Mechanisms and Control of Nutrient Uptake in Plants

Robert Reid and Julie Hayes
Department of Environmental Biology, University of Adelaide, Adelaide 5005, Australia

This review is a distillation of the vast amount of physiological and molecular data on plant membrane transport, to provide a concise overview of the main processes involved in the uptake of mineral nutrients in plants. Emphasis has been placed on transport across the plasma membrane, and on the primary uptake from soil into roots, or in the case of aquatic plants, from their aqueous environment. Control of uptake has been mainly considered in terms of local effects on the rate of transport and not in terms of long-distance signaling. The general picture emerging is of a large array of membrane transporters, few of which display any strong selectivity for individual nutrients. Instead, many transporters allow low-affinity uptake of several different nutrients. These features, plus the huge number of potential transporter genes that has been revealed by sequencing of plant genomes, raise some interesting questions about their evolution and likely function.

KEY WORDS: Ion channels, Membrane transport, Nutrient uptake, Plant nutrition, Plasma membrane, Transporter genes.

I. Introduction

A review of the uptake of all plant nutrients would seem to require a whole volume of this series rather than a single chapter, since there are many levels at which nutrient acquisition could be considered. There are about 19 different elements that are considered essential for plant growth (Epstein, 1972, 1999) and of these many are absorbed into plants in multiple forms (e.g., N as NO_3, NH_4, amino acids). Then there is the question of uptake into different tissues with varying absorption structures, not to mention the diversity that

exists among plants and the differences in nutritional requirements that this imposes. Fortunately, there are some simplifications that can be made, since C, H, and O are usually obtained from gases or from water and the pathways for their uptake are uncomplicated and have been well described. The remaining 16 or so are considered mineral elements and are mostly obtained either from soil in the case of terrestrial plants, or from the bathing medium in the case of aquatic plants. A further simplification comes from the fact that during evolution, many transport systems were conserved and can be found across a wide range of plant and animal types (Geisler *et al*., 2000; Guerinot, 2000; Martinoia *et al*., 2002). There is also a relatively small range of transport mechanisms, the selection of which is largely determined by electrochemical considerations: central to these are H^+-ATPases in the plasma membrane.

The mechanisms for the uptake of most macronutrients (K, Ca, Mg, S, P, N) are now reasonably well understood. Unfortunately this is not the case with any of the micronutrients except Cl, and perhaps Fe. Understanding how transport of both micro- and macronutrients is regulated has also proved to be a very difficult task. Thus, the length of a review of nutrient uptake in plants can be shortened because of the evolutionary economy in design of transporters and by the gaps in our knowledge as to how uptake is controlled. This review will focus on the primary mechanisms for uptake across the plasma membrane of cells and will mainly consider the uptake systems applying to mineral nutrients.

II. Basic Features of Plant Membrane Transport

A. Electrochemical Gradients and Driving Forces

Nutrient uptake into plant cells involves accumulation of nutrient molecules to higher concentrations than in the surrounding medium, with few exceptions. This accumulation represents a significant investment of energy, needed to generate the driving forces for uptake. The coupling of ATP produced by metabolism to transport across the plasma membrane is achieved by H^+-ATPases that establish electrical and proton gradients that in turn drive uptake of both cations and anions. Direct energization by adenosine triphosphate (ATP) of transport across the plasma membrane (e.g., Ca^{2+}-ATPase) is rare, with most transport depending on chemiosmotic mechanisms.

Electrogenic pumping by plasma membrane H^+-ATPases produces transmembrane electrical potential differences (PDs) commonly in the range -100 to -200 mV, depending on the location of the cell and the composition of the bathing solution. A PD of -180 mV is sufficient to drive uptake of monovalent cations to internal concentrations three orders of magnitude

higher than outside. For divalent cations, this PD allows a 10^6-fold accumulation within the cell. For anions, however, the negative PD represents a strong repulsive force that must be overcome for internal accumulation to occur.

B. Transport Mechanisms

Inorganic mineral nutrients, with the exception of B, are all charged to varying degrees. Therefore membrane voltages will have a significant impact on their movements into cells. The electrochemical gradients applying to transport of a particular nutrient largely define the possible mechanisms for uptake. Division on thermodynamic grounds into active and passive transport tends to obscure the fact that any accumulation involves an energy cost. Passive uptake through ion channels is possible only because of the energy previously expended by electrogenic pumps in setting up the voltage gradient that drives the ion movement through the channel. Active uptake is usually interpreted as movement of a nutrient against its electrochemical gradient, the gradient as it applies in a normal energized cell in which a membrane PD has already been established. By this definition uptake of nutrient cations will almost always be passive, and uptake of anionic nutrients will be active.

Three main transport mechanisms accommodate most known nutrient transport systems in the plasma membrane of plants—channels, carriers, and cotransporters. Uptake of macronutrient cations Ca and K (and probably also NH_4 and Mg) occurs via channels, although there is ongoing debate about whether K uptake can be mediated by channels under conditions in which K is supplied at micromolar concentrations (see discussion in Section III.A). Uptake of nutrient anions NO_3, PO_4, and SO_4 is driven by cotransport with H^+. Micronutrient uptake systems are less well defined, in part due to problems associated with measurement of their small fluxes, usually from small external concentrations. The micronutrient cations are di- or trivalent and therefore strong electrical gradients exist for passive uptake, but due to the small currents associated with their fluxes, it is difficult to ascertain whether ion channels or other transporters are responsible for uptake.

C. Kinetic Analysis of Transport

It was implicit in most of the early studies of membrane transport that the rate could be described in terms equivalent to an enzyme reaction, except that substrate and product now became transport substrate on either side of a membrane. In many cases nutrient transport did appear to follow simple saturation kinetics that could conveniently be described by K_m and V_{max}. In other cases, however, the relationship between rate and substrate

concentration was more complex, leading to the supposition that more than one transporter was involved. The simplest way to resolve the complexity was to attribute transport to both high- and low-affinity transporters, with the low-affinity system being constitutive and the high-affinity system inducible. This so-called "dual isotherm" model appeared satisfactory for many nutrients but others displayed unusually complex patterns that could not easily be dissected into individual components. These "bumpy isotherms" were interpreted by some as representing concentration-dependent phase changes in a single carrier that possessed two binding sites, one a transport site and the other a transition site. The multiphasic carrier concept, championed by Nissen (1991), has not been discounted but awaits more thorough investigation.

It is now well established that not only do electrical potential differences exist across membranes, but also between the membrane surface and the bathing solution. Whereas the PD across the plasma membrane is generated mainly by electrogenic pumping, the surface potential is due to excess negative charges on phospholipids and ionization of acidic side groups on membrane proteins. In effect, the membrane surface is like a charged plate that electrostatically attracts or repels ions. The significance of the surface potential for membrane transport is that transporters almost certainly "see" the concentration of substrate ions adjacent to the surface, not the concentration in the bulk solution. Measurements on protoplasts have shown that under low ionic conditions, the surface potential could be more negative than −60 mV (Nagata and Melchers, 1978; Møller *et al*., 1984; Abe and Takeda, 1988), a potential at which monovalent and divalent cations would be, respectively, 10-fold or 100-fold higher than in the bulk solution. Clearly this will have major implications for measurements of the K_m of a transport system. Because the surface potential is strongly dependent on the ionic composition of the medium, depolarizing with increasing ionic strength, and is sensitive to pH, becoming more negative with increasing pH due to dissociation of acidic amino acids on membrane proteins, the K_m will be related to the conditions under which it was measured. Moreover, failure to take into account the electrostatic nature of the membrane will lead to large errors in estimating K_ms. This also means the comparisons between K_m values for ionic solutes will be valid only for measurements made under similar solution conditions. Readers unfamiliar with the concept of membrane surface charge are referred to recent articles by Kinraide (2001) and Zhang *et al*. (2001).

