Annu. Rev. Plant Physiol. Plant Mol. Biol. 1998. 49:669–96

MOLECULAR BIOLOGY OF CATION TRANSPORT IN PLANTS

Tama Christine Fox and Mary Lou Guerinot
6044 Gilman, Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755; e-mail: mary.lou.guerinot@dartmouth.edu

KEY WORDS: calcium, copper, iron, manganese, potassium, zinc

ABSTRACT

This review summarizes current knowledge about genes whose products function in the transport of various cationic macronutrients (K, Ca) and micronutrients (Cu, Fe, Mn, and Zn) in plants. Such genes have been identified on the basis of function, via complementation of yeast mutants, or on the basis of sequence similarity, via database analysis, degenerate PCR, or low stringency hybridization. Not surprisingly, many of these genes belong to previously described transporter families, including those encoding Shaker-type K^+ channels, P-type ATPases, and Nramp proteins. ZIP, a novel cation transporter family first identified in plants, also seems to be ubiquitous; members of this family are found in protozoa, yeast, nematodes, and humans. Emerging information on where in the plant each transporter functions and how each is controlled in response to nutrient availability may allow creation of food crops with enhanced mineral content as well as crops that bioaccumulate or exclude toxic metals.

CONTENTS

INTRODUCTION 670
POTASSIUM 671
High-Affinity K^+ Transport 671
Low-Affinity K^+ Transport 673
CALCIUM 678
P-type Ca-ATPases 678
Calcium/Proton Antiporters 681
COPPER 682

1040-2519/98/0601-0669$08.00

Uptake 682
Intracellular Transport 683
Efflux 684
MANGANESE 684
Uptake 684
Intracellular Transport 686
IRON 686
Strategy I 686
Strategy II 688
ZINC 689
Uptake 689
CONCLUSIONS 690

INTRODUCTION

Ions need to be transported from the soil solution into the root and then distributed throughout the plant, crossing both cellular and organellar membranes. Transport across the plant plasma membrane is driven by an electrochemical gradient of protons generated by plasma membrane H^+-ATPases (76, 106). These primary transporters pump protons out of the cell, thereby creating pH and electrical potential differences across the plasma membrane. Secondary transport systems then utilize these gradients for many functions, including nutrient uptake, phloem loading, and stomatal opening. Since the first reports on the cloning of H^+-ATPase genes from plants in 1989 (40, 88), a number of genes have been identified that encode various types of primary and secondary transporters (as reviewed in 3, 66). This review focuses on a group of plant cation transporters that has been identified either functionally or via sequence similarity. We highlight transporters for two macronutrient cations, K and Ca, and four micronutrient cations, Cu, Fe, Mn, and Zn. These are the mineral cations for which we currently have the most information at the molecular level.

When we think about cation transport, it is important to keep in mind that many cations, although essential, can also be toxic when present in excess (e.g. 39). Thus, plants may regulate uptake and efflux systems in order to control their intracellular cation concentrations. With cloned genes in hand, it now becomes possible not only to carry out structure-function studies on how various transporters move cations but also to investigate how transporters respond to changing cation levels. For most of the transporters we discuss, definitive proof of where they function within the plant has not yet been obtained. Nonetheless, what is becoming increasingly clear is that plant transporters belong to a number of well-described families, thus narrowing the perceived gap between the biology of animals and that of plants and allowing us to apply knowledge gained in one system directly to another.

Throughout this review, the term transporter is used inclusively to refer to both channels and carriers. Ion channels are distinguished from carriers by their several orders of magnitude higher flux rates and by their mediation of the passive flux of ions across membranes. The electrochemical gradient across the membrane in which the channel resides determines whether, and in which direction, ions move. Carriers may move ions either with or against their substrate concentration gradients and may function as uniporters or as cotransporters.

POTASSIUM

K^+ is the most abundant cation in higher plant cells. K^+ plays central roles in plant growth and development, including maintenance of turgor pressure, leaf and stomatal movement, and cell elongation (68, 69, 102, 111). Whereas K^+ levels in soil solution range from 1 μM to 10 mM, with many soils falling in the range of 0.3 to 5.0 mM, intracellular K^+ levels are maintained at 100–200 mM (56). Thus, uptake of K^+ from the soil must move K^+ against its concentration gradient. Recent articles have provided excellent overviews of K^+ transport in plants (10, 68, 69, 98, 102). We discuss genes identified to date that encode plant K^+ transporters. These transporters can be grouped into four categories: (*a*) high-affinity cotransporters, (*b*) inwardly rectifying K^+ channel α subunits, (*c*) K^+ channel β subunits, and (*d*) an outwardly rectifying K^+ channel (Table 1).

High-Affinity K^+ Transport

High-affinity K^+ transport occurs when external K^+ concentrations range from approximately 1–200 μM (56). The first high-affinity transporter, HKT1, was identified by complementation of a mutant yeast strain defective for K^+ uptake with a wheat (*Triticum aestivum*) root cDNA expression library. *HKT1* restores the ability of the yeast *trk1trk2* mutant to grow on low K^+-containing medium (96). In situ hybridization experiments show that *HKT1* mRNA localizes to the root cortex as well as to leaf and stem vascular tissue. *HKT1* confers high-affinity K^+ transport when expressed in yeast and in *Xenopus* oocytes (96).

Although originally thought to be a H^+-K^+ symporter, further investigations suggest that HKT1 may function as a K^+-Na^+ symporter (34, 92). Nontoxic (μM) Na^+ concentrations stimulate K^+ transport when *HKT1* is heterologously expressed in yeast or in *Xenopus* oocytes (92). Even more striking, HKT1 can mediate low-affinity Na^+ influx into oocytes. This Na^+ influx can be stimulated by external K^+. Furthermore, high (10–100 mM) external Na^+ concentrations inhibit K^+ influx into oocytes, probably via competition between Na^+ and K^+ for binding to the K^+ coupling site of HKT1 (92). The dual activities expressed

Table 1 Plant K^+ transporters and associated subunits

Gene	Species	Cloning approach	Direction of transport	Proposed function	Reference
High-Affinity K^+ Carriers					
HKT1	*T. aestivum*	Complementation of a yeast *trk1 trk2* mutant	Inward	K^+-Na^+(H^+)-symporter	96
KEA1	*A. thaliana*	PCR	Inward	Putative K^+-H^+ or Na^+-H^+ antiporter	115
Low-Affinity K^+ Channels					
LCT1	*T. aestivum*	Complementation of a yeast *trk1 trk2* mutant	Inward	K^+, Na^+ transport	95
KAT1	*A. thaliana*	Complementation of a yeast *trk1 trk2* mutant	Inward	Channel	97
KST1	*S. tuberosum*	DNA hybridization based on *KAT1* sequences	Inward	Channel	80
AKT1	*A. thaliana*	Complementation of a yeast *trk1 trk2* mutant	Inward	Channel	99
AKT 2/3	*A. thaliana*	DNA hybridization based on *KAT1* or *Shaker* sequences	Inward	Channel	16, 52
SKT2	*S. tuberosum*	Yeast two-hybrid screen using *KST1* as bait	Inward	Channel	28
SKT3	*S. tuberosum*	Yeast two-hybrid screen using *KST1* as bait	Inward	Channel	28
KCO1	*A. thaliana*	EST database searched for P-domain sequences	Outward	Channel	27
K^+ Channel β Subunits					
KAB1	*A. thaliana*	EST database searched for animal β subunits sequences		Modulates channels	109

by HKT1—beneficial high-affinity K^+-Na^+ uptake under non-Na^+ stress conditions and detrimental low-affinity Na^+ uptake under Na^+ stress conditions—point out the importance of dissecting the selectivity of transporters. Na^+ stress conditions are frequently found in irrigated soils, and HKT1 may provide one of the pathways for Na^+ uptake, making it a potential target for engineering Na^+ tolerance in plants. Indeed, genetic selection in yeast has allowed the isolation of mutations in HKT1 that confer reduced Na^+ uptake and improved salt tolerance relative to wild-type HKT1. The next step is to test such mutant forms in plants. It is crucial that the observations obtained via heterologous expression in yeast and oocytes be verified experimentally in plants. To date, physiological studies of high affinity K^+ uptake in intact roots have not supported the importance of Na^+-coupled K^+ transport in roots of terrestrial plants (69a).

Another gene, *LCT1*, was identified in the same screen that identified *HKT1* (95). LCT1 mediates low-affinity Na^+, Rb^+, and possibly Ca^{2+} transport in yeast. The physiological role of LCT1 in plants is uncertain, but the competitive inhibition of cation uptake by Ca^{2+} fits well with studies that have shown the importance of Ca^{2+} in reducing Na^+ uptake and ameliorating Na^+ toxicity. Thus, LCT1, like HKT1, may be a good target for lowering Na^+ influx in plants to increase salinity tolerance (95). *LCT1* is expressed at a low level in roots and leaves. LCT1 is predicted to have 6 or 7 transmembrane domains and a hydrophilic N-terminus with two PEST sequences. There are currently no related proteins in the databases.

Finally, a cDNA—*KEA1*—has been cloned from Arabidopsis that shares 22% identity with bacterial K^+-H^+ and Na^+-H^+ transporters and mediates K^+-dependent inward currents in oocytes (115). It is unknown whether KEA1 also mediates Na^+ transport.

