resolved by Studies with Intact Roots

Because of the importance of high-affinity K^+ uptake for plant growth, the molecular bases and transport mechanisms used to accumulate K^+ from micromolar K^+ concentrations are of particular interest. In *N. crassa*, H^+–K^+ symport activity is responsible for high-affinity K^+ uptake (Rodriguez-Navarro et al., 1986). The physiological mechanisms for high-affinity K^+ nutrition in plants have, however, remained more elusive. For example, Kochian, Lucas, and colleagues pursued elegant characterizations of high-affinity K^+ uptake in maize roots using tracers, intracellular K^+ and pH electrodes, extracellular K^+-gradient sensing (vibrating)

electrodes, and membrane potential recordings (Newman et al., 1987; Kochian et al., 1989). The K_m for K^+ uptake in maize roots was 4 to 6 μM and that for Rb^+ uptake was 30 μM, indicating a higher apparent affinity for K^+ than for Rb^+ (Newman et al., 1987). Exposure to extracellular micromolar K^+ concentrations produced a rapid depolarization of the plasma membrane, suggesting that the K^+ uptake mechanism is not electroneutral and that uptake of the positively charged K^+ ions contributes to this depolarization (Newman et al., 1987). Therefore, an electroneutral H^+–K^+ exchange is unlikely to be the mechanism for high-affinity K^+ uptake. Interestingly, however, in these detailed studies with intact roots, no single K^+ transport mechanism could be identified that mediates high-affinity K^+ uptake. For example, no proton stimulation or evidence for proton–K^+ exchange could be obtained with regard to the high-affinity system, and ATP-dependent K^+ uptake pumping also was thought to be unlikely (Newman et al., 1987; Kochian et al., 1989). Studies with barley roots also show no proton stimulation of high-affinity K^+ uptake (Glass and Siddiqi, 1982).

Other nutrients, including ammonium, nitrate, phosphate, and sucrose, show a clear stimulation of nutrient uptake by the physiological proton gradient in intact organs (McClure et al., 1990; Meharg and Blatt, 1995). However, no proton stimulation of K^+ uptake has yet been reported in intact roots. Proton stimulation of high-affinity K^+ uptake was indicated in subtractive current–voltage analyses of Arabidopsis root cells (Maathuis and Sanders, 1994), but this activity (~5 to 15 pA per cell) was observed in ⩽10% of patch clamp experiments (Maathuis and Sanders, 1997), and the relative contribution of this activity to whole-root uptake has not yet been determined. Disruption of genes for other mechanisms of K^+ uptake could provide an approach to resolve the proposed proton-stimulated high-affinity K^+ uptake in roots.

Does Sodium Play a Role in Potassium Transport?

The finding that the wheat *HKT1* cDNA encodes a Na^+–K^+ symporter when expressed in yeast and *Xenopus* ooctyes was surprising. This is because other nutrients in plants are transported via symport with protons rather than Na^+ (Rubio et al., 1995) and because coupling of high-affinity K^+ uptake to Na^+ uptake was not found in wheat roots, where the gene is expressed (Maathuis et al., 1996). At low concentrations, Na^+ is known to stimulate root growth (Flowers and Läuchli, 1983, and references therein), but the mechanisms remain unknown. It is interesting that, in certain aquatic plants (e.g., Charophyte algae, *Egeria* and *Elodea* roots, and in *Vallisneria* and *Egeria* leaves), Na^+–K^+ symport has been identified as a high-affinity K^+ uptake mechanism (Smith and Walker, 1989; Walker and Sanders, 1991; Maathuis et al., 1996). These data show that Na^+-coupled high-affinity K^+ uptake activity exists in these plants. Whether the underlying transporters are related to *HKT* genes has not been analyzed. It is possible that in these aquatic plants, a single K^+ transport mechanism could be resolved because one major type of K^+ uptake transporter is responsible for the bulk of high-affinity K^+ uptake, whereas high-affinity K^+ uptake in terrestrial plants has been proposed to require many types of transporters to adapt to environmental and soil conditions (Rubio et al., 1996).