D. Insights from Molecular Biology

The completion of sequencing of the *Arabidopsis thaliana* genome (Arabidopsis Genome Initiative, 2000) resulted in the identification of genes coding for 24,470 proteins. Of these, 18% (4589) were predicted to

have at least two membrane-spanning domains that would identify them as membrane integral proteins (Ward, 2001). Within this group, 70% (3208) could be clustered into 628 families, and 30% existed as unique sequences. Known function or homology to proteins of known function in other eukaryotes could be assigned to 211 families accounting for 1764 proteins. In addition to *Arabidopsis*, many of the gene sequences encoding transporters in rice (http://www.cbs.umn.edu/rice/) have also been determined.

The presence of large numbers of quite similar genes raises questions concerning their respective functions. In *Arabidopsis* there is significant duplication (24 duplicated segments, each in excess of 100 kb, comprise over half of the genome) and many genes may also have become redundant. However, subtle differences between similar transport genes may reflect differences in expression pattern or differences in regulation in varying cell and tissue types. For example, there are 10 plasma membrane H^+-ATPases in *Arabidopsis* (Arango *et al*., 2003); all carry out the same basic reaction. The electrochemical gradients that they establish are used to energize a wide range of secondary transport processes, so it is reasonable to propose that regulation of different H^+-ATPases by signals from individual solute levels or transporters would be desirable to optimize the efficiency of both ATP utilization and nutrient transport.

In the following sections on mineral nutrient uptake, it has been necessary to restrict the descriptions of transporter genes to those for which some functional information has been obtained. More details can be obtained from the reviews cited for each nutrient. The functions of only a handful of the transporters listed in Table I have been confirmed. Even for macronutrients, the nature of the dominant transporter in plants remains uncertain in most cases. There is also a very large group of genes encoding *A*TP *b*inding *c*assette (ABC) transporters, many of which could potentially mediate directly energized pumping of nutrients into cells, but evidence presented so far points to their main role being either detoxification or the regulation of other transporters (Martinoia *et al*., 2002).

III. Macronutrients

A. Potassium

K is the most abundant cation in plant cells. Its compatibility with enzymes allows it to be used at high concentrations in the cytosol as an osmoticum, unlike most other cations that inhibit enzymic reactions at high concentrations (e.g., Na) or precipitate important metabolites (e.g., Ca). K also has a dominant role in the regulation of the electrical PDs across membranes.

TABLE I

Membrane Proteins from *Arabidopsis thaliana*, with Known or Putative Functions in Membrane Transport[a]

Category	No. of families	No. of family members	No. of single sequences	Examples (No. of family members)
ABC transporters	6	92	1	
Antiporters	8	57	1	Four putative Na^+/H^+ antiporter families (36)
				Low-affinity Ca antiporter CAX2 family (6)
				KEA1 K^+/H^+ antiporter family (3)
Aquaporins	2	38	0	
Inorganic solute cotransporters	13	92	6	K^+ transporter HAK5 family (14)
				Sulfate transporter family (12)
				P_i transporter family (11)
				Na^+-dependent P_i transporter-like family (6)
				NH_4^+ transporter family (5)
				High-affinity NO_3^- transporter family (7)
				Putative Fe(II) transport protein family (14)
				Putative Zn transporter (ZIP2)-like family (2)
				Zn transporter (ZAT) family (6)
				Cu transport protein-like family (6)
Ion channels	5	60	1	
Organic solute cotransporters	30	354	8	
Primary pumps (ATPases)	7	57	3	Plasma membrane H^+-ATPase family (12)
				Putative P-type ATPase family (12)
				Vacuolar H^+-ATPase 16-kDa proteolipid family (7)
				Putative vacuolar H^+-ATPase subunit 1 family (3)
				Autoinhibited Ca-ATPase (ACA) family (16)

[a]Information was obtained from the *Arabidopsis* Membrane Protein Library (http://www.cbs.umn.edu/arabidopsis/).

Early work by Epstein *et al.* (1963) showed that K uptake into roots could be kinetically dissected into high- and low-affinity uptake systems. Further physiological and electrical studies indicated that the low-affinity transport was due to K^+-selective channels whereas the high-affinity transport could most likely be attributed to the action of K^+/H^+ or K^+/Na^+ symport (Maathuis and Sanders, 1997). Recent molecular studies appear to have clouded rather than clarified this simple description of K uptake. At least seven families of K^+-permeable membrane proteins have been identified in plants, and within these families there are approximately 55 different transporters varying in both selectivity for K and regulatory mechanisms (Mäser *et al.*, 2002). The first K channels cloned from plants were AKT1 (Sentenac *et al.*, 1992) and KAT1 (Anderson *et al.*, 1992), members of the "Shaker" superfamily that currently includes nine genes for K channels (Mäser *et al.*, 2002). Two other K-permeable channel types have been shown to be expressed in plants, the so-called "two-pore" channels and the cyclic nucleotide-gated channels, but their contribution to K uptake is unclear at present. Similarly the significance of the K^+/H^+ exchanger and various high-affinity K uptake transporters, KUPs (KUP/KAK/KT) or the K/Na (K/H) symporter HKT1, have yet to be established in plants (Mäser *et al.*, 2001).

As with most molecular studies, functional analysis has lagged well behind gene discovery. Establishing the physiological significance of members of this vast array of possible K uptake proteins is still in the early stages. Three studies in which individual genes have been knocked out causing impaired growth and reduced K uptake suggest that K uptake may be mediated by one or two dominant genes in each cell type rather than by a composite of many genes expressing multiple transporters. Disruption of *AKT1* in roots of *Arabidopsis* significantly reduced plant growth (Hirsch *et al.*, 1998; Dennison *et al.*, 2001). Growth of the mutant was most strongly inhibited in the presence of NH_4, which appeared to indicate that a parallel uptake system existed that was less selective for K than AKT1 and was blocked by high concentrations of NH_4 (Spalding *et al.*, 1999). Disruption of another Shaker-type K channel, *SPIK*, was shown to impair the growth of pollen tubes in *Arabidopsis* (Mouline *et al.*, 2002) whereas mutation of *TRH1*, a member of the *KUP* family, caused loss of tip growth in root hairs (Rigas *et al.*, 2001).

An issue that has raised considerable debate is whether channels could be responsible for high-affinity uptake. The cytosolic concentration of K is often in excess of 100 m*M*, yet plants are able to grow in the presence of low micromolar concentrations in the external medium. For example, Box and Schachtman (2000) found that the early growth of wheat at 20 μ*M* K was similar to that at 1 m*M* K. Channel-mediated uptake of K is a passive process driven by the electrical PD across the membrane. Uptake from a medium containing 10 μ*M* K into a cytosol containing 100 m*M* K would require a membrane PD of around −240 mV, a potential that is rarely

observed in electrophysiological studies. However, Hirsch *et al.* (1998) found that growth and K uptake in the AKT1-disrupted mutant was reduced at 10 μ*M* K, from which they concluded that a channel could indeed contribute to K uptake in the high-affinity range. A similar conclusion was reached by Mouline *et al.* (2002) for the SPIK channel, based on the concentration dependence of pollen tube growth in normal and mutant pollen. Measurements of membrane PDs in apical root cells of *Arabidopsis* showed that around 20% of cells were sufficiently hyperpolarized to drive net K uptake through channels (Hirsch *et al.*, 1998). It is quite feasible that because K fluxes usually represent a large fraction of the depolarizing membrane conductance, K starvation could induce hyperpolarization beyond the PD needed for passive K uptake. The cytosolic K concentrations used in these calculations may also be overestimates of the true concentrations under low external K conditions, and this would reduce the degree of hyperpolarization needed to support uptake via channels.

Regulation of K uptake is still poorly understood. It has been shown that plants respond to low concentrations of K by increasing the capacity for high-affinity K transport (White, 1997), which might be due either to an increase in the number of transporters or to a higher activity of existing transporters. However, the signal that evokes this increase in transport activity is unclear. The cytosolic K concentration is relatively unresponsive to variations in K supply (Walker *et al.*, 1996) and so the signal must come either from changes in the external or vacuolar K concentrations or from parameters related to plant K status, most likely mediated by hormones. In the case of Shaker-type K channels, these signals must ultimately be translated into changes in membrane voltage that regulate the opening of both influx (by hyperpolarization) and efflux (by depolarization) channels (Maathuis and Sanders, 1997).