Low-Affinity K^+ Transport

Low-affinity K^+ transport occurs at mM external K^+ concentrations and is nonsaturating at physiologically relevant K^+ concentrations. Low-affinity K^+ transport is carried out by voltage-gated channels that allow the passive flow of K^+ down its electrochemical gradient (10, 69, 102). All of the known plant K^+ inward channels show amino acid similarity to the animal Shaker K^+-channel family (58). Family members have six conserved transmembrane domains (called S1–S6). An amphipathic S4 domain is involved in voltage sensing, and a hydrophobic hairpin region between S4 and S5, called the H5 or P(pore) domain, is proposed to form the channel pore and contain ion binding sites (Figure 1). Shaker channels are composed of four α-subunits, with the P domain from each subunit lining the channel pore (13). Some Shaker channels have additional β subunits as well (discussed below). All the plant K^+ inward channels, and many animal Shaker channels, contain a cyclic nucleotide binding

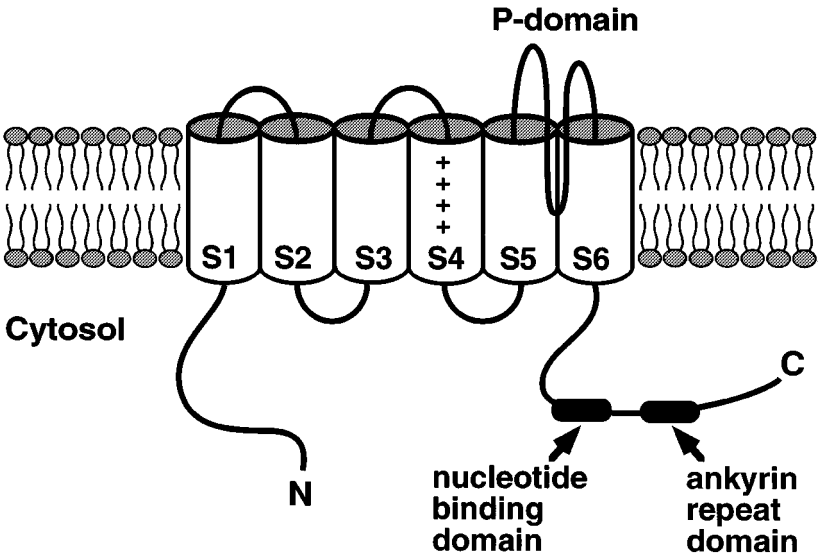

Figure 1 Plant inward-rectifying K channels all contain six transmembrane domains, a voltage sensing S4 domain, a nucleotide binding domain and a P domain. Some also have an ankyrin-repeat domain at the C terminus.

domain (1, 16, 52, 99) and some of the plant channels have an ankyrin repeat domain (16, 99).

Structural similarity does not predict direction of ion flux. Animal Shaker channels are all outward-rectifying. Outward-rectifying channels open upon depolarization of the membrane potential, thereby allowing K^+ efflux. The only plant outward-rectifying K^+ channel identified so far, *KCO1*, has four transmembrane domains and is more similar to human and yeast inward-rectifying channels than to the 6-transmembrane Shaker channels (27). In contrast, the plant 6-transmembrane Shaker channels are all inward-rectifying and thus activate K^+ influx upon membrane potential hyperpolarization. Animal inward-rectifying K^+ channels have only two transmembrane domains (11). Further details on specific plant K^+ channels are presented below.

KAT1/KST1 The Arabidopsis *KAT1* gene was identified by its ability to restore the growth of a yeast *trk1trk2* mutant strain on low K^+ media. KAT1 transports K^+ when expressed in *Xenopus* oocytes (97) and insect Sf9 cells (35, 72). KAT1-GUS constructs are expressed in guard cells and the vascular tissue of the stem and root of Arabidopsis (82). Thus, KAT1 is likely to have a role in stomatal opening and the transport of K^+ into vascular cells rather than the direct

uptake of soil K^+. Further evidence that KAT1 functions in vivo as predicted comes from studies on transgenic plants expressing a mutant form of KAT1 that is resistant to Cs^+ blockage (48). These plants showed inward K^+ currents that were less sensitive to blockage by Cs^+ than controls. In addition, the Cs^+ inhibition of light-induced stomatal opening observed in wild-type plants was not seen in the transgenic plants; stomata in these plants opened in the presence of external Cs^+. This study clearly provides molecular biological evidence that K^+ channels constitute an important pathway for physiological K^+ uptake during stomatal opening.

The tripeptide $G^{262}Y^{263}G^{264}$ is conserved in the P domain of KAT1 and in animal inward-rectifying K^+ channels. This sequence may create selectivity by occupying the narrowest region of an hour glass–shaped pore (58). Normally, ion permeability through KAT1 in oocytes is in the order $K^+ > NH4^+ > Rb^+ \gg Na^+ \sim Li^+ \sim Cs^+$ (97). Altering the GYG tripeptide region or a T proposed to interact with GYG changed selectivity and/or converted acid activation to inactivation (81, 112). In addition, mutations were found that increased the sensitivity of yeast to $NH4^+$ or Na^+. KAT1 has an additional 14 amino acids in the P domain as compared to Drosophila Shaker channels. Mutation analyses suggest that the additional KAT1 amino acids are involved in pore selectivity and inhibition by Cs^+ and Ca^{2+} (6). Both plant inward- and animal outward-rectifying K^+ channels appear to have the same orientation in membranes, with the P domain on the extracellular side (Figure 1) (49). Evidence for conserved membrane orientation includes (*a*) chimeric channels containing portions of KAT1 and the *Xenopus* K^+ channel, Xsha2, are capable of transporting K^+ into *Xenopus* oocytes, suggesting that the two channels have the same orientation in the membrane (15), and (*b*) the KAT1 P domain mutants (H^{267} to T, and E^{269} to V) are less sensitive to Cs^+ and TEA blockage (without changing selectivity), suggesting that the P domain is accessible to the external side of the membrane as in animal Shaker channels (49). If inward- and outward-rectifying channels have the same orientation, then what determines whether a channel is inward or outward rectifying? The S4 domain appears to be involved; altering three amino acids in the S4 domain converts the Shaker B channel from outward to inward rectification (77).

A gene encoding a guard cell K^+ channel, *KST1*, was also cloned from a *Solanum tuberosum* cDNA library based on its similarity to *KAT1* (80). While both *KAT1* and *KST1* mRNA are both predominantly expressed in guard cells, *KST1* mRNA is present at low levels in flowers, whereas *KAT1* mRNA is present in the vascular tissue of stems and roots (82).

The yeast two-hybrid system was used to screen for proteins that interact with KST1 (28). Because transmembrane domains cannot be used efficiently in the two-hybrid system, C-terminal regions of KST1 predicted to be cytosolic were

used as bait. However, instead of identifying genes encoding proteins that might be involved in channel regulation or localization, two putative K^+ channel genes, *SKT2* and *SKT3*, were isolated. SKT2 and SKT3 are 88% similar to each other and both show similarity to Arabidopsis AKT2 (81% amino acid similarity for SKT2 and 77% similarity for SKT3; see next section for discussion of AKT2). A comparison of KST1, SKT2, and SKT3 with other K^+ channels revealed two conserved regions at the C-terminus that are found exclusively in all of the plant inward-rectifying K^+ channels. These regions, termed K_{HA} domains, are enriched for hydrophobic and acidic amino acid residues (28). Using insect cells expressing a GFP-KST1 fusion, clusters of K^+ channels could be visualized within the plasma membrane. Deletion of the K_{HA} domain resulted in an even distribution of the GFP-KST1ΔK_{HA} fluorescence pattern, suggesting that the K_{HA} domain is required for the clustering of KST1. KST1 lacking the K_{HA} domain is electrophysiologically active in insect cells, indicating that the K_{HA} domain is not required for assembly into α-subunit tetramers (28). All the potato inward-rectifying K^+ channels interact with each other indiscriminately in vitro, suggesting that the tissue-specific expression of these transporters likely influences which proteins interact to form channels.

AKT1 The *Arabidopsis AKT1* gene was cloned by complementing the yeast *trk1trk2* mutant (99). *AKT1* transports K^+ into transfected insect cells but not into *Xenopus* oocytes (35). Northern blot analyses indicate that *AKT1* is expressed mainly in roots (5, 59). Transgenic Arabidopsis expressing an *AKT1* promoter-GUS fusion exhibit GUS activity in the peripheral cell layers of mature root regions as well as in leaf primordia and hydathodes (59). *AKT1* expression in roots is consistent with a role in K^+ influx from the soil solution. This was confirmed using a T-DNA insertional mutant of *AKT1* called *akt1-1* (44). In *akt1-1* root cells, no inward K^+ currents could be detected. These *akt1-1* plants grew poorly on low K^+ medium and exhibited reduced high- and low-affinity Rb^+ uptake.

AKT2, which is approximately 60% identical to *AKT1* and *KAT1*, was identified using a *KAT1* PCR product to probe an Arabidopsis cDNA library (16). The same gene was also identified by using a degenerate oligonucleotide made from the conserved P domain of Shaker K^+ channels to probe an Arabidopsis genomic library (52). Although *AKT2* did not complement the yeast *trk1trk2* mutant (16), *AKT2* can function as an inward-rectifying K^+ channel in oocytes (52). *AKT2* was found by Northern blot analysis to be highly expressed in leaves (16, 52). This contrasts with the root expression seen for *AKT1* (5, 59). Both *AKT1* and *AKT2* genes contain similar C-terminal ankyrin-binding sequences. Ankyrin-binding sites facilitate the binding of proteins to the cytoskeleton, either to localize proteins to specific locations on the PM, or to promote protein-protein

interactions (reviewed in 52). This cytoskeletal interaction is different from that proposed for KAT1, which has a C-terminal microtubule-binding site rather than ankyrin-binding sites. Colchicine, which destabilizes microtubules, decreases the current amplitude in oocytes expressing KAT1 but has not been tested on AKT1 or AKT2.

KCO1 The first plant outward-rectifying K^+ channel, KCO1 (for K^+ channel, Ca^{2+} activated, *outward-rectifying*), was found by searching the Arabidopsis EST database for sequences containing the conserved P domain of Shaker channels (27). KCO1 belongs to a new class of K^+ channels, recently described in yeast and in humans, that contain not one but two P domains and have four transmembrane segments. Sequences with similarity to KCO1 have also been identified in other plant species (79). When expressed in insect cells, KCO1 directs outward-rectifying K^+ currents that are strongly dependent on the presence of nanomolar concentrations of cytosolic free Ca^{2+}. Indeed, two EF-hand motifs (Ca^{2+}-binding sites) are present at the C-terminal end of KCO1. KCO1 is activated by depolarization of the plasma membrane. This depolarization is affected by external K^+ concentration. *KCO1* expression can be detected in seedlings, as well as in the leaves, and flowers of older plants, albeit at a low level, requiring the use of RT-PCR for detection. This level of localization offers few clues about KCO1 function. However, physiological studies have identified a number of outwardly rectifying K^+ channels and it is hoped that we will soon be in a position to correlate KCO1 expression with one of these known K^+ channel activities.