Numerous cDNAs Encode Proposed K^+ Transporters

Three gene families have been identified and proposed to contribute to high-affinity K^+ transport in plants, including the Arabidopsis root inward-rectifying K^+ channel *AKT1* (Sentenac et al., 1992; Hirsch et al., 1998), the wheat root Na^+–K^+ transporter *HKT1* (Schachtman and Schroeder, 1994), and the recently identified gene family of *HAK/KUP* transporters in barley (*HvHAK*) and Arabidopsis (*AtKUP* or *AtKT*) (Quintero and Blatt, 1997; Santa-Maria et al., 1997; Fu and Luan, 1998; Kim et al., 1998). Promoter–GUS studies suggest that *AKT1* is predominantly expressed in the epidermis, cortex, and endodermis of mature Arabidopsis roots (Lagarde et al., 1996). As seen in Figure 4, mRNA in situ hybridization analyses show *HKT1* expression in the cortex of wheat roots and in cells surrounding the vasculature of wheat leaves (Schachtman and Schroeder, 1994). In rice roots, an *HKT1* homolog is expressed in the epidermis and endodermis (Golldack et al., 1997).

HKT1 is related to *TRK1* and *TRK2*, the yeast genes encoding plasma membrane high-affinity K^+ uptake transporters (Schachtman and Schroeder, 1994). Similarly, the *HAK/KUP* genes in barley and Arabidopsis show similarity to the bacterial low-affinity *Kup* and the *Schwanniomyces occidentalis* high-affinity *HAK1* K^+ uptake transporters (Schleyer and Bakker, 1993; Banuelos et al., 1995). The *HAK/KUP* transporters represent a large gene family in Arabidopsis (Quintero and Blatt, 1997; Kim et al., 1998), and genome database searches indicate that this species possesses at least 10 members. The transport mechanisms of the *HAK/KUP* transporter family members remain uncharacterized.

Expression of the Arabidopsis *HAK/KUP* homolog *AtKUP2* in yeast results in low-affinity Rb^+ uptake (Quintero and Blatt, 1997). Expression of the barley homolog *HvHAK* in yeast and expression of an Arabidopsis homolog *AtKUP1* in Arabidopsis suspension cells results in high-affinity Rb^+ uptake (Santa-Maria et al., 1997; Kim et al., 1998). In addition, AtKUP1 can show both high- and low-affinity Rb^+ uptake activity, as discussed below (Fu and Luan, 1998; Kim et al., 1998). Interestingly, *HvHAK* and *AtKUP3* mRNA levels increase markedly in barley and Arabidopsis roots, respectively, after K^+ starvation, indicating a putative contribution of *HAK/KUP* gene products to inducible high-affinity K^+ uptake (Santa-Maria et al., 1997; Kim et al., 1998). In barley, wheat, and rice, *HKT1* mRNA levels are also enhanced in response to K^+ withdrawal, with rapid induction kinetics (Golldack et al., 1997; T.-B. Wang et al., 1998). It is therefore possible that *HvHAK*, *AtKUP3*, and *HKT1* contribute to

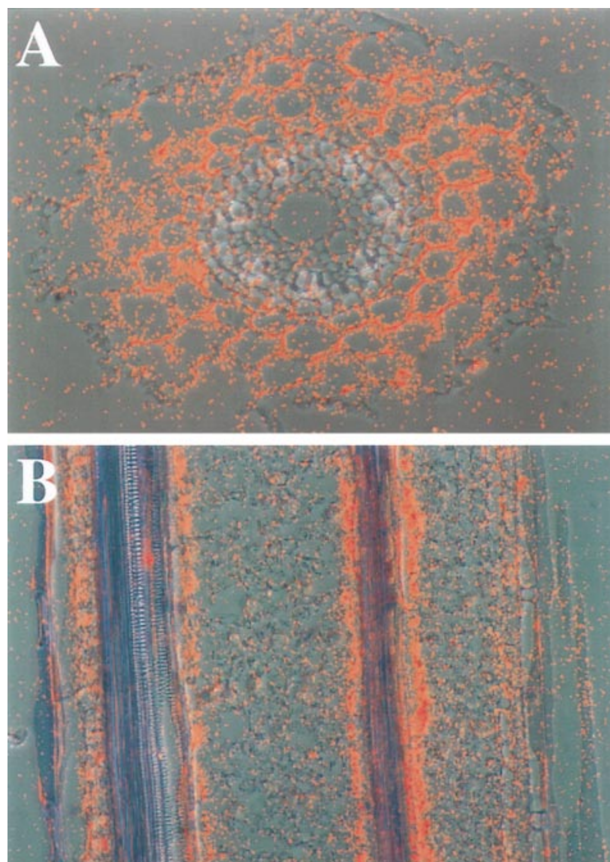

Figure 4. Expression of *HKT1* in 4-Day-Old Wheat Seedlings.