B. Calcium

Calcium has chemical attributes that give it a special role in controlling many aspects of plant growth and development. High Ca concentrations are incompatible with metabolism because of the insolubility of Ca-P (Sanders *et al.*, 1999). A major consequence of this is that symplastic movement of Ca is restricted. Uptake by roots occurs preferentially at the root apex where the lack of a tight endodermal barrier allows apoplastic movement of Ca into the xylem (Harrison-Murray and Clarkson, 1973), from which it is distributed to the rest of the plant. Subsequent symplastic redistribution of Ca in phloem to developing reproductive structures is often inadequate, leading to the development of a range of physiological disorders induced by Ca deficiency (Shear, 1975).

Ca is rather unusual among plant nutrients in that most Ca is localized outside of the cell where it has an important role in stabilizing cell walls by cross-linking acidic side groups on adjacent pectic polysaccharide molecules. In plants without pectin in cell walls, essentiality for Ca is difficult to demonstrate (O'Kelley, 1968). This low intracellular requirement emphasizes the fact that the most important role for Ca, in signaling, depends more on a lack of Ca rather than on its abundance. Ca is normally maintained at submicromolar levels and small variations in these levels trigger physiological responses mediated via Ca-binding proteins (Sanders *et al.*, 1999). Changes in cytosolic Ca have been observed in plants in response to numerous environmental stimuli including mechanical disturbance, red light, cold, water status, and hormones (Sanders *et al.*, 1999). It has been proposed that differences in amplitude, duration, frequency, and spatial distribution of Ca signals are used to generate a range of different signals that activate specific cellular responses (Allen *et al.*, 2001).

The potency with which the cytosolic Ca level can dictate physiological responses requires control mechanisms that are able to be activated within the relevant time frames, as well as the flexibility to generate a complex range of Ca levels. Rapid increases in cytosolic Ca are effected by opening of ion channels in the plasma membrane as well as by similar channels on endomembranes that release Ca stored in organelles. Return to the low resting Ca level is achieved by Ca pumps, again operating on both plasma membrane and organellar membranes (Sze *et al.*, 2000).

Ca uptake must represent a balance between supply for nutritional purposes and short-term fluxes needed to satisfy signaling requirements. There is a vast range of cation channels in plant plasma membranes, many of which allow the permeation of a range of metal cations (Demidchik *et al.*, 2002b; Very and Sentenac, 2002). These so-called *n*on*s*elective *c*ation *c*hannels (NSCCs) include various classes of voltage-sensitive channels, channels gated by cyclic nucleotides or glutamate, as well as mechanosensitive channels, most of which have been reported to allow entry of Ca (Demidchik *et al.*, 2002b). The primary classification of these channels relates to their voltage sensitivity: hyperpolarization activated (HACCs), depolarization activated (DACCs), or voltage independent (VICCs). Each type of channel is permeable to a wide range of both mono- and divalent cations. Consideration of normal resting PDs in cells suggests that basal influx of Ca for nutritional purposes occurs through VICCs that open at these potentials. Higher demand for Ca in active cells with more hyperpolarized potentials may be met by influx through HACCs, while membrane depolarization would allow entry through DACCs for signaling purposes (Demidchik *et al.*, 2002a). Candidate genes for all three types of channels have been identified, but more work will be required to demonstrate their true roles (White *et al.*, 2002). Genes for highly Ca-selective channels on the plasma membrane

have not been identified and indeed may not exist in plants. The current situation is summed up by Demidchik *et al.* (2002b), who note that "there is no evidence in plants for the evolution of any highly selective cation channels except K^+ channels."

Measurements of Ca fluxes in intact plant cells are rare, mainly because of the problems associated with the extensive binding of Ca in cell walls that tends to obscure actual entry of Ca into the symplasm. Reid *et al.* (1997) circumvented these methodological difficulties by using giant-celled charophyte algae, which permit easy separation of cell wall from cell contents. Membrane depolarization by either voltage-clamp or high K concentrations resulted in a rapid increase in ^{45}Ca influx at PDs more positive than about −100 mV (Fig. 1). This stimulated influx began to turn off again as the membrane was depolarized to values more positive than about −50 mV (Reid *et al.*, 1997), which is broadly consistent with the pattern of electrical currents observed during activation and deactivation of depolarization-activated channels in patch-clamp studies (Miedema *et al.*, 2001). The K_m for Ca uptake into *Chara* cells depolarized by K was 1.23 m*M* (Reid *et al.*, 1997).

Chara cells are also electrically excitable and show quantized fluxes during action potentials that exceed the resting flux by up to 2000-fold (Reid *et al.*, 1997), which suggests that in normal (hyperpolarized) cells only a very small fraction (<0.1%) of the available Ca channels would be open. The data on Ca fluxes in mature *Chara* cells collected from two studies (Reid and Smith, 1992; Reid *et al.*, 1997) were used to construct a model of cellular Ca fluxes

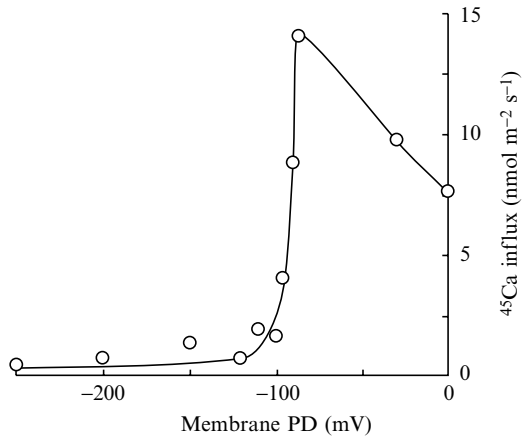

FIG. 1 Voltage dependence of ^{45}Ca influx into cells of the giant alga *Chara*. The membrane PD was shifted using voltage-clamp techniques. (Redrawn from data in Reid *et al.*, 1997.)

(Fig. 2). A notable feature of the model was the efflux of Ca across the plasma membrane that accounted for 75% of influx. This large efflux is consistent with a pump and leak system for regulating net Ca fluxes. Efflux from the cell occurs against both strong electrical and concentration gradients and is mediated by Ca-ATPases. These Ca pumps generally have high affinity for Ca ($K_m = 0.1$–$0.2\ \mu M$) but have low capacity (Sze *et al.*, 2000). This latter feature would make them unsuitable for resisting rapid changes in cytosolic Ca level, but may be sufficient to compensate for continual slow leakage of Ca across the plasma membrane. More rapid removal of Ca from the cytosol probably occurs through H^+/Ca^{2+} antiporters on the tonoplast and endoplasmic reticulum membranes. H^+-coupled antiporters have a much lower affinity ($K_m = 10$–$15\ \mu M$) but a much higher Ca transport capacity (Schumaker and Sze, 1986). These transporters may operate in concert with the tonoplast SV channel that allows a range of metal cations into the cytosol (Pottosin *et al.*, 2001).

Molecular studies have identified a number of candidate genes for both Ca-ATPase and antiporter type proteins. H^+/Ca^{2+} *ca*lcium e*x*changer genes (CAX) with similar sequences and properties have been isolated from *Arabidopsis*, maize, and mung bean (Hirschi, 2001). There are at least 11 genes for Ca-ATPases in *Arabidopsis*, but assigning these proteins to active transport activities on specific membranes is difficult (Geisler *et al.*, 2000).

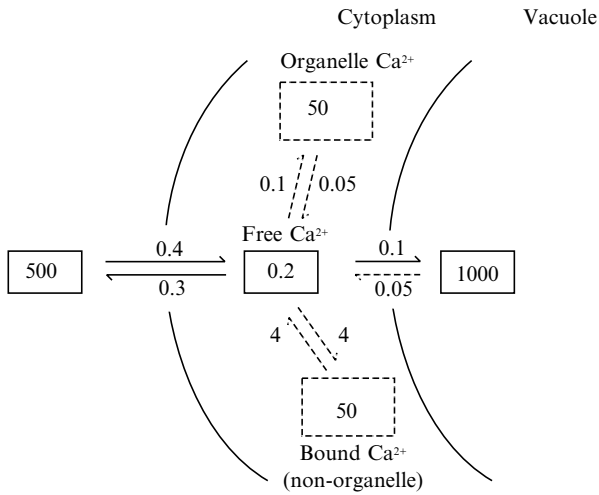

FIG. 2 Theoretical model for Ca^{2+} fluxes in normal (hyperpolarized) cells of *Chara*. Solid arrows and boxes refer to measured fluxes (in nmol $m^{-2}\ s^{-1}$) or concentrations (in μM), respectively. Dashed lines and boxes refer to fluxes and concentrations inferred by adjusting parameters to fit the flux data given in Reid and Smith (1992) and Reid *et al.* (1997).