KAB1 An Arabidopsis EST was found to encode a protein with 49% identity to rat and bovine K^+ channel β subunits (74, 109). Co-expression of certain β subunits with Shaker α subunits can convert these channels from noninactivating to fast-inactivating. The rat β subunit, while hydrophilic, co-localizes with native α subunits isolated from brain membranes, suggesting that α and β subunits may interact (reviewed in 58). β subunits have been proposed to modulate K^+ channels by (*a*) blocking the pore sterically, causing channels to be fast inactivating (58); (*b*) reducing α subunits, based on the similarity of β subunits to NAD(P)H oxidoreductases (22, 74); and/or (*c*) modulating the maturation and direction of α/β complexes to membranes (100).

KAB1 RNA is highly expressed in Arabidopsis leaf (especially guard) cells and to a lesser extent in root cells, as shown by in situ hybridization (109). Western blot analyses demonstrated the presence of KAB1 in both soluble and membrane protein fractions of leaves, flowers, and roots (110). Immunocytochemical analyses show that KAB1 is present in the plasma membrane, tonoplast, chloroplast inner envelope, and mitochondrial inner membrane. The staining

pattern suggests that the native polypeptide is present in regularly spaced individual protein complexes (H Tang, AC Vasconcelos & GA Berkowitz, submitted manuscript). KAB1 physically associates with KAT1 α subunits; in vitro translated ^{35}S-KAT1 binds specifically to an affinity column made from immobilized affinity-purified KAB1 and polyclonal antibodies directed against KAB1 specifically immunoprecipitate both KAB1 and ^{35}S-KAT1. The widespread expression of KAB1 mRNA in various tissues suggests that KAB1 may interact with multiple K^+ channels rather than exclusively with shoot-localized KAT1 (110).

CALCIUM

Ca is an abundant element in soils and is usually present in sufficient amounts to meet plant needs. Ca constitutes 0.1–2.0% dry weight of plants and structurally stabilizes membranes and cell walls (71). Ca is also an important signaling molecule: stimulation by red light, gravity, touch, cold shock, fungal elicitors, hormones, or salt stress induces the opening of gated Ca^{2+} channels (reviewed in 14). Ca^{2+} is transiently transported from organelles and the apoplasm into the cytosol, thereby activating signal transduction pathways. In resting plant cells, Ca^{2+} is actively exported by high-affinity Ca^{2+}-ATPases and Ca^{2+}/H^+ antiporters out of the cell or into organelles to maintain cytosolic $Ca^{2+} \leq 0.1\ \mu$M. This prevents the activation of signal transduction pathways until a stimulus is sensed (reviewed in 14).

P-type Ca-ATPases

High-affinity Ca^{2+}-ATPases localize to the plant plasma membrane (2, 31) and endomembranes (J Harper, personal communication; 91), including the ER (63, 65), the Golgi Apparatus (65), the tonoplast (2, 20, 30, 31, 70), and the plastid envelope (47). These transporters remove cytosolic Ca^{2+} into organelles or the apoplasm. All the plant Ca^{2+}-ATPases cloned are P-type ATPases that are characterized by a phosphorylated intermediate, inhibition by vanadate, a requirement for Mg^{2+}, and a high substrate specificity for MgATP (47). Alignment of eukaryotic Ca^{2+}-ATPases shows that the plant and animal genes are related (70) and can be divided into "PM-type" pumps that are calmodulin-stimulated and "ER-type" pumps that are not (78).

CALMODULIN-STIMULATED CA^{2+}-ATPASES At present, three genes encoding calmodulin-stimulated Ca^{2+}-ATPases have been cloned from plants (Table 2). Although similar to animal PM-type pumps, none localizes to the PM. *PEA1* [(47), later called *ACA1* for Arabidopsis Ca^{2+}-ATPase; (70)], was cloned from Arabidopsis using antibodies generated against spinach chloroplast envelope proteins (47). ACA1 is 40–44% identical to mammalian PM-type Ca^{2+}-ATPases

Table 2 Ca^{2+} transporters in plants

Gene	Species	Cloning approach	Location	Reference
Calmodulin-Stimulated Ca^{2+}-ATPases				
PEA1/ACA1	*A. thaliana*	Antibody to chloroplast protein	Chloroplast inner envelope	47
ACA2	*A. thaliana*	PCR using degenerate primers	Endomembranes	40a
BCA1	*B. oleracea*	Calmodulin-binding protein micro-sequencing	Tonoplast	70
Calmodulin-Insensitive Ca^{2+}-ATPases				
LCA1	*L. esculentum*	PCR using degenerate primers	Tonoplast and plasma membrane	113
pH27	*N. tabacum*	PCR using degenerate primers	Endomembrane	90
DCBA1	*D. bioculata*	PCR using degenerate primers	Endomembrane	91
ACA3/ECA1	*A. thaliana*	PCR using degenerate primers (*ACA3*) and DNA hybridization using pH27 as a DNA probe (*ECA1*)	Endoplasmic reticulum	63
OsCa-atpase	*O. sativum*	Differential display +/− gibberellin	Endomembrane	21
Ca^{2+}/H^{+}-Antiporters				
CAX1	*A. thaliana*	Complementation of a yeast *vcx1pmc1* mutant	Tonoplast	45
CAX2	*A. thaliana*	Complementation of a yeast *vcx1pmc1* mutant	Tonoplast	45

but lacks the C-terminal calmodulin-binding domain. ACA1 has been proposed to export Ca^{2+} from the plastids into the cytosol because (*a*) ATP-dependent Ca^{2+} import could not be shown in spinach leaf chloroplasts (albeit *ACA1* expression is lower in leaves than in roots); (*b*) the evolutionary relationship of plastids to cyanobacteria, which export Ca^{2+} out of the cytoplasmic membrane; and (*c*) the low activity of Ca^{2+} in the stroma as compared with total plastid Ca^{2+} concentration (47).

A vacuolar calmodulin-affinity purified Ca^{2+}-ATPase from *Brassica oleracea* was microsequenced, and the gene, *BCA1*, was cloned (70). BCA1

shares 62% identity with ACA1 and >80% identity with proteins specified by three Arabidopsis EST sequences. BCA1 contains a potential calmodulin-binding, amphipathic helix at the N-terminus (instead of at the C-terminus as in animal PM-type ATPases). Immunoblotting confirmed that BCA1 is in the tonoplast, and isolated vacuoles were shown to have calmodulin-stimulated, ATP-dependent Ca^{2+} influx (2).

An Arabidopsis cDNA, *ACA2*, was identified using primers designed against sequences conserved among P-type ATPases (40a). ACA2 is 78% and 62% identical to ACA1 and BCA1, respectively, at the amino acid level. Biochemical assays confirm that ACA2 encodes a calmodulin-regulated Ca^{2+}-ATPase and provide evidence that the N-terminal domain of ACA2 functions as a calmodulin-regulated autoinhibitor. Removal of this autoinhibitory domain results in constitutively high ACA2 activity. Only the truncated pump allows a yeast mutant defective in calcium homeostasis (lacking both tonoplast and Golgi apparatus Ca^{2+}-ATPases as well as calcineurin) to grow on Ca^{2+}-depleted medium. Anti-ACA2 antibodies recognize a protein that is most abundant in root and flower endomembranes.

CALMODULIN-INSENSITIVE CA^{2+}-ATPASES *LCA1*, a *Lycopersicon esculentum* Ca^{2+}-ATPase, was identified by using a degenerate sequence of the conserved ATP-binding domain of P-type ATPases to probe a root cDNA library (113). LCA1 shares over 60% amino acid identity with regions of animal ER-type Ca^{2+}-ATPases. Northern blots of tomato identified three *LCA1* transcripts in roots and one in leaves. *LCA1* expression was induced by 50 mM NaCl in both leaves and roots (113), consistent with a model in which salt stress perturbs intracellular Ca^{2+} levels (62). A similar Ca^{2+}-ATPase (encoded by a cDNA called pH27) was identified in *Nicotiana tabacum* (90). Expression of the *N. tabacum* gene was highest in cultured cells and stems, intermediate in roots, and lowest in leaves. High NaCl induces expression of this Ca^{2+}-ATPase in cultured cells. Both LCA1 and the protein encoded by pH27 were initially proposed to localize to the ER (90, 113). However, antibodies raised against LCA1 (31) reacted instead with tonoplast and PM vesicle proteins. ATP-dependent $^{45}Ca^{2+}$ transport occurred only in those vesicles containing LCA1 and was inhibited by the anti-LCA1 antibodies (31).

A Ca^{2+}-ATPase, DCBA1, with 54% identity to mammalian ER-type Ca^{2+}-ATPases was identified from the highly salt-tolerant alga *Dunaliella bioculata* using a conserved region of the *Dunaliella* P-type H^+-ATPase gene, *DBPMA1*, to probe a cDNA library (91). *DCBA1* is expressed at very low levels as shown by Northern blots.

An Arabidopsis Ca^{2+}-ATPase gene, *ACA3/ECA1* (for ER-type Ca^{2+}-ATPase), was cloned using the same screen that identified *ACA2* (Y Wang, B Hong &

JF Harper, submitted manuscript) and by probing a cDNA library with *pH27* (63). ECA1 is 69% identical to OsCa-atpase (see below), 64% identical to LCA1 and 42% identical to a rabbit ER-type Ca^{2+}-ATPase. Western blots showed highest expression of ECA1 in roots and flowers versus shoots and siliques. ECA1 is located mainly in the ER (63). ECA1 restores growth to both a *pmr1* yeast mutant (defective in a Golgi Apparatus Ca^{2+}-pump), and a *pmr1pmc1cnb1* mutant (lacking also the vacuolar Ca^{2+}-pump and calcineurin) on low Ca^{2+}-containing medium (63). ECA1 may also transport Mn^{2+} (discussed in the Mn section; 63).

A differential display approach was used to compare gene expression in rice aleurone cells treated $\pm$ gibberellin for 1 h (21). A gibberellin-inducible Ca^{2+}-ATPase, OsCa-atpase, was identified that, when transiently expressed in aleurone cells, bypasses the gibberellin requirement for stimulating α-amylase. Thus, OsCa-atpase may be an early downstream component of the gibberellin signal transduction pathway.