(A) Cross-section of a root.
(B) Longitudinal section of a leaf.
RNA in situ hybridization (in red) with an *HKT1* probe shows high levels of *HKT1* expression in root cortical cells **(A)** and in cell layers surrounding the vascular bundles of leaves **(B)** (D.P. Schachtman and J.I. Schroeder, unpublished data). The expression pattern of *HKT1* may indicate a role for HKT1 in leaf and root Na^+ and K^+ transport, as discussed in the text.

K^+ starvation–induced K^+ transport in the analyzed plant species.

How are the activities of K^+ transport proteins regulated? In addition to transcriptional regulation, post-translational regulation seems likely to play an important role in K^+ uptake. Several mechanisms for post-translational regulation of K^+_{in} channels in guard cells and other plant cells have indeed been reported (reviewed in Müller-Röber et al., 1998). In addition, genetic studies of *sos* mutants in Arabidopsis indicate that single genetic loci may regulate multiple K^+ and Na^+ transport components in parallel, suggesting that central regulation mechanisms exist for K^+ transport (Wu et al., 1996; Liu and Zhu, 1997). The Na^+ sensitivity mutant *sos3* regulates K^+ (Rb^+) and Na^+ uptake and was recently shown to encode a homolog of a Ca^{2+}-sensing regulatory subunit that modulates protein phosphatases or kinases in animal cells (Liu and Zhu, 1998). In fact, ectopic expression of constitutively active mutants of the yeast protein phosphatase calcineurin showed enhanced NaCl tolerance (Pardo et al., 1998). These data demonstrate the importance of post-translational regulation for K^+ and Na^+ transport. Further biochemical, electrophysiological, and genetic analyses will help to determine the regulation and physiological contributions of individual K^+ transporters in vivo.

Disruption of the Inward K^+ Channel AKT1 Indicates that Single Transporters May Mediate Both Low- and High-Affinity K^+ Uptake

The classical separation of low- and high-affinity K^+ transport components is being reconsidered based on recent findings. Most strikingly, a loss-of-function mutation of the *AKT1* K^+_{in} channel gene (*akt1-1*) has recently been shown to cause reduced growth of Arabidopsis seedlings at micromolar extracellular K^+ concentrations (Hirsch et al., 1998). Moreover, Rb^+ uptake analyses, membrane potential recordings, patch clamp recordings, plant growth assays, and genetic segregation analysis suggest that the AKT1 K^+ channel, previously thought to constitute a low-affinity K^+ transporter, can in fact function in high-affinity K^+ uptake. Previous theoretical discussions have also led to the suggestion that high- and low-affinity components may be mediated by the same transporter (Nandi et al., 1987) and from guard cell studies that K^+ channels could contribute to high-affinity K^+ uptake when other transporters are inactivated (Schroeder et al., 1994). Expression of *AKT1* in yeast also allows dual-affinity Rb^+ uptake at micromolar concentrations as well as at millimolar K^+ concentrations (Sentenac et al., 1992).

One explanation for a "dual-affinity" nature of single transporters could lie in the fact that extracellular ions, particularly K^+ ions, have a significant influence on the membrane potential of cells. For example, at micromolar extracellular K^+ concentrations, the membrane potential is extremely negative (−230 mV) (Kochian et al., 1989; Hirsch et al., 1998), thus facilitating proton pump–driven K^+ uptake through channels. When other high-affinity K^+ transporters are inactivated or blocked, K^+ channels allow high-affinity K^+ uptake (Hirsch et al., 1998). For example, addition of ammonium to the growth medium is necessary to retard growth of the *akt-1-1* mutant at micromolar K^+ concentrations. NH_4^+ inhibits Rb^+ uptake in Arabidopsis roots (Hirsch et al., 1998), and it also inhibits Rb^+ uptake by the HvHAK1 transporter (Santa-Maria et al., 1997) and by the AtKUP1, 2, and 3 transporters (E.J. Kim and J.I. Schroeder, unpublished data). Together, these data indicate that repression or NH_4^+ inhibition of some of the high-affinity K^+ uptake pathways results in conditions whereby AKT1 contributes to growth at micromolar K^+ concentrations (Hirsch et al., 1998). Further-

more, the finding that disruption of the *AKT1* gene reduces Arabidopsis growth at micromolar K^+ concentrations in the presence of NH_4^+ provides a new experimental system in which to further examine hypotheses regarding multiple affinities by an individual transporter in vivo.