C. Magnesium

Magnesium is present in plant cells in high concentrations and has a range of important functions: it is a cofactor in reactions involving ATP, stabilizes DNA and RNA molecules and cellular membranes, and is a component of chlorophyll. Despite this, our understanding of Mg uptake is very poor, with almost nothing reported in the literature about the mechanism of Mg entry into plant cells.

As with Ca, Mg is understood to enter plants largely via apoplastic routes and most uptake of Mg may occur at root apices where the endodermis is unsuberized and allows apoplastic movement into the xylem (Ferguson and Clarkson, 1976). The predominance of apoplastic movement was recently demonstrated by Kuhn *et al.* (2000) for both Mg and Ca, using sensitive analyses of cryosubstituted cross sections of root for stable radioisotopes. ^{25}Mg and ^{44}Ca in the cortical apoplast and the external solution equilibrated rapidly with a half-time of about 3 min whereas the half-time for movement into the stele was comparatively very slow (100–120 min), and entry into the cortical cytosol was undetectable (Kuhn *et al.*, 2000).

Presumably, Mg enters cells via low-selectivity channels in a manner similar to Ca. Some of the characterized Ca channels on the plasma membrane are reported to also show permeability to Mg, whereas others do not (White, 2000). There are very few reports of investigations into the mechanism of Mg movement across plant cell membranes, and all of these relate to vacuolar membranes. Pfeiffer and Hager (1993) identified a Mg^{2+}/H^{+} antiporter on tonoplast membranes of *Zea mays*. Previously, this had been characterized as a Ca^{2+}/H^{+} antiporter, but it was considered unlikely that Ca^{2+}/H^{+} antiport activity would occur across the tonoplast membrane *in vivo*, given that cytosolic Ca concentrations are usually very low. Significant Mg^{2+}/H^{+} antiport activity, however, could be demonstrated across a range of Mg concentrations that are physiologically relevant (Pfeiffer and Hager, 1993). Similarly, Amalou *et al.* (1992) characterized $Mg^{2+}/2H^{+}$ antiport activity in tonoplast membrane vesicles from *Hevea brasiliensis*, the latex rubber plant. They were able to physically separate Mg from Ca transport to show that different proteins were responsible for uptake of the two cations (Amalou *et al.*, 1994). However, because the cytosol of *H. brasiliensis* is highly specialized for accumulation of latex, it is uncertain whether the findings of Amalou and co-workers can be generalized to describe Mg uptake activity across the tonoplast membrane for all higher plants.

In support of the biochemical studies, a gene encoding a vacuolar Mg^{2+}/H^{+} exchanger, *AtMHX*, was cloned and characterized from *Arabidopsis* (Shaul *et al.*, 1999). Gene expression in tobacco cell lines resulted in significant electrogenic Mg^{2+}/H^{+} and Zn^{2+}/H^{+} exchange activities in vacuolar

patches. AtMHX was considered to be involved in the control of Mg and Zn distribution within the plant (Shaul *et al.*, 1999). Recently, a novel family of at least 10 putative Mg transporter genes was identified in *Arabidopsis* (Li *et al.*, 2001). The *AtMGT1* gene appeared to localize to the plasma membrane of plant cells, and conferred high-affinity Mg transport activity when expressed in bacteria, similar to that of the well-characterized CorA, a high-affinity Mg transporter in *Salmonella typhimurium* (Smith and Maguire, 1998). It will be interesting to see if the *AtMGT* genes also encode high-affinity Mg transport activity across the plasma membrane *in planta*, to identify the tissues in which they are expressed, and to determine the relative importance of a high-affinity transport system for Mg uptake. Although there is a vast lack of physiological knowledge about Mg uptake and transport in plants, further characterization of the *AtMGT* family may help to remedy this.

D. Phosphorus and Sulfur

Uptake of the macronutrient anions phosphate (P_i) and sulfate are active processes, each involving membrane-spanning, proton-coupled symporters acting against very large electrical and concentration gradients (Smith *et al.*, 1995; Daram *et al.*, 1998). Phosphate concentrations in the soil solution, for example, are typically only 1–10 μM, whereas cellular concentrations may be more than 10 mM (Mimura, 1999). The driving force required to overcome such large electrochemical gradients is provided by the coupling of symport activity with ATP-driven H^+-pumps in the plasma membrane. For both anions, kinetic studies have identified dual-affinity uptake systems that appear to result from activities of different transporters. Correspondingly, genes encoding both high- and low-affinity P_i and sulfate transporters have been identified. The high-affinity transporters may be of particular importance at the level of influx across root cell membranes, whereas low-affinity transporters are thought to be primarily involved in the redistribution of P_i and sulfate within the plant (Smith, 2001). Investigations into the specific localization of expression of high- and low-affinity transporter genes generally support this hypothesis.

There are large numbers of plant sulfate and P_i transporters. In *Arabidopsis*, for example, there are at least 12 genes encoding putative sulfate transporters and 11 gene sequences that are putative phosphate transporters (http://www.cbs.umn.edu/arabidopsis/). Although there may be some redundancy, many of the characterized genes have unique patterns of expression (e.g., Table II), suggesting specific roles in the uptake, transport, and redistribution of sulfate and P_i for each transporter protein. Several P_i transporters have been shown to be expressed exclusively in the periarbuscular

TABLE II

Patterns of Expression in *Arabidopsis* for Nine Members of the *Arabidopsis* Phosphate Transporter Gene Family, *AtPht1*[a,b]

	Roots		Tissue type				
Gene name	+P	−P	Cotyledons	Young leaves	Old leaves	Flowers	Siliques
Pht1;1[c]	++	+++	+	+	−	−	−
Pht1;2	+	+++	−	−	−	−	−
Pht1;3	+	+++	−	+	−	−	−
Pht1;4[c]	+	+++	+	−	−	+	+
Pht1;5	−	+	++	−	+	+	−
Pht1;6	−	−	+	−	−	++	−
Pht1;7	−	+	−	−	−	++	−
Pht1;8	−	+	−	−	−	−	−
Pht1;9	−	+	−	−	−	−	−

[a]Extracted from Mudge *et al.* (2002).

[b]Expression was detected either by reverse transcription polymerase chain reaction (RT-PCR) or visual analyses of promoter-reporter gene (β-glucuronidase or green fluorescent protein) fusion experiments. −, expression not detected; +, low expression; +++, high expression.

[c]Only *Pht1;1* and *Pht1;4* have been functionally characterized as P_i transporters.

membranes of root cells infected with mycorrhizas (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002), implying that these transporters function in the transfer of P_i from the mycorrhizal fungus to the plant. Recently, a Na^+–P_i cotransport system was identified and characterized in the giant-celled alga *Chara corallina* (Reid *et al.*, 2000). Although Na^+-P_i symporters are known to occur in yeast and animal systems, this is the first report of Na-dependent P_i uptake in plant cells. Genes of putative Na^+-P_i cotransporters have been reported in *Arabidopsis*. The Pht2 family of P_i transporters shows strong sequence similarity to Na^+-P_i genes of known function from other organisms (Daram *et al.*, 1999; Versaw and Harrison, 2002). However, Pht2;1 from *Arabidopsis* and all other P_i transporters from higher plants that have so far been functionally characterized are H^+-P_i symporters. Similarly, functional analyses of sulfate transporters indicate that these are H^+-sulfate cotransporters (Smith *et al.*, 1995; 1997).