Calcium/Proton Antiporters

Previously, high-affinity vacuolar Ca^{2+}-ATPases were thought to lower Ca^{2+} activities in the cytosol of resting cells, whereas Ca^{2+}/H^+-antiporters were thought to pump Ca^{2+} from the cytosol only when cytosolic Ca^{2+} levels were elevated (30). However, genes encoding two high- and low-affinity Ca^{2+}-H^+-antiporters, *CAX1* and *CAX2*, have now been cloned from Arabidopsis (45) via their ability to suppress the Ca^{2+} hypersensitivity of a *Saccharomyces cerevisiae vcx1pmc1* double mutant that lacks both the vacuolar P-type Ca^{2+}-ATPase and the Ca^{2+}/H^+-antiporter (25, 26; Table 2). CAX1 and CAX2 share a central acidic motif, and 11 proposed membrane-spanning domains with Ca^{2+}/H^+-antiporters identified in *S. cerevisiae* and *E. coli*. CAX1 and CAX2 catalyze ΔpH-dependent Ca^{2+} transport. CAX1-mediated Ca^{2+} transport is concentration dependent and exhibits Michaelis-Menton kinetics with a K_m of 13 μM. Thus CAX1, along with Ca^{2+}-ATPases, is proposed to keep cytosolic Ca^{2+} activities below 1 μM in resting plant cells (45). However, Cd, Hg, and La strongly inhibit ^{45}Ca uptake, suggesting that CAX1 may transport other cations (121). In support of this, CAX1 mediates Na^+-H^+ antiport as well as Cd transport (121). CAX2-mediated Ca^{2+} transport was also concentration dependent but exhibits a lower affinity ($Km > 100$ μM) for Ca^{2+} (45). The high K_m suggests that CAX2 does not normally function in Ca^{2+} transport. Rather, preliminary experiments suggest that CAX2 may function as a high-affinity H^+/heavy metal cation antiporter with a role in metal homeostasis rather than Ca^{2+} signaling (K Hirschi, personal communication). *CAX1* and *CAX2* RNA are both expressed in Arabidopsis roots, leaves, stems, flowers and siliques.

COPPER

Uptake

Cu is an essential redox component required for a wide variety of processes, including the electron transfer reactions of respiration (cytochrome *c* oxidase, alternate oxidase) and photosynthesis (plastocyanin), the detoxification of superoxide radicals (Cu-Zn superoxide dismutase) and lignification of plant cell walls (laccase). Cu levels in soils range from 10^{-4} to 10^{-9} M, with up to 98% of the Cu in soil solution complexed to low molecular weight organic compounds (71). Plants require from 5 to 20 μg gDW^{-1} Cu, depending on the species. Whether Cu needs to be reduced before transport, as has been shown to be the case for yeast (41), is still an open question (see section below on Fe).

A putative Cu transporter from Arabidopsis, encoded by the *COPT1* gene (Table 3), can suppress the growth defects of a yeast *ctr1-3* strain that lacks high-affinity Cu uptake (51). *COPT1* is expressed in flowers, stems, and leaves but is undetectable in roots. The lack of root expression may indicate that COPT1 is not responsible for Cu uptake from soil; however, it is not known whether Cu deficiency causes an increase in the expression of *COPT1* in roots (or in any other tissue). Although originally reported to be present as a single copy in the genome, several ESTs in the database encode proteins with similarity to COPT1, suggesting that *COPT1* may be part of a small gene family in Arabidopsis.

COPT1 is similar to Ctr1p (49% similarity as predicted by the GAP program) and to a newly described human copper transporter, hCTR1 (56% similarity as predicted by the GAP program), that was also identified by its ability to rescue a yeast *ctr1* mutant (122). COPT1 (169 aa) and hCTR1 (190 aa) are significantly smaller than Ctr1p (406 aa), mainly due to truncations at the N- and C-termini. However, COPT1, like Ctr1p and hCTR1, has three potential transmembrane domains, and all three proteins contain an N-terminal putative metal-binding domain rich in methionine and serine residues. This metal-binding domain is predicted to lie on the extracellular surface and is similar to those found in several bacterial copper-binding proteins, including the *Enterococcus hirae* copper ATPase (CopB) and the CopA and CopB proteins from *Pseudomonas syringae* (101). Two other genes in yeast (*CTR2* and *CTR3*; see 51, 53) and one other gene in humans (h*CTR2*; see 122) are similar to *COPT1*. Ctr2p and hCTR2 were originally identified as being similar to COPT1 and to hCTR1, respectively, in database searches (51). Neither *CTR2* nor h*CTR2* can rescue a *ctr1-3* mutant, and neither has a recognizable metal-binding motif (51, 122). However, *CTR2* overexpression confers sensitivity to Cu, and *ctr2* mutants are more resistant to Cu, suggesting that Ctr2p functions as a low-affinity Cu transporter. Ctr3p is a small, integral membrane protein identified by its ability to restore high-affinity Cu uptake to a *ctr1-3*–deficient yeast mutant (53). Mutations in

Table 3 Genes involved in micronutrient transport in plants

Gene	Species	Cloning approach	Proposed function	Reference
COPT1	*A. thaliana*	Complementation of a yeast *ctr1* mutant	Cu transport	51
PAA1	*A. thaliana*	PCR using degenerate primers	Cu efflux from cytosol	107
AMA1	*A. thaliana*	PCR using degenerate primers	Cu efflux from cytosol	J Harper, personal communication
OsNramp1	*O. sativa*	BLAST search for Nramp ESTs and DNA probing of a cDNA library	Mn transport, roots	8
OsNramp2	*O. sativa*	BLAST search for Nramp ESTs and DNA probing of a cDNA library	Mn transport, leaves	8
OsNramp3	*O. sativa*	BLAST search for Nramp ESTs and DNA probing of a cDNA library	Mn transport, roots and leaves	8
frohA, frohB, frohC, frohD	*A. thaliana*	PCR using primers based on yeast ferric reductases	*b*-type cytochrome; Fe(III)-chelate reductase	91a
IRT1	*A. thaliana*	Complementation of a yeast *fet3fet4* mutant	Fe(II) transport Mn(II) transport	29; H Pakrasi, personal communication
ZIP1, ZIP2, ZIP3	*A. thaliana*	Complementation of a yeast *zrt1zrt2* mutant	Zn transport	ML Guerinot, unpublished data
NA synthase	*H. vulgare*	Protein purification; cDNA has not been identified	Synthesis of phytosiderophores	42
NAAT	*H. vulgare*	PCR with degenerate primers based on microsequencing data	Synthesis of phytosiderophores	108
IDS2, IDS3	*H. vulgare*	Subtractive hybridization of +/− Fe roots	Synthesis of phytosiderophores	83, 85

both Ctr1p and Ctr3p are required to completely eliminate high-affinity copper uptake. Interestingly, in many laboratory strains, *CTR3* is interrupted by a transposable element (53). The exact role that Ctr3p plays in Cu transport is not entirely clear. Ctr3p is thought to function in an endocytic copper transport pathway, based on its localization pattern (53).

Intracellular Transport

It is likely that multiple Cu-trafficking pathways come into play after transport of Cu across the plasma membrane. Cu must be delivered to the mitochondria, to a compartment needed for activation of cytosolic Cu proteins and to the secretory pathway. In *S. cerevisiae*, *ATX1* encodes a 73 amino acid polypeptide that is believed to act in the intracellular transport of Cu to the secretory system (64). Atx1p contains the highly conserved metal-binding motif MTCXXC

that functions as the mercury binding site in the *Escherichia coli* MerP and MerA proteins and as the putative copper-binding site for the Cu-ATPases from *E. hirae* (CopA), *S. cerevisiae* (Ccc2p), and humans (the Wilson and Menkes proteins). Two plant ESTs, from Arabidopsis and rice, encode peptides having greater than 60% identity to Atx1p; each contains a presumptive metal-binding motif, MXCXXC. Although the role of these Atx1p-like proteins in metal homeostasis in plants has not yet been established, their similarity to Atx1p suggests a similar function and makes these genes interesting targets for further investigation.

Efflux

P-type ATPases belong to a large superfamily of ATP-driven pumps involved in the transmembrane transport of a variety of cations across cell membranes. A recent analysis of 159 P-type ATPases demonstrates that these proteins can be organized in a phylogenetic tree with five major branches according to substrate specificity and not according to the evolutionary relationship of parent species. This indicates that abrupt changes in the rate of sequence evolution are accompanied by the acquisition of new substrate specificities (4). Analysis of the Arabidopsis EST database reveals that there are at least 18 different P-type ATPases represented.

Using degenerate oligonucleotides based on residues conserved among metal-transporting P-type ATPases, an Arabidopsis cDNA has been identified encoding a protein, PAA1, that is 42.6% identical to the PacS Cu-ATPase from *Synechococcus* and 37.9% identical to the Wilson Cu-ATPase (107). PAA1 contains all four motifs commonly found in P-type ATPases, a phosphatase region (TGES), an ion transduction region (xPC), a phosphorylation site (DKTGT), and an ATP binding domain (GDGxNDxP). It also contains the highly conserved MTCXXC metal-binding motif in its N terminal domain. *PAA1* transcripts are not very abundant in either roots or shoots and are not copper inducible. Another P-type ATPase, AMA1, has also been identified from Arabidopsis using degenerate PCR. There is also an Arabidopsis gene, recently sequenced as part of the genome effort, that has up to 80% similarity to Ccc2p, Menkes protein, and Wilson's protein (ML Guerinot, unpublished data). All three of these proteins probably function in the export of Cu from the cytosol into an extracytosolic compartment (118).

MANGANESE

Uptake

Mn is required for a number of essential processes in plants, including oxygen evolution in photosynthesis (water-splitting enzyme S in the Hill reaction),

detoxification of oxygen-free radicals (Mn-superoxide dismutase), and CO2 fixation in C4 and CAM plants (PEP carboxylase). Mn is taken into plants mainly as the free Mn^{2+} ion. Concentrations of Mn can vary greatly in the soil, ranging from less than 0.1 μM in well-aerated alkaline soils to greater than 400 μM in submerged soils (54). The Mn concentration required by plants also spans a wide range, from 0.01 to 50 μM. Although there are a large number of reports linking Fe and Mn nutrition, almost no work has been done at the molecular level on the transport of Mn in plants. A number of proteins similar to Nramp may function as Mn transporters (see below). In addition, *IRT1*, identified originally by its ability to complement a yeast mutant with a defect in Fe uptake (see section on Fe), has been shown to rescue a *smf1* mutant of yeast that has a defect in high-affinity Mn uptake (H Pakrasi, personal communication). There is accumulating evidence from both yeast and Arabidopsis that Ca and Mn may be substrates for the same intracellular transporter (60, 63).