Evidence for dual-affinity K^+ uptake by a single transporter was also found for a member of the HAK/KUP transporter family. Expression of the *AtKUP1* cDNA in Arabidopsis suspension cells (Kim et al., 1998) or in yeast cells (Fu and Luan, 1998) gives rise to simultaneous high- and low-affinity Rb^+ uptake fluxes.

On the other hand, kinetic studies of HKT1-induced Rb^+ and K^+ uptake in yeast show only a saturable high-affinity component for uptake (Rubio et al., 1995; Gassmann et al., 1996). However, for Na^+ ions, the HKT1 transporter has been shown to mediate high-affinity and low-affinity Na^+ uptake in yeast and *Xenopus* oocytes (Rubio et al., 1995; Gassmann et al., 1996). In this case, the high- and low-affinity Na^+ uptake components can be mechanistically separated: high-affinity Na^+ uptake occurs via Na^+–K^+ symport, whereas low-affinity Na^+ uptake is possibly more channel-like and does not require cotransport of K^+ (Rubio et al., 1995). Recent studies on K^+ channels, HAK/KUPs, and HKT1 point to an emerging theme in which single transporters may function physiologically over broad substrate concentration ranges depending on growth conditions.

The capacity of HKT1 to function as an Na^+ uptake pathway at millimolar Na^+ concentrations indicates possible roles for HKT1 in physiological Na^+ transport and Na^+ toxicity in root and leaf cells (Rubio et al., 1995). Note that Na^+ influx data in roots also show multiple components for low-affinity Na^+ uptake (Epstein, 1972). As for K^+ uptake, models assuming one major pathway for low-affinity Na^+ uptake are unlikely to survive in the wake of findings of multiple gene families mediating cation uptake.

Why Do Multiple Nutrient Uptake Transport Mechanisms Exist?

The physiological reasons for the existence of large gene families for the transport of plant nutrients have not yet been experimentally determined. However, several considerations provide plausible explanations. First, redundancy in the essential mechanisms for nutrient accumulation may be important for the survival of plants. Second, as discussed above, the large physiological variation in soil and apoplastic concentrations of nutrients and toxic competitor ions may call for an array of nutrient transport mechanisms to ensure condition-dependent nutrient uptake. Third, expression in different membranes (e.g., plasma membrane versus tonoplast) and tissues (e.g., root hairs versus phloem) is important and has not yet been analyzed in detail, particularly for K^+ transporters. Fourth, constitutive and inducible high-affinity uptake components can respond to changing nutrient availabilities and ionic conditions. For example, AKT1 expression levels were reported not to be affected by K^+ starvation in Arabidopsis roots (Lagarde et al., 1996). Therefore, AKT1 has been hypothesized to contribute to constitutive K^+ uptake in Arabidopsis (Lagarde et al., 1996). Studies in the past three years and earlier research by Kochian et al. (1989) show that the older models assuming one major pathway for high-affinity K^+ uptake are not adequate to analyze the underlying biological complexity of K^+ nutrition.

Unsolved Problems in Potassium Transport

Significant advances have been made in characterizing cDNAs and in analyzing the biophysical transport mechanisms of individual plant K^+ transporters. Recent findings nevertheless point to many unresolved central questions regarding integrated K^+ nutrient uptake in the plant. The inability thus far to resolve individual high-affinity K^+ uptake mechanisms in whole roots of terrestrial plants suggests that the transporter families discussed above, and perhaps others as yet unknown, may function in parallel. Loss-of-function mutations, such as *chl1* and *akt1-1*, can provide insight into the contributions of individual genes to nutrient uptake under different growth conditions. Important open questions, including tissue-specific expression, membrane targeting, post-translational regulatory mechanisms, and regulatory responses to differing environmental conditions, are currently being addressed. The ability to disrupt individual genes in plant species such as Arabidopsis and maize, together with interdisciplinary analyses, will provide approaches for determining the roles of individual K^+ transporters to many essential plant physiological processes.

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