Regulation of the uptake of sulfate and P_i appears to be via the internal nutrient status of the plant. Gene expression and kinetics studies both suggest that the high-affinity P_i and sulfate transporters are more responsive

to nutrient status than low-affinity transporters. When plants are deprived of a supply of external S, there is a rapid increase in the capacity for uptake of sulfate, coupled with increased levels of mRNA encoding high-affinity sulfate transporters (Smith *et al.*, 1997; Vidmar *et al.*, 1999). Glutathione is a product of S assimilation that is phloem mobile and an important signal for the S nutrition status of the plant (Herschbach and Rennenberg, 2001). High levels of S or glutathione in the plant lead to a decrease in sulfate uptake, resulting in a tight, regulatory feedback mechanism of control of uptake. Split root studies in solution culture in which a part of the root is subjected to a change in S supply suggest that there is systemic regulation of the uptake of sulfate, and that a long-distance signaling compound such as glutathione or cysteine is involved, leading to changes in sulfate uptake across the whole root system (Lapparteint and Touraine, 1996). Sulfate transporters have also been shown to be up-regulated by increased levels of precursor molecules for S assimilation (Smith *et al.*, 1997).

Similarly, the capacity for P_i uptake and the expression of P_i transporter genes (Dong *et al.*, 1999; Mudge *et al.*, 2002) both increase following removal of P from the growth medium. The signals involved in controlling P_i uptake are presently not known, although plant hormones have been suggested as possible candidates (Abel *et al.*, 2002). There is good evidence for control of uptake both at the level of gene expression and physiologically via inhibition and activation of the activities of P_i transporters and the H^+-pump (Mimura, 2001). In contrast to S, there is often a delay of several days between removal of P from the external medium and gene induction or increased P_i uptake (Dong *et al.*, 1999), supporting the general hypothesis that P_i uptake is regulated by the internal P status of the plant but that a sustained period of starvation is necessary to deplete vacuolar P_i levels.

There is some confusion, however, as to whether the uptake of P_i is controlled only by the nutrient status of the whole plant, or if there is also localized regulation. Split root experiments have shown that when part of the root system is exposed to P, P_i uptake in roots not exposed to P also changes (Drew and Saker, 1984; Liu *et al.*, 1998). This implies that there is systemic control of uptake. In soil, however, P-deficient plants of *Artemisia tridentata* and two *Agropyron* species showed rapid local increases in P_i uptake capacity where roots were in contact with P-rich soil patches (Jackson *et al.*, 1990). Recent experiments with *Chara* cells also showed that a very low concentration of external P_i initially induced higher P_i uptake activities than complete P starvation (Mimura *et al.*, 2002). One possible explanation is that there is a dual mechanism of control: that plants of very low nutrient status can perceive low concentrations of external P_i and respond via a local increase in uptake capacity, but that a high internal nutrient status in the plant represses uptake and overrides localized control. Further research into the mechanisms of regulation of P_i uptake is

warranted, to determine the extent to which local changes in nutrient supply can influence uptake.

The uptake of P_i is further mediated by root-derived processes that increase the availability of mineral and organic forms of soil P in the rhizosphere. Like P_i uptake itself, these processes are responsive to P deficiency and include rhizosphere acidification and the exudation of organic acids and phosphatases that can solubilize precipitated forms of P and release P_i from organic P compounds, respectively.

E. Nitrogen

Of all of the essential mineral nutrients, plants require N in the greatest amounts. Nitrogen is largely taken up by plants in either of two forms: nitrate (NO_3^-) or ammonium (NH_4^+). Small amounts of the free base NH_3 may also move into cells by passive diffusion in parallel with NH_4^+ transport, but at physiological pH the protonated form, NH_4^+, predominates. Although NO_3^- is normally the most abundant species of inorganic nitrogen in soil, plants can acquire N from the two sources simultaneously using different transport systems. When both forms of N are available in similar quantities, there is often a preference for uptake of NH_4^+ over NO_3^- (Kronzucker *et al.*, 1997; Garnett and Smethurst, 1999; Rroço and Mengel, 2000). This may reflect the lower energy requirement for assimilation of NH_4^+.

Nitrogen can also be taken up directly in reduced forms, such as amino acids, peptides, purines, or urea (Williams and Miller, 2001). Organic N may be an important source of N in tundra, forests, and other environments where there is a limited supply of inorganic N (Kielland, 1994; Nasholm *et al.*, 1998). Despite its potential importance, very little is presently known about either the mechanism or the control of uptake of organic N. Recently, an active transporter for urea was cloned from Arabidopsis (Liu *et al.*, 2003). Kinetic studies show that there is biphasic uptake of NH_4^+ into cells, with high- and low-affinity components. The high-affinity system is saturable with low K_m values for NH_4^+ (7–50 μM NH_4^+, Shelden *et al.*, 2001), whereas the low-affinity system is linear for concentrations higher than ~1 mM (Wang *et al.*, 1994; Kronzucker *et al.*, 1996). There is considerable debate with respect to the need for active uptake of NH_4^+ at low extracellular concentrations. The controversy centers around measurements of cytosolic concentrations of NH_4^+. Howitt and Udvardi (2000) suggest that cytosolic NH_4^+ is likely to be in the micromolar range based upon the high affinity of the primary assimilatory enzyme, glutamine synthetase (K_m <10 μM NH_4^+; Lea, 1993), and a single report of low cytosolic NH_4^+ concentrations (≥15 μM for external concentrations of <5 mM NH_4^+; Roberts and Pang, 1992).

They propose that NH_4^+ uptake occurs via a uniport system (Howitt and Udvardi, 2000). Highly selective amine uniports with K_m values in the low micromolar range have been described in *Chara* (Walker *et al*., 1979a,b) and also in tomato (Ludewig *et al*., 2002). On the other hand, Britto *et al*. (2001a) argue that active transport of NH_4^+ is necessary if cytosolic NH_4^+ concentrations are in the millimolar range, as is mostly reported (Wang *et al*., 1993; Kronzucker *et al*., 1995; Wells and Miller, 2000).

Six putative NH_4^+ transporter genes have been identified in the *Arabidopsis* genome (http://www.cbs.umn.edu/arabidopsis/). Those that have been characterized show specific patterns of expression in different tissue types and in response to environmental conditions, and different substrate affinities when expressed in yeast mutants (Gazzarrini *et al*., 1999), indicating unique roles for each transporter in NH_4^+ nutrition. One transporter gene, *AtAMT1;2*, shows dual affinity uptake when expressed in yeast (Shelden *et al*., 2001) and may facilitate NH_3 diffusion. Howitt and Udvardi (2000) have suggested that whereas the high-affinity system transports NH_4^+, the low-affinity component may represent passive movement of NH_3 across the plasma membrane. Given that very low concentrations of free NH_3 exist at normal growth pH, however, it would seem more likely that low-affinity NH_4^+ uptake is mediated by cation channels on the plasma membrane, as proposed by White (1996). Various studies have identified nonselective cation channels that show permeability to NH_4^+ similar to that of other macronutrient cations such as K and Ca. Voltage-independent cation channels from roots of both rye (White and Tester, 1992) and wheat (Davenport and Tester, 2000) have been studied in artificial planar lipid bilayers and have been shown to be permeable to a range of monovalent cations with the selectivity sequence $NH_4^+ > Rb^+ \geq K^+ > Na^+$. Similar permeabilities were obtained in a patch-clamp study of *Arabidopsis* root protoplasts (Demidchik and Tester, 2002). Ammonium channels have also been detected on the symbiosome membrane of legumes (Roberts and Tyerman, 2002), where they serve to deliver fixed N from bacteria to plant cells. Finally, aquaporins may also facilitate NH_3 transport (Tyerman *et al*., 2002).

The uptake of NO_3^- occurs via NO_3^-/H^+ cotransporters, which are dependent on maintenance of the proton motive force generated by H^+-ATPases. Cellular NO_3^- must first be reduced to NH_4^+ before assimilation into organic compounds—this extra metabolic step may explain the preference by many plants for NH_4^+ uptake when both forms of N are supplied. Some of the NO_3^- taken up is assimilated immediately in the roots, or it can be transported to the shoot (in contrast, NH_4^+ assimilation occurs exclusively in the roots). NO_3^- may also be stored in the vacuole, which may serve to buffer cytosolic concentrations within a relatively constant range (Miller and Smith, 1996). However, there is currently little agreement as to the actual level of NO_3^- in the cytosol (Britto and Kronzucker, 2003).