Members of the Nramp (*N*atural *r*esistance *a*ssociated *m*acrophage *p*rotein) family have now been implicated in Mn transport in *S. cerevisiae* (104) and in Fe transport in mammals (32, 38). Two ESTs from *O. sativa* and one from Arabidopsis show strong similarity (60% as determined by a BLAST search) to mammalian Nramp (7). Using the ESTs as hybridization probes, three different cDNAs have been isolated from rice (8). Northern blots indicate that *OsNramp1* is expressed primarily in roots, whereas *OsNramp2* is primarily expressed in leaves. *OsNramp3* is expressed in both tissues. As yet, there is no functional proof of what substrate(s) the plant Nramp proteins are transporting. We would also like to know whether any of the plant Nramp proteins respond to a deficiency of any particular metal. For example, dietary Fe deficiency upregulates the expression of *DCT1*, the rat isoform of human Nramp2, possibly via an iron responsive element found in the 3′ UTR of *DCT1* (38). DCT1 has an unusually broad substrate range that includes Fe, Mn, Co, Cd, Cu, Ni, and Pb. It is not known whether other dietary deficiencies can also upregulate the expression of this transporter.

The Nramp family has been highly conserved throughout evolution with representatives found in yeast, birds, Drosophila, *Caenorhabditis elegans* and bacteria in addition to those found in mammals and plants (17, 18). Nramp proteins have 10 conserved transmembrane domains, a glycosylated loop (between TM6 and TM7), and a sequence signature (TMT[X]4G[D/Q[X]4GF in the TM8 to TM9 interval) that shares similarity to the permeation pore of the K^+ channel family (17). Originally, Nramp1 was identified in mice because a mutation in this gene leads to susceptibility to intracellular pathogens such as *Mycobacterium*, *Salmonella*, and *Listeria*. If all members of the Nramp family function as metal transporters, this could nicely explain the original observations on Nramp1 (for models, see 38, 105). Briefly, Nramp is proposed

to transport certain divalent cations, possibly Mn and/or Fe, from the extracellular milieu into the cytoplasm of a macrophage, and after the generation of a phagosome removes these divalent cations from the organelle, thus depriving the invading microorganism of divalent cations needed for production of defense enzymes such as superoxide dismutase (Mn) and catalase (Fe). It is also possible that Fe^{2+} uptake by macrophages may allow production of toxic hydroxyl radicals via the Fenton reaction, killing pathogens in the phagosome as part of the defense mechanism.

Intracellular Transport

ECA1 encodes a Ca^{2+}-ATPase in Arabidopsis that localizes mainly to the ER (63). In addition to being able to rescue the Ca pumping defect of a *pmr1* mutant of *S. cerevisiae*, ECA1 could also restore the growth of the *pmr1* mutant in Mn-containing medium, suggesting that this Ca pump may also catalyze Mn transport. Mn has been shown to stimulate the formation of a phosphorylated intermediate of ECA1 in microsomes prepared from yeast expressing ECA1. Mn is likely to be sequestered in endomembrane compartments; ECA1 may be responsible for transport of both Ca and Mn into the lumen of the ER or the Golgi. Indeed, Pmr1p has been implicated in supplying both Mn and Ca to the Golgi in yeast (60). An Arabidopsis line with a T-DNA insertion in *ACA3* (identical to *ECA1*) can now be used to examine the role of this pump in plants (J Harper, personal communication). For example, one would predict that such a line would be more sensitive than wild type to high levels of Mn.

IRON

Fe is found in the soil mainly as insoluble oxyhydroxide polymers of the general composition FeOOH. In an aerobic, aqueous environment at neutral pH, free Fe^{3+} is limited to an equilibrium concentration of approximately 10^{-17} M, a value far below that required for the optimal growth of plants (10^{-6} M). Thus, before plants can take up Fe for transport into the root, they must somehow solubilize these Fe(III) oxides. A recent summary of plant mutants affected in iron uptake (12) will provide additional information to that covered here on components of iron uptake systems now identified at the molecular level.

Strategy I

All plants except the grasses use a strategy (termed Strategy I) to acquire Fe that is similar to the system used by *S. cerevisiae* (37). The initial reduction of Fe(III), carried out by a plasma membrane–bound Fe(III) chelate reductase, is followed by transport of Fe(II) across the root epidermal cell membrane. Both the Fe(III) chelate reductase (117) and the Fe(II) transport activities

(33) are enhanced under Fe deficiency. Several candidate genes (*frohA*, *frohB*, *frohC*, and *frohD*) that may encode Fe(III) reductases have been identified in *Arabidopsis* using degenerate PCR with primers designed against motifs common to the yeast Fe(III) reductase proteins, Fre1p, Fre2p, and Frp1 (91a, 93). Each of these *froh* genes encodes a *b*-type cytochrome belonging to a larger family whose other members include the respiratory burst oxidase of mammalian neutrophils (gp91-phox). Arabidopsis mutants—*frd1* and *frd3*—that exhibit defects in Fe(III) reduction have also been identified (116, 117). We are now testing to see whether the *frohA* or *frohB* genes can complement *frd1* mutants, because these two genes map to the same location as *FRD1* (Q Groom, C Procter, E Connolly, ML Guerinot & N Robinson, unpublished data). Whereas there is good evidence that Fe is required in its reduced form for uptake (19, 117), it is still not clear whether Cu needs to be reduced before transport and whether Fe(III) chelate reductases also reduce Cu(II) chelates. Because Arabidopsis has a Cu transporter (COPT1) similar to the yeast Cu transporter Ctr1p that uses Cu(I) as a substrate, it may indeed turn out that plants also reduce Cu before transport. Cu deficiency has been shown to induce Fe(III) chelate reductase activity in pea plants; deficiencies of other cations (K, Mg, Ca, Mn, and Zn) do not elicit a similar response (23). Furthermore, *frd1* mutants that do not show induction of Fe(III) chelate reductase activity under iron-deficient growth conditions have also lost the ability to reduce Cu(II) chelates (117).

Proton release is also enhanced under Fe deficiency, which lowers rhizosphere pH and thereby increases the solubility of Fe(III). There is a large family of H^+-ATPase genes in Arabidopsis. One of these, *AHA2*, is upregulated in response to Fe deficiency (BA Parry & ML Guerinot, unpublished data).

The Arabidopsis *IRT1* gene (for *I*ron *R*egulated *T*ransporter) was isolated because its expression in yeast could restore iron-limited growth to a yeast *fet3fet4* mutant defective in iron uptake (29). Consistent with its proposed role as a metal ion transporter, yeast expressing *IRT1* possess a novel iron uptake system that is specific for Fe(II) over Fe(III). Moreover, IRT1 is specific for iron over other potential substrates; Fe(II) uptake was not greatly inhibited by high concentrations of other physiologically relevant metal ions such as Cu(I), Cu(II), Mn(II), and Zn(II). Most interestingly, Cd has been shown to inhibit iron uptake by IRT1. This suggests that Cd may serve as a substrate for this transporter. This will need to be tested directly using radiolabeled Cd. In Arabidopsis, *IRT1* is expressed in roots and is induced by iron-deficient growth conditions. Furthermore, its expression is altered in mutant strains with defects in regulation of the root Fe(III)-chelate reductase. Based on these results, we proposed that IRT1 is an Fe(II) transporter that takes up iron from the soil.

The significance of IRT1 in the field of metal uptake research is twofold. *IRT1* is the first Fe transporter gene to be isolated from plants, and it provides a

useful handle on the mechanism and regulation of Fe uptake in plants. Second, IRT1 has led to the discovery of a family of transporters involved in metal ion uptake. We have named this group the "ZIP" gene family (for ZRT/IRT-related proteins) for the first three members to be isolated and characterized, ZRT1, ZRT2, and IRT1. *ZRT1* and *ZRT2* encode Zn transporters in *S. cerevisiae* (119, 120). *ZIP* family genes are found in a diverse array of eukaryotic organisms (eight genes in Arabidopsis, one in rice, one gene in trypanosomes, two genes in *S. cerevisiae*, four genes in nematodes, and two in humans). Based on our studies of *IRT1* and the two *ZRT* genes, we propose that the other genes in this family also function as metal transporters.

All ZIP proteins are predicted to have eight transmembrane domains. These proteins range from 309 to 476 amino acids in length; this difference is largely due to the length between transmembrane domains III and IV, designated the "variable region." This region is particularly intriguing because in all but two members this domain contains a histidine-rich motif that may serve as a metal-binding site. Similar domains have also been found in the Zn efflux transporters ZRC1 (50), COT1 (24), ZnT-1 (87), and ZnT-2 (86).

Strategy II

Strategy II plants, the grasses, release phytosiderophores, low molecular weight Fe(III)-specific ligands, in response to iron deficiency. These molecules are nonproteinogenic amino acids synthesized from methionine via nicotianamine (NA) to give mugineic acids that efficiently chelate Fe(III) with their amino and carboxyl groups (67). The Fe(III)–mugineic acid complexes are then believed to be internalized by specific transport systems that have yet to be characterized at the molecular level. A similar chelating strategy is used by a wide variety of bacteria and fungi (36).

The first step in phytosiderophore synthesis is the combination of three molecules of *S*-adenosylmethionine to form NA via NA synthase. Genes encoding *S*-adenosylmethionine synthetase have been cloned from a number of species, including Arabidopsis. NA synthase has been purified from iron-deficient barley roots, and efforts are now under way to identify cDNAs encoding this enzyme (42). Besides serving as a precursor for mugineic biosynthesis, NA is also thought to play a role in long-distance metal transport in Strategy I plants (103). Based on electrophoretic and potentiometric techniques, NA is predicted to transport Cu and Zn in the xylem and Cu, Zn, and Fe(II) in the phloem (43).

After formation of NA, NA aminotransferase (NAAT) is then thought to transfer an amino group to produce an unstable intermediate that is rapidly reduced to form deoxymugineic acid. cDNAs from barley-encoding NAAT have been identified; NAAT is strongly induced under iron deficiency (108). Using a

subtractive hybridization approach, two other barley genes—*ids2* and *ids3*—that may function in synthesis of mugineic acid have been identified (83, 85). Each encodes a protein with some similarity to 2-oxoglutarate dioxygenases, making them good candidates for the conversion of deoxymugineic acid and mugineic acid to epihydroxymugineic acid via hydroxylation.

In order to identify the Fe(III)–mugineic acid transporter, a yeast *ctr1* mutant that is unable to grow on iron-deficient media was transformed with a barley cDNA expression library, and clones that could use Fe(III)–mugineic acid as an iron source were isolated. One clone, designated *SFD1* (*S*uppressor of *F*errous uptake *D*efect), can restore the ability of a *ctr1* mutant of yeast to grow on iron-deficient media when either Fe(III) mugineic acid or Fe(III) citrate are provided (114). SFD1 has no similarity to any protein of known function, and the exact mechanism by which the growth arrest is bypassed remains to be determined.

ZINC

Uptake

Zn is taken up from the soil solution as a divalent cation (71). Once taken up, Zn is neither oxidized nor reduced; thus, the role of Zn in cells is based on its behavior as a divalent cation that has a strong tendency to form tetrahedral complexes (for a review, see 9). Zn is an essential catalytic component of over 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase, and carbonic anhydrase. Zn also plays a critical structural role in many proteins. For example, several motifs found in transcriptional regulatory proteins are stabilized by Zn, including the Zn finger, Zn cluster, and RING finger domains. Proteins containing these domains are very common; it has been estimated that as many as 2% of all yeast gene products contain Zn-binding domains. Despite the importance of Zn as an essential micronutrient for plant growth, relatively few studies have examined the mechanisms and regulation of Zn absorption by roots. Currently, there is little agreement on whether Zn enters via ion channels or via a divalent cation carrier and whether there is a link between uptake and metabolic energy transduction (55). Attention has mainly been focused on hyperaccumulators, i.e. plants that can grow in soils containing high levels of Zn and accumulate high concentrations of Zn in their shoots. Certain populations of *Thlaspi caerulescens* can tolerate up to 40,000 μg Zn g^{-1} tissue in their shoots; for most plants, optimal Zn concentration is between 20 and 100 μg g^{-1} tissue. Radiotracer studies with *T. caerulescens* and a nonhyperaccumulating related species, *T. arvense*, have shown that the *V*max for the uptake of Zn was 4.5-fold greater for *T. caerulescens* than for the nonhyperaccumulator *T. arvense*, while their *K*m values were not significantly different (61). This suggests that Zn uptake is controlled by regulating the number of transporters

in the membrane (61). Once in the shoot, Zn is believed to be stored in the vacuoles of leaf cells, preventing the buildup of toxic levels in the cytoplasm.

Using a method similar to the one used to isolate *IRT1*, we isolated the *ZIP1*, *ZIP2*, and *ZIP3* genes of Arabidopsis by functional expression cloning in a *zrt1zrt2* mutant yeast strain; expression of these genes in yeast restored Zn-limited growth to this strain (N Grotz, T Fox, E Connolly, ML Guerinot, W Park & D Eide, submitted manuscript). Biochemical analysis of metal uptake has demonstrated that these genes encode Zn transporters. Yeast expressing *ZIP1*, *ZIP2*, and *ZIP3* each have a different time-, temperature-, and concentration-dependent Zn uptake activity with apparent *K*m values between 10 and 100 nM Zn(II). These values are similar to the levels of free Zn available in the rhizosphere (84). Moreover, no Fe uptake activity has been detected with any of these proteins in uptake experiments using ^{55}Fe. We propose that each of these three genes plays a role in Zn transport in the plant. These represent the first Zn transporter genes to be cloned from any plant species.

CONCLUSIONS

DNA-based strategies (as opposed to biochemical approaches) have successfully identified a number of genes involved in cation transport in plants. Despite this success, we must keep in mind that such approaches may not identify all the relevant transporters. For example, yeast complementation may not allow us to identify multimeric transporters if yeast cannot provide the appropriate partner proteins. And although plant genes are well represented in several distinctive families of cation transporters such as P-type ATPases, there are currently no members of the RND (resistance/nodulation/cell division) (94), the CDF (cation diffusion facilitator) (89), or ABC (ATP-binding cassette) transporter families that have been implicated in cation transport in plants. Searching for family members will undoubtedly continue; this search will surely be facilitated by ongoing efforts to complete the sequence of Arabidopsis and other plant (rice, maize) genomes. Space limitations did not allow us to cover another possible avenue for identifying transporter genes, namely searching for mutants with transporter defects.

With many cloned genes already in hand, the obvious challenge now is to decipher the role of each of the transporters encoded by these genes. For most transporters, physiologically important information such as expression pattern and mechanism of regulation is still lacking. Various molecular approaches ultimately can tell not only in what tissue and cell types certain transporters are expressed but where within a cell each is expressed. They can also tell whether gene expression is directly influenced by changes in cation concentrations. We are also now in a position to identify plant mutants carrying insertions

in particular transporter genes (57, 75); this will greatly help in assigning functions. Having cloned genes is also allowing us to undertake structure-function studies on the encoded proteins themselves. Many transporter activities have been well characterized at the electrophysiological level. Our ability to now combine such information with structural information about the proteins will hopefully lead to an understanding of the molecular mechanisms of transport. Finally, moving beyond how any one transporter functions, we need to keep in mind that ultimately we want to understand cation transport at the whole plant level and to use such knowledge to create plants with enhanced mineral content as well as plants that bioaccumulate or exclude toxic cations such as cadmium and lead.

ACKNOWLEDGMENTS

We thank Erin Connolly, David Eide, Natasha Grotz, and Rob McClung for critical reading of the text. We also thank numerous colleagues for sharing unpublished or prepublication materials. Research in the Guerinot lab is supported by grants from the National Science Foundation, the United States Department of Agriculture, and the Department of Energy.

Literature Cited

1. Anderson JA, Huprikar SS, Kochian LV, Lucas WJ, Gaber RF. 1992. Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 89:3736–40
2. Askerlund P. 1997. Calmodulin-stimulated Ca^{2+}-ATPases in the vacuolar and plasma membranes in cauliflower. *Plant Physiol.* 114:999–1007
3. Assmann SM, Haubrick LL. 1996. Transport proteins of the plant plasma membrane. *Curr. Opin. Cell Biol.* 8:458–67
4. Axelsen KB, Palmgren MG. 1998. Evolution of substrate specificities in the P-type ATPase superfamily. *J. Mol. Evol.* 46:84–101
5. Basset M, Conejero G, Lepetit M, Fourcroy P, Sentenac H. 1995. Organization and expression of the gene coding for the potassium transport system AKT1 of *Arabidopsis thaliana*. *Plant Mol. Biol.* 29: 947–58
6. Becker D, Dreyer I, Hoth S, Reid JD, Busch H, et al. 1996. Changes in voltage activation, Cs^+ sensitivity, and ion permeability in H5 mutants of the plant K^+ channel KAT1. *Proc. Natl. Acad. Sci. USA* 93:8123–28
7. Belouchi A, Cellier M, Kwan T, Saini HS, Leroux G, Gros P. 1995. The macrophage specific membrane protein Nramp controlling natural resistance in mice has homologues expressed in the root systems of plants. *Plant Mol. Biol.* 29:1181–96
8. Belouchi A, Kwan T, Gros P. 1997. Cloning and characterization of the *Os-Nramp* family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. *Plant Mol. Biol.* 33:1085–92
9. Berg JM, Shi Y. 1996. The galvanization of biology: a growing appreciation for the roles of zinc. *Science* 271:1081–85
10. Berkowitz GA, Ma J, Tang H, Zhang X, Fang Z, Vasconcelos AC. 1998. Molecular characterization of plant potassium channels. In *Frontiers in Potassium Nutrition: New Perspectives on the Effects of Potassium on Crop Plant Physiology*, ed. D Oostechnis, GA Berkowitz. Madison, Wis: Am. Soc. Agron.