Physiological studies point to there being three systems for uptake of NO_3^-, two high-affinity systems (one is inducible by initial exposure to low levels of NO_3^-, whereas the other is constitutive) and a low-affinity component. On the molecular front, two families of NO_3^- transporter genes have been identified in plants (Forde, 2002). There are seven members of the *NRT2* family of transporters in *Arabidopsis* (Orsel *et al.*, 2002) believed to encode high-affinity, NO_3^--inducible transporters on the basis of correlations between uptake activity and patterns of gene expression in response to N treatment and analyses of generated mutants (Zhuo *et al.*, 1999; Vidmar *et al.*, 2000b; Crezo *et al.*, 2001). As yet, expression of plant *NRT2* genes in heterologous systems to demonstrate NO_3^- transport activity has not been reported. Members of the NRT1 family of transporters have been shown to have low-affinity uptake activities in heterologous expression systems (Lin *et al.*, 2000), although dual-affinity uptake has been identified for some *NRT1* genes (Liu *et al.*, 1999). The general characteristics of NH_4^+ and NO_3^- transporters are shown in Table III.

Regulation of N uptake is complex, with carefully controlled integration of NH_4^+ and NO_3^- uptake, and occurs at both genetic and physiological levels. In leguminous plants, where most of the required N is obtained from close associations with N_2-fixing bacteria in root nodules, the regulatory system is even more complicated and is likely to involve proteins and signals specific for the transfer of NH_4^+ across the peribacteroid membrane (Tyerman *et al.*, 1995; Marini *et al.*, 2000).

As with the uptake of other nutrients, NH_4^+ and NO_3^- uptake activities are both strongly down-regulated by a high plant N status. Although evidence in the literature is contradictory, recent conclusions are that downstream metabolites of NO_3^- and NH_4^+ uptake are the effectors of this down-regulation, rather than high levels of the ions themselves (Glass *et al.*, 2002). Glutamine was found to be the most likely candidate for regulating expression of NRT2 (Vidmar *et al.*, 2000b), but other amino acids have also been found to influence uptake (Muller and Touraine, 1992). One striking feature of NO_3^- uptake is that it is stimulated, by as much as 30-fold (Siddiqi *et al.*, 1990), on exposure to low levels of NO_3^-. Prolonged NO_3^- exposure raises the N status of the plant so that NO_3^- uptake rates are soon down-regulated as a consequence of tight, negative feedback control of uptake. However, initial activation of the NO_3^- uptake system seems to require preexposure to NO_3^- itself. The question posed is whether this phenomenon is unique to N because the plant encounters choices in terms of what form of N is taken up (i.e., NH_4^+, NO_3^-, organic N, etc.), or is there a similar induction of transporters by low levels of other nutrient ions, as we have suggested may be the case for P_i?

Both NH_4^+ and NO_3^- transport are also regulated diurnally, with maximum uptake rates recorded during the light period (Gazzarrini *et al.*, 1999;

Ono *et al.*, 2000). Because addition of sucrose to the growing medium can reduce the diurnal effect (Lejay *et al.*, 1999), the supply of carbon precursors is thought to influence uptake of N to some degree. Regulation may further be achieved through the efflux of NH_4^+ and NO_3^-, as significant efflux of both N species from plant roots is reported (Forde and Clarkson, 1999; Lejay *et al.*, 1999; Mata *et al.*, 2000; Britto *et al.*, 2001b). Currently, little is known about when and how N efflux occurs, and the exact role this plays.

IV. Micronutrients

A. Micronutrient Metals

1. Low-Affinity Uptake

Uptake of micronutrient metals (Fe, Cu, Mn, Zn, and Ni) has not been well characterized. Their low internal requirements and low extracellular concentrations mean that membrane fluxes will be correspondingly low. This presents various technical difficulties in their measurement, not the least of which is distinguishing between the considerable binding to cell walls and the fluxes across the plasma membrane. As a result, there exist few reliable data on trace metal uptake into intact plant cells, and evidence for particular transport mechanisms is therefore largely indirect. The nonselective channels discussed above in relation to uptake of Ca also allow entry of other divalent cations and may represent the dominant pathway for uptake of trace metals in the low-affinity range. Both HACCs and DACCs from roots have been shown to transport various trace metals (see catalogue of studies in Demidchik *et al.*, 2002); permeation through VICCs has not been extensively studied, but because these are the only NSCCs open at normal resting PDs, their involvement in trace metal nutrition seems highly probable.

Reid *et al.* (1996) described the characteristics of uptake of Zn into giant algal cells. They obtained evidence for both high-affinity and low-affinity Zn uptake systems and characterized the latter. At concentrations higher than about 1 μM, Zn influx was nonsaturating up to at least 200 μM, and was inhibited by various cations including Ca, Na, K, Mn, and Fe(II). Zn influx was insensitive to membrane PD and was not significantly increased by opening of depolarization-activated Ca channels, or inhibited by blockers of Ca and K channels (Reid *et al.*, 1996). All of these features are consistent with low-affinity uptake of Zn through voltage-insensitive NSCCs.

There are many other examples of competition between trace metals for uptake and also between trace metals and macronutrient cations. For example, Mn was shown to strongly inhibit Mg uptake and vice versa, whereas high K inhibited both Mn and Mg uptake (Heenan and Campbell,

TABLE III
Characteristics of Some Identified Plant NO_3^- and NH_4^+ Transporters

Gene family	Species	Gene	Expression pattern in tissues[a]								K_m[b]	Reference
			Roots				Shoots					
			Expressed?	Low N	NO_3^-	NH_4^+	Expressed?	Low N	NO_3^-	NH_4^+		
AMT1	*Arabidopsis thaliana*	*AtAMT1;1*	Yes	+			Yes				8–32 μ*M* MA[c]	Gazzarrini *et al.* (1999); Shelden *et al.* (2001)
											16–25 μm NH_4^+	Sohlenkamp *et al.* (2002)
	A. thaliana	*AtAMT1;2*	Yes		Not inducible		No				24–36 μ*M* MA/3.0 m*M* MA[d]	Gazzarrini *et al.* (1999); Shelden *et al.* (2001)
	A. thaliana	*AtAMT1;3*	Yes	+			No				11 μ*M* MA	Gazzarrini *et al.* (1999)
	Lycopersicon esculentum	*LeAMT1;1*	Yes (especially root hairs)	+			No				8–22 μ*M* NH_4^+	von Wirén *et al.* (2000); Ludewig *et al.* (2002)
	L. esculentum	*LeAMT1;2*	Yes (especially root hairs)		+	+	Yes					von Wirén *et al.* (2000)
	L. esculentum	*LeAMT1;3*	No				Yes (leaves)		+			von Wirén *et al.* (2000)

AMT2	*A. thaliana*	*AtAMT2*	Yes	+			Yes			20 μ*M* NH_4^+	Sohlenkamp *et al.* (2002)
NRT1	*A. thaliana*	*AtNRT1.1*	Yes		+		?			50 μ*M* NO_3^-/ 4–8.5 m*M* NO_3^-	Huang *et al.* (1996); Liu *et al.* (1999)
	A. thaliana	*AtNRT1.2*	Yes		Not inducible		?			5.9 m*M* NO_3^-	Huang *et al.* (1999)
	L. esculentum	*LeNRT1-1*	Yes		Not inducible		?				Lauter *et al.* (1996)
	L. esculentum	*LeNRT1-2*	Yes (root hairs)			+	?				Lauter *et al.* (1996)
	Oryza sativa	*OsNRT1*	Yes		Not inducible		No			9 m*M* NO_3^-	Lin *et al.* (2000)
	Brassica napus	*BnNRT1;2*	Yes		+		?			4–14 m*M* NO_3^-/ 1.5–25 m*M* histidine	Zhou *et al.* (1998)
NRT2	*A. thaliana*	*AtNRT2.1*	Yes (older seedlings)	+	+	–	?				Zhou *et al.* (1999); Orsel *et al.* (2002)
	A. thaliana	*AtNRT2.2*	N/D[e]				N/D				Orsel *et al.* (2002)
	A. thaliana	*AtNRT2.3*	Yes	+			Yes	+			Orsel *et al.* (2002)
	A. thaliana	*AtNRT2.4*	Yes	+			No				Orsel *et al.* (2002)
	A. thaliana	*AtNRT2.5*	Yes	+			No				Orsel *et al.* (2002)