11. Blatt MR. 1997. Plant potassium channels double up. *Trends Plant Sci.* 2:244–46
12. Briat J-F, Lobréaux S. 1997. Iron transport and storage in plants. *Trends Plant Sci.* 2:187–93
13. Brown AM. 1993. Functional bases for interpreting amino acid sequences of voltage-dependent K^+ channels. *Annu. Rev. Biophys. Biomol. Struct.* 22:173–98
14. Bush DS. 1995. Calcium regulation in plant cells and its role in signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46:95–122
15. Cao Y, Crawford NM, Schroeder JI. 1995. Amino terminus and the first four membrane-spanning segments of the *Arabidopsis* K^+ channel KAT1 confer inward-rectification property of plant-animal chimeric channels. *J. Biol. Chem.* 270:17697–701
16. Cao Y, Ward JM, Kelly WB, Ichida AM, Gaber RF, et al. 1995. Multiple genes, tissue specificity, and expression-dependent modulation contribute to the functional diversity of potassium channels in *Arabidopsis thaliana*. *Plant Physiol.* 109:1093–106
17. Cellier M, Belouchi A, Gros P. 1996. Resistance to intracellular infections: comparative genomic analysis of *Nramp*. *Trends Genet.* 12:201–4
18. Cellier M, Privé G, Belouchi A, Kwan T, Rodrigues V, et al. 1995. Nramp defines a family of membrane proteins. *Proc. Natl. Acad. Sci. USA* 92:10089–93
19. Chaney RL, Brown JC, Tiffin LO. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. *Plant Physiol.* 50:208–13
20. Chanson A. 1993. Active transport of proton and calcium in higher plants. *Plant Physiol. Biochem.* 31:943–55
21. Chen XF, Chang MC, Wang BY, Wu R. 1997. Cloning of a Ca^{2+}-ATPase gene and the role of cytosolic Ca^{2+} in the gibberellin-dependent signaling pathway in aleurone cells. *Plant J.* 11:363–71
22. Chouinard SW, Wilson GF, Schlimgen AK, Ganetzky B. 1995. A potassium channel β subunit related to the aldo-keto reductase superfamily is encoded by the *Drosophila* hyperkinetic locus. *Proc. Natl. Acad. Sci. USA* 92:6763–67
23. Cohen CK, Norvell WA, Kochian LV. 1997. Induction of the root cell plasma membrane ferric reductase. *Plant Physiol.* 114:1061–69
24. Conklin DS, McMaster JA, Culbertson MR, Kung C. 1992. *COT1*, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12:3678–88
25. Cunningham KW, Fink GR. 1994. Calcineurin dependent growth control in *Saccharomyces cerevisiae* mutants lacking *PMC1*, a homolog of plasma membrane Ca^{2+} ATPases. *J. Cell Biol.* 124:351–63
26. Cunningham KW, Fink GR. 1996. Calcineurin inhibits *VCX1*-dependent H^+/Ca^{2+} and induces Ca^{2+} ATPases in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 16:2226–37
27. Czempinski K, Zimmermann S, Ehrhardt T, Müller-Röber B. 1997. New structure and function in plant K^+ channels: KCO1, an outward rectifier with a steep Ca^{2+} dependency. *EMBO J.* 16:2565–75
28. Ehrhardt T, Zimmermann S, Müller-Röber B. 1997. Association of plant K^+in channels is mediated by conserved C-termini and does not affect subunit assembly. *FEBS Lett.* 409:166–70
29. Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc. Natl. Acad. Sci. USA* 93:5624–28
30. Evans DE, Briars S-A, Williams LE, Chanson A. 1991. Active transport of proton and calcium in higher plant cells. *J. Exp. Bot.* 42:285–303
31. Ferrol N, Bennett AB. 1996. A single gene may encode differentially localized Ca^{2+}-ATPases in tomato. *Plant Cell* 8:1159–69
32. Fleming MD, Trenor CC, Su MA, Foernzler D, Beier DR, et al. 1997. Microcytic anaemia mice have a mutation in *Nramp2*, a candidate iron transporter gene. *Nat. Genet.* 16:383–86
33. Fox TC, Shaff JE, Grusak MA, Norvell WA, Chen Y, et al. 1996. Direct measurement of 59 labeled Fe^{2+} influx in roots of *Pisum sativum* using a chelator buffer system to control free Fe^{2+} in solution. *Plant Physiol.* 111:93–100
34. Gassmann W, Rubio F, Schroeder JI. 1996. Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. *Plant J.* 10:869–82
35. Gaymard F, Cerutti M, Horeau C, Lemaillet G, Urbach S, et al. 1996. The baculovirus/insect cell system as an alternative to *Xenopus* oocytes; first characterization of the AKT1 K^+ channel from *Arabidopsis thaliana*. *J. Biol. Chem.* 271: 22863–870
36. Guerinot ML. 1994. Microbial iron transport. *Annu. Rev. Microbiol.* 48:743–72
37. Guerinot ML, Yi Y. 1994. Iron: nutritious,

noxious, and not readily available. *Plant Physiol.* 104:815–20
38. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, et al. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482–88
39. Halliwell B, Gutteridge JMC. 1992. Biologically relevant metal ion-dependent hydroxyl radical generation. *FEBS Lett.* 307:108–12
40. Harper JF, Surowy TK, Sussman MR. 1989. Molecular cloning and sequence of cDNA encoding the membrane proton pump (H^+-ATPase) of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 86: 1234–38
40a. Harper JF, Hong B, Hwang I, Guo HQ, Stoddard R, et al. 1998. A novel calmodulin-regulated Ca^{2+}-ATPase (*ACA2*) from *Arabidopsis* with an N-terminal autoinhibitory domain. *J. Biol. Chem.* 273:1099–1106
41. Hassett R, Kosman DJ. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisiae*. *J. Biol. Chem.* 270:128–34
42. Herbik A, Giritch A, Bäumlein H, Stephan UW, Mock H-P. 1997. *Characterization and purification of nicotianamine synthase*. Presented at Int. Symp. Iron Nutr. Interact. Plants, 9th, Stuttgart, Ger.
43. Hider RC, von Wiren N, Leigh R, Bansal S, Briat JF. 1997. *Physicochemical characterization of Fe-nicotianamine complexes and their physiological implications*. Presented at Int. Symp. Iron Nutr. Interact. Plants, 9th, Stuttgart, Ger.
44. Hirsch RE, Lewis B, Spalding E, Sussman MR. 1997. *Characterization of an Arabidopsis potassium channel mutant*. Presented at Int. Conf. Arabidopsis Res., 8th, Madison, Wis.
45. Hirschi KD, Zhen R-G, Cunningham KW, Rea PA, Fink GR. 1996. CAX1, an H^+/Ca^{2+} antiporter from *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 93:8782–86
46. Deleted in proof
47. Huang LQ, Berkelman T, Franklin AE, Hoffman NE. 1993. Characterization of a gene encoding a Ca^{2+}- ATPase-like protein in the plastid envelope. *Proc. Natl. Acad. Sci. USA* 90:10066–70
48. Ichida AM, Pei Z-M, Turner KJ, Schroeder JI. 1997. Expression of a Cs^+ resistant guard cell K^+ channel confers Cs^+ resistant light-induced stomatal opening in transgenic *Arabidopsis*. *Plant Cell* 9(10):1843–57
49. Ichida AM, Schroeder JI. 1996. Increased resistance to extracellular cation block by mutation of the pore domain of the *Arabidopsis* inward-rectifying K^+ channel KAT1. *J. Membr. Biol.* 151:53–62
50. Kamizono A, Nishizawa M, Teranishi Y, Murata K, Kimura A. 1989. Identification of a gene conferring resistance to zinc and cadmium ions in the yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 219:161–67
51. Kampfenkel K, Kushnir S, Babiychuk E, Inzé D, Van Montagu M. 1995. Molecular characterization of a putative *Arabidopsis thaliana* copper transporter and its yeast homologue. *J. Biol. Chem.* 270:28479–86
52. Ketchum KA, Slayman CW. 1996. Isolation of an ion channel gene from *Arabidopsis thaliana* using the H5 signature sequence from voltage-dependent K^+ channels. *FEBS Lett.* 378:19–26
53. Knight SAB, Labbé S, Kwon LF, Kosman DJ, Thiele DJ. 1996. A widespread transposable element masks expression of a yeast copper transport gene. *Genes Dev.* 10:1917–29
54. Kochian LV. 1991. Mechanisms of micronutrient uptake and translocation in plants. In *Micronutrients in Agriculture*, ed. JJ Mortvedt, FR Cox, LM Shuman, RM Welch, pp. 229–96. Madison, Wis: Soil Soc. Am. 2nd ed.
55. Kochian LV. 1993. Zinc absorption from hydroponic solutions by plant roots. In *Zinc in Soils and Plants*, ed. AD Robson, pp. 45–57. Boston/Dordrecht: Kluwer
56. Kochian LV, Lucas WJ. 1988. Potassium transport in roots. *Adv. Bot. Res.* 15:93–178
57. Krysan PJ, Young JC, Tax F, Sussman MR. 1996. Identification of transferred DNA insertions within *Arabidopsis* genes involved in signal transduction and ion transport. *Proc. Natl. Acad. Sci. USA* 93: 8145–50
58. Kukuljan M, Labarca P, Latorre R. 1995. Molecular determinants of ion conduction and inactivation in K^+ channels. *Am. J. Physiol.* 268:C535–56
59. Lagarde D, Basset M, Lepetit M, Conejero G, Gaymard F, et al. 1996. Tissue-specific expression of *Arabidopsis AKT1* gene is consistent with a role in K^+ nutrition. *Plant J.* 9:195–203
60. Lapinskas PJ, Cunningham KW, Liu XF, Fink GR, Culotta VC. 1995. Mutations in *PMR1* suppress oxidative damage in yeast cells lacking superoxide dismutase. *Mol. Cell Biol.* 15:1382–88
61. Lasat MM, Baker AJM, Kochian LV. 1996. Physiological characterization of root Zn^{2+} absorption and translocation