(continued)

TABLE III (*continued*)

Gene family	Species	Gene	Expression pattern in tissues[a]								K_m[b]	Reference
			Roots				Shoots					
			Expressed?	Low N	NO_3^-	NH_4^+	Expressed?	Low N	NO_3^-	NH_4^+		
	A. thaliana	*AtNRT2.6*	Yes	+			No					Orsel *et al.* (2002)
	A. thaliana	*AtNRT2.7*	No				Yes	+				Orsel *et al.* (2002)
	Hordeum vulgare	*HvNRT2*	Yes		+	–	No					Vidmar *et al.* (2000a)
	Glycine max	*GmNRT2*	Yes	+	+	–	?					Amarasinghe *et al.* (1998)
	Nicotiana plumbaginifolia	*NpNRT2.1*	Yes		+	–	No					Krapp *et al.* (1998)

[a] +, enhanced gene expression; –, reduced gene expression.

[b] K_m values were obtained in heterologous expression systems, which may or may not reflect *in planta* characteristics. Kinetic data have not yet been obtained for the NRT2 gene family (Forde, 2000).

[c] MA, methylammonium (analogue of NH_4^+). Electrical measurements indicate that NH_4^+ transporters are likely to have a lower affinity for MA compared to NH_4^+ (Shelden *et al.*, 2001; Ludewig *et al.*, 2002).

[d] Shelden *et al.* (2001) reported dual affinity uptake activity for AtAMT1;2, whereas Gazzarrini *et al.* (1999) measured only a single K_m.

[e] N/D, not detected. RT-PCR methods failed to detect transcripts of the *AtNRT2.2* gene.

1981). Such interactions also point to the involvement of a nonselective cation uptake mechanism that facilitates entry of a broad spectrum of essential and nonessential metals.

This lack of selectivity has consequences both for regulation of intracellular metal concentrations and for the unintended uptake of toxic species such as Co, Cd, and Pb. Liu (1998) showed that Co uptake might occur through three transport systems, with both saturating and nonsaturating kinetics. Uptake through the low-affinity system was sensitive to most other divalent metals (Fig. 3), suggesting that uptake was mediated by a generic, divalent cation transporter.

Broad-spectrum transporters also bring into question the mechanism of control of uptake for individual nutrients. For a selective channel or carrier, it is easy to envisage feedback regulation linked to some internal or external level but in the case of multiple substrates such feedback signals could easily conflict. Moreover, competition for transport sites would favor the uptake of those nutrients at the higher concentrations, at the expense of those whose supply was limiting, thereby exacerbating nutrient deficiencies. In fact, this is precisely what seems to happen, and unbalanced nutrient supply is commonly

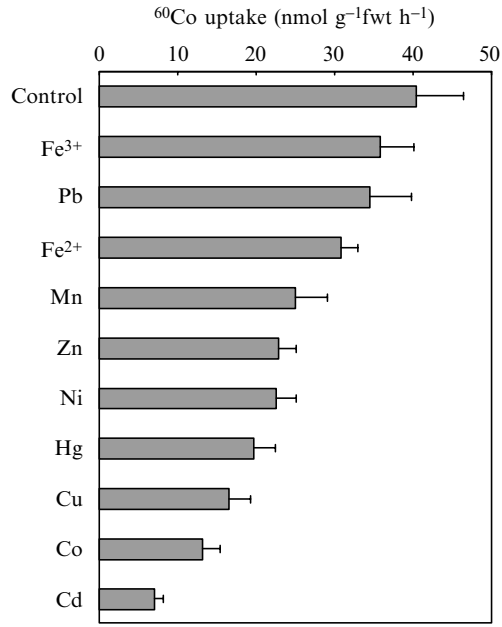

FIG. 3 Apparent competition between Co and other trace metals for uptake into roots of mung beans. Uptake was measured in a solution containing 1 μ*M* ^{60}Co over 4 h. Other trace metals were present at 5 μ*M*.

translated into internal imbalances that impact on plant growth. Assuming that low-affinity uptake of trace metals does indeed occur through a non-selective permease on the plasma membrane, two principal mechanisms for regulation of cytosolic metal concentrations exist—sequestration into the vacuole by metal/H^+ antiport or export across the plasma membrane by metal-pumping P-type ATPases (Williams *et al*., 2000). However, the widespread occurrence of toxicity of trace metals suggests that mechanisms for efflux across the plasma membrane are inadequate. Tonoplast metal cation antiporters capable of transporting Ca (Bush, 1995; Gonzalez *et al*., 1999), Cd (Salt and Wagner, 1993; Gonzalez *et al*., 1999), Mg (Amalou *et al*., 1994), and Zn and Mn (Gonzalez *et al*., 1999) have been identified in plants. The driving force for uptake into the vacuole is generated by ATPases on the tonoplast membrane. Internal detoxification by binding to phytochelatins has only been demonstrated for Cd, whereas the role of metallothioneins in heavy metal detoxification and homeostasis is still unclear (Cobbett and Goldsbrough, 2002).

2. High-Affinity Uptake

When supply of micronutrient cations is in the adequate range, uptake via a general cation carrier may be sufficient to meet nutritional requirements. However, when supply of one or more micronutrients is limited, a different strategy may be required to prevent the development of deficiency. There is increasing molecular and physiological evidence for the induction of high-affinity trace metal transport systems in response to deficiency. Three main groups have been identified—IRT, ZIP, and Nramp proteins.

IRT1 (Eide *et al*., 1996; Korshunova *et al*., 1999) expression was shown to be localized to the plasma membrane of root epidermal cells of *Arabidopsis* and was induced by Fe deficiency (Vert *et al*., 2002). An *IRT1* knockout mutant of *Arabidopsis* was chlorotic but could be rescued by high concentrations of Fe, indicating that an alternative low-affinity pathway for Fe existed in parallel with IRT1. Fe deficiency also strongly stimulated uptake of Mn, Zn, and Co but not Cu, and this stimulated uptake was absent in the mutant. Again there was evidence for a bypass pathway for uptake of these metals (Vert *et al*., 2002). These results are consistent with observations that Fe-deficient plants accumulate high concentrations of these metals (Cohen *et al*., 1998). Thus *IRT1* appears to encode a multispecific metal cation transporter that is functional in roots for high-affinity Fe uptake and is regulated by Fe status both transcriptionally and at the protein level (Connolly *et al*., 2002).

The ZIP proteins are closely related to IRT1 and appear to function as high-affinity Zn transporters controlled by genes that are up-regulated by Zn deficiency. In *Arabidopsis ZIP1, ZIP3*, and *ZIP4* are expressed in the roots

of Zn-deficient plants, whereas *ZIP4* is expressed in both shoots and roots (Grotz *et al.*, 1998). None of the ZIP proteins transports Fe (Guerinot, 2000) but ZIP1, ZIP2, and ZIP3 are inhibited by Cu and Cd, and ZIP3 also by Mn and Co (Grotz *et al.*, 1998), and could potentially act as high-affinity transporters for these other cations.

Nramps are members of a multigene family found across a broad range of organisms. Heterologous expression of the rat Nramp2 isologue DCT1 in *Xenopus* oocytes indicated that it could transport Mn, Zn, Ni, Cu, Fe, Co, and Cd (Gunshin *et al.*, 1997). Nramp homologues in yeast, SMF1 and SMF2, are primarily involved in transport of Fe and Mn (Supek *et al.*, 1996; Chen *et al.*, 1999). In plants, Nramp proteins have been implicated in the transport of Fe and Cd (Curie *et al.*, 2000; Thomine *et al.*, 2000). Five Nramp genes have been identified in *Arabidopsis*, and three in rice. In *Arabidopsis*, AtNramp1, AtNramp3, and AtNramp4 were expressed in roots and AtNramp3 and AtNramp4 in shoots (Thomine *et al.*, 2000). Each of these genes was expressed in nonstarved plants and expression in roots was increased by Fe starvation (Thomine *et al.*, 2000), suggesting that they may play a role in both low-affinity as well as high-affinity Fe uptake.

Opinions as to the likely location of Nramp proteins differ. Curie *et al.* (2000) found that overexpression of AtNramp1 protected against Fe toxicity and proposed that the transport activity was confined to endomembranes where it mediated transport and compartmentation of Fe in organelles. On the other hand, Thomine *et al.* (2000) found that overexpression of AtNramp3 increased the sensitivity of *Arabidopsis* plants to Cd toxicity, which was consistent with it being located on the plasma membrane. In the original investigation of the kinetics of DCT1, Gunshin *et al.* (1997) established that transport of Fe was associated with inward (positive) current and intracellular acidification, consistent with it being a metal/H^+ symporter. Such a mechanism in plants could take advantage of the driving force provided by the negative membrane PD and would be more effective at acid pH where the proton gradient would be inwardly directed. For micronutrient cations, the extra driving force due to coupling with H^+ would allow uptake from extremely low external concentrations. Calculations of thermodynamic gradients required for uptake of trace metals indicate that an active mechanism may be unnecessary. For example, the concentration of Zn (total) in the cytosol of *Chara* grown in medium containing 0.1 μ*M* Zn was 56 μ*M* in cells in which the membrane PD would be expected to more negative than −120 mV, a PD that theoretically could support electrophoretic uptake of Zn to 1 m*M* (Reid *et al.*, 1996). Even if the external concentration was 1 n*M*, which is the lower limit of Zn in a typical soil solution (Welch, 1995), such a PD could generate a cytosolic concentration of 10 μ*M*, and a slightly more hyperpolarized cell could easily reach the concentration measured by Reid *et al.* (1996). This calculation does not take into account the fact that the free

concentration of Zn is likely to be much lower than the total concentration because of binding of Zn to cytosolic molecules, especially proteins.

If it is assumed that all Nramps are metal/H^+ symporters, then it is difficult to envisage any role for transport at the tonoplast except to release metals back into the cytosol, since both electrical and pH gradients would favor transport out of the vacuole.

For IRT1, other ZIPs, and Nramps, there appears to be a preferred substrate and several "unintended" substrates. An interesting insight into the structural aspects of transporters that influence selectivity was provided by Rogers *et al*. (2000), who manipulated the amino acid sequence of IRT1 in *Arabidopsis* and examined transport characteristics of the mutants in yeast. The unmodified IRT1 transported Fe, Mn, and Zn and conferred Cd sensitivity. Single amino acid substitutions generated mutations in which single or multiple transport activities were knocked out, but none of the substitutions generated a mutant capable of Fe transport alone. This work highlights the sensitivity of transporters to subtle changes in protein conformation but also raises the possibility of improving the characteristics of transporters, particularly selectivity, by manipulation of amino acid sequences.

Little is known about uptake mechanisms for Ni or for Cu. A gene from *Arabidopsis* that complemented a yeast mutant deficient in Cu transport has been reported (Kamffenkel *et al*., 1995) but the location and function of the encoded protein, COPT1, have not been determined.

3. Mobilization and Reduction of Fe

For some trace metals, the major obstacle for absorption by plants is not transport across membranes but extraction of sufficient metal from the rhizosphere where complexation by soil minerals and organic matter can greatly reduce availability. This is particularly true for Fe, which in well-aerated soils occurs predominantly as Fe III, oxides of which have very low solubility (Kamffenkel *et al*., 1995). Apart from low availability, uptake of Fe is complicated by the fact that the metal transporters mentioned above require Fe to be present as the divalent cation, and Fe^{3+} must first be reduced by reductases located on the external face of the cell membrane.

Various strategies have been developed by plants to mobilize Fe and other sparingly soluble metals in the rhizosphere and to transport them into roots. Higher plants employ one of two mechanisms for the uptake of Fe. Dicotyledonous species and nongraminaceous monocots use Fe^{2+} transporters that are coupled to ferric chelate-reductase (FC-R) activity (Strategy I). Fe(III) chelate compounds are reduced by plasma membrane-bound FC-R proteins and Fe^{2+} is immediately taken into the plant. Together with Fe^{2+} transport activity, FC-R activity is enhanced under conditions of Fe deficiency (Grusak *et al*., 1990; Grusak and Pezeshgi, 1996), and FRO2, a low Fe-inducible

FC-R, has recently been identified in *Arabidopsis* (Robinson *et al.*, 1999). Proton efflux is a further component of Fe uptake in Strategy I plants. Protons are released by H^+-ATPases in the plasma membrane of root cells to acidify the rhizosphere and apoplasm, which serves both to increase the solubility of Fe(III) compounds and to stimulate FC-R activity (Toulon *et al.*, 1992). H^+-ATPase activity is stimulated under conditions of Fe deficiency, and it was also recently shown that H^+-ATPase mRNA and protein levels increase in the roots of Fe-deficient plants (Dell'Orto *et al.*, 2002).

Graminaceous monocot species employ a different uptake mechanism, known as Strategy II. Phytosiderophores (nonprotein amino acids, of which the best-known include mugeneic acid, avenic acid, and nicotianamine) are released from the roots and form soluble complexes with Fe^{3+}. Subsequently, the Fe(III)-phytosiderophore is taken up via specialized transport systems. Phytosiderophore efflux and Fe-phytosiderophore uptake are stimulated under conditions of Fe deficiency (Römheld and Marschner, 1990). Although this is the major mechanism for Fe uptake in Strategy II plants, there is also evidence for phytochelate-linked transport of other trace metals (Welch, 1995; Gries *et al.*, 1998; Hopkins *et al.*, 1998). The mechanism for Fe-phytosiderophore uptake is presently not known. YS1 was recently identified in *Zea mays* (a Strategy II species) as a membrane protein that mediates Fe uptake (Curie *et al.*, 2001). The predicted structure of YS1 is consistent with that of a transporter protein and its expression is enhanced by Fe deficiency, but it has not yet been shown to confer Fe-phytosiderophore transport activity.

Significant efflux of Fe from plant cells is not reported and the uptake of Fe must therefore be tightly regulated to avoid Fe toxicity. Fe uptake processes are regulated on many levels (Schmidt, 1999). At present it is still unclear whether the Fe mobilization and reduction processes are regulated together with uptake as tightly coordinated sets of responses to Fe deficiency. Alternatively, the various responses of Strategy I and Strategy II plants may be only loosely associated and respond independently to regulatory signals. A further complication is that Strategy I and Strategy II may not be mutually exclusive in plants, given that homologues of *YS1* have been found in *Arabidopsis*, a Strategy I species (Curie *et al.*, 2001), and that some species of Strategy II plants have plasma membrane-associated FC-R activity (Bagnaresi and Pupillo, 1995).

B. Chlorine

The essentiality of Cl in plants is due to its involvement in the water-splitting system of photosynthesis. The amounts required to fulfill this function are low and consequently it is classified as a micronutrient. Cl is also commonly

employed for osmotic and electrical balance but under low Cl conditions; other anions, particularly organic acids, are effective substitutes.

The thermodynamic gradients applying to Cl^- are similar to those of other anions such as NO_3^- and PO_4^-, and it is therefore not surprising that a similar mechanism exists for transport. Studies by a number of groups have established that Cl^- is symported with protons, with a stoichiometry of either $1H^+/Cl^-$ or $2H^+/Cl^-$ depending on the conditions (Smith and Walker, 1976). In *Chara*, Cl influx is acutely sensitive to cytosolic pH, increasing as the cytosol becomes more alkaline (Sanders, 1980b; Reid and Walker, 1984). However, at pH greater than about 7.5, the driving force provided by H^+ becomes insufficient and influx declines (Sanders, 1980b). Cl influx is also sensitive to the internal Cl status; reduction of the concentration of Cl in the cytosol by starvation