to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiol.* 112:1715–22
62. Lauchli A. 1990. Calcium, salinity and the plasma membrane. In *Calcium in Plant Growth and Development*, ed. RT Leonard, PK Hepler, pp. 26-35. Rockville: Am. Soc. Plant Physiol.
63. Liang F, Cunningham KW, Harper JF, Sze H. 1997. *ECA1* complements yeast mutants defective in Ca^{2+} pumps and encodes an endoplasmic reticulum-type Ca^{2+}-ATPase in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 94:8579–84
64. Lin S-J, Pufahl RA, Dancis A, O'Halloran TV, Culotta VC. 1997. A role for the *Saccharomyces cerevisiae ATX1* gene in copper trafficking and iron transport. *J. Biol. Chem.* 272:9215–20
65. Logan DC, Venis MA. 1995. Characterisation and immunological identification of a calmodulin-stimulated Ca^{2+}- ATPase from maize shoots. *J. Plant Physiol.* 145:702–10
66. Logan H, Basset M, Véry A-A, Sentenac H. 1997. Plasma membrane transport systems in higher plants: from black boxes to molecular physiology. *Physiol. Plant.* 100:1–15
67. Ma JF, Nomoto K. 1996. Effective regulation of iron acquisition in graminaceous plants. The role of mugineic acids as phytosiderophores. *Physiol. Plant.* 97:609–17
68. Maathuis FJM, Ichida AM, Sanders D, Schroeder JI. 1997. Roles of higher plant K^+ channels. *Plant Physiol.* 114:1141–49
69. Maathuis FJM, Sanders D. 1996. Mechanisms of potassium absorption by higher plant roots. *Physiol. Plant.* 96:158–68
69a. Maathuis FJM, Verlin D, Smith FA, Sanders D, Fernandez JA, Walker NA. 1996. The physiological relevance of Na^+-coupled K^+ transport. *Plant Physiol.* 112: 1609–16
70. Malmström S, Askerlund P, Palmgren MG. 1997. A calmodulin-stimulated Ca^{2+}-ATPase from plant vacuolar membranes with a putative regulatory domain at its N-terminus. *FEBS Lett.* 400:324–28
71. Marschner H. 1995. *Mineral Nutrition of Higher Plants*. Boston: Academic. 2nd ed.
72. Marten I, Gaymard F, Lemaillet G, Thibaud J-B, Sentenac H, Hedrich R. 1996. Functional expression of the plant K^+ channel KAT1 in insect cells. *FEBS Lett.* 380:229–32
73. Deleted in proof
74. McCormack T, McCormack K. 1994. *Shaker* K^+ channel β subunits belong to an NAD(P)H-dependent oxidoreductase superfamily. *Cell* 79:1133–35
75. McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, et al. 1995. Sequence-based identification of T-DNA insertion mutations in *Arabidopsis*: actin mutants *act2-1* and *act4-1*. *Plant J.* 8: 613–22
76. Michelet B, Boutry M. 1995. The plasma membrane H^+-ATPase. *Plant Physiol.* 108:1–6
77. Miller AG, Aldrich RW. 1996. Conversion of a delayed rectifier K^+ channel to a voltage-gated inward rectifier K^+ channel by three amino acid substitutions. *Neuron* 16:853–58
78. Møller JV, Juul B, le Maire M. 1996. Structural organization, ion transport and energy transduction of P-type ATPases. *Biochim. Biophys. Acta* 1286:1–51
79. Müller-Röber B, Czempinski K, Ehrhardt T, Zimmermann S. 1997. *Properties of a cloned Arabidopsis outward rectifying K^+ channel, KCO1, indicate a role in Ca^{2+}-mediated signal transduction*. Presented at Int. Conf. Arabidopsis Res., 8th, Madison, Wis.
80. Müller-Röber B, Ellenberg J, Provart N, Willmitzer L, Busch H, et al. 1995. Cloning and electrophysiological analysis of KST1, an inward rectifying K^+ channel expressed in potato guard cells. *EMBO J.* 14:2409–16
81. Nakamura RL, Anderson JA, Gaber RF. 1997. Determination of key structural requirements of a K^+ channel pore. *J. Biol. Chem.* 272:1011–18
82. Nakamura RL, McKendree WL, Hirsch RE, Sedbrook JC, Gaber RF, Sussman MR. 1995. Expression of an *Arabidopsis* potassium channel gene in guard cells. *Plant Physiol.* 109:371–74
83. Nakanishi H, Okumura N, Umehara Y, Nishizawa N-K, Chino M, Mori S. 1993. Expression of a gene specific for iron deficiency (*Ids3*) in the roots of *Hordeum vulgare*. *Plant Cell Physiol.* 34:401–10
84. Norvell WA, Welch RM. 1993. Growth and nutrient uptake by barley (*Hordeum vulgare* L. cv. Herta): studies using an N-(2–hydroxyethyl)ethylenedinitrilotriacetic acid-buffered nutrient solution technique. I. Zinc ion requirements. *Plant Physiol.* 101:619–25
85. Okumura N, Nishizawa N-K, Umehara Y, Ohata T, Nakanishi H, et al. 1994. A dioxygenase gene (*Ids2*) expressed under iron deficiency conditions in the roots of

Hordeum vulgare. *Plant Mol. Biol.* 25: 705–19
86. Palmiter RD, Cole TB, Findley SD. 1996. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *EMBO J.* 15:1784–91
87. Palmiter RD, Findley SD. 1995. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J.* 14:639–49
88. Pardo JM, Serrano R. 1989. Structure of a plasma membrane H^+-ATPase gene from the plant *Arabidopsis thaliana*. *J. Biol. Chem.* 264:8557–62
89. Paulsen LT, Saier MH. 1997. A novel family of ubiquitous heavy metal ion transport proteins. *J. Membr. Biol.* 156:99–103
90. Perez-Prat E, Narasimhan ML, Binzel ML, Botella MA, Chen Z, et al. 1992. Induction of a putative Ca^{2+}-ATPase mRNA in NaCl-adapted cells. *Plant Physiol.* 100:1471–78
91. Raschke BC, Wolf AH. 1996. Molecular cloning of a P-type Ca^{2+}-ATPase from the halotolerant alga *Dunaliella bioculata*. *Planta* 200:78–84
91a. Robinson NJ, Sadjuga MR, Groom QJ. 1997. The *froh* gene family from *Arabidopsis thaliana*: putative iron-chelate reductases. In *Plant Nutrition- for sustainable food production and environment*, ed. T Ando, 191–94. Kluwer Academic Pub.
92. Rubio F, Gassmann W, Schroeder JI. 1995. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* 270:1660–63
93. Sadjuga MR, Procter CM, Robinson NJ, Groom QJ. 1997. Isolation and analysis of genes encoding novel NAD(P)H oxidases from *Arabidopsis thaliana*: their putative role(s) as ferric/cupric reductases. *J. Exp. Bot.* 48:101 (Suppl.)
94. Saier MH, Tam R, Reizer A, Reizer J. 1994. Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. *Mol. Microbiol.* 11:841–47
95. Schachtman DP, Kumar R, Schroeder JI, Marsh EL. 1997. Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. *Proc. Natl. Acad. Sci. USA* 94:11079–84
96. Schachtman DP, Schroeder JI. 1994. Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. *Nature* 370:655–58
97. Schachtman DP, Schroeder JI, Lucas WJ, Anderson JA, Gaber RF. 1992. Expression of an inward-rectifying potassium channel by the *Arabidopsis KAT1* cDNA. *Science* 258:1654–58
98. Schroeder JI, Ward JM, Gassmann W. 1994. Perspectives on the physiology and structure of inward-rectifying K^+ channels in higher plants. *Annu. Rev. Biophys. Biomol. Struct.* 23:441–71
99. Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon J-M, et al. 1992. Cloning and expression in yeast of a plant potassium ion transport system. *Science* 256:663–65
100. Shi G, Nakahira K, Hammond S, Rhodes KJ, Schechter LE, Trimmer JS. 1996. β subunits promote K^+ channel surface expression through effects early in biosynthesis. *Neuron* 16:843–52
101. Silver S, Phung LT. 1996. Bacterial heavy metal resistance: new surprises. *Annu. Rev. Microbiol.* 50:753–89
102. Smart CJ, Garvin DF, Prince JP, Lucas WJ, Kochian LV. 1996. The molecular basis of potassium nutrition in plants. *Plant Soil* 187:81–89
103. Stephan UW, Schmidke I, Stephan VW, Scholz G. 1996. The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. *BioMetals* 9:84–90
104. Supek F, Supekova L, Nelson H, Nelson N. 1996. A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proc. Natl. Acad. Sci. USA* 93: 5105–10
105. Supek F, Supekova L, Nelson H, Nelson N. 1997. Function of metal-ion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. *J. Exp. Biol.* 200:321–30
106. Sussman MR. 1994. Molecular analysis of proteins in the plant plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:211–34
107. Tabata K, Kashiwagi S, Mori H, Ueguchi C, Mizuno T. 1997. Cloning of a cDNA encoding a putative metal-transporting P-type ATPase from *Arabidopsis thaliana*. *Biochim. Biophys. Acta* 1326:1–6
108. Takahashi M, Yamaguchi H, Nakanishi H, Kanazawa K, Shioiri T, et al. 1997. *Cloning and sequencing of nicotianamine aminotransferase gene (Naat)—a key enzyme for the synthesis of mugineic acid-family phytosiderophores*. Presented at Int. Symp. Iron Nutr. Interact. Plants, 9th, Stuttgart, Ger.
109. Tang HX, Vasconcelos AC, Berkowitz

GA. 1995. Evidence that plant K^+ channel proteins have two different types of subunits. *Plant Physiol.* 109:327–30
110. Tang HX, Vasconcelos AC, Berkowitz GA. 1996. Physical association of KAB1 with plant K^+ channel α subunits. *Plant Cell* 8:1545–53
111. Thiel G, Wolf AH. 1997. Operation of K^+-channels in stomatal movement. *Trends Plant Sci.* 2:339–45
112. Uozumi N, Gassmann W, Cao Y, Schroeder JI. 1995. Identification of strong modifications in cation selectivity in an *Arabidopsis* inward rectifying potassium channel by mutant selection in yeast. *J. Biol. Chem.* 270:24276–81
113. Wimmers LE, Ewing NN, Bennett AB. 1992. Higher plant Ca^{2+}-ATPase: primary structure and regulation of mRNA abundance by salt. *Proc. Natl. Acad. Sci. USA* 89:9205–9
114. Yamaguchi H, Shioiri T, Mori S. 1997. *Isolation* and *characterization of a barley cDNA clone which restores growth defect of yeast ferrous uptake mutant*, ctr1. Presented at Int. Symp. Iron Nutr. Interact. Plants, 9th, Stuttgart, Ger.
115. Yao W, Hadjeb N, Berkowitz GA. 1997. Molecular cloning and characterization of the first plant K(Na)/proton antiporter (Abstract No. 999). *Plant Physiol.* 114: S200
116. Yi Y. 1995. Iron uptake in *Arabidopsis thaliana*. PhD thesis. Dartmouth Coll., Hanover, NH
117. Yi Y, Guerinot ML. 1996. Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency. *Plant J.* 10: 835–44
118. Yuan DS, Stearman R, Dancis A, Dunn T, Beeler T, Klausner RD. 1995. The Menkes/Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-like oxidase required for iron uptake. *Proc. Natl. Acad. Sci. USA* 92:2632–36
119. Zhao H, Eide D. 1996. The yeast *ZRT1* gene encodes the zinc transporter of a high affinity uptake system induced by zinc limitation. *Proc. Natl. Acad. Sci. USA* 93:2454–58
120. Zhao H, Eide D. 1996. The ZRT2 gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 271:23203–10
121. Zhen R-G, Hirschi KD, Chapman D, Fink GR, Rea PA. 1997. Cax1p: an H^+/Ca^{2+}, H^+/Cd^{2+} and/or Na^+/H^+ antiporter from *Arabidopsis*? (Abstract No. 51). *Plant Physiol.* 114:S28
122. Zhou B, Gitschier J. 1997. *hCTR1*: a human gene for copper uptake identified by complementation in yeast. *Proc. Natl. Acad. Sci. USA* 94:7481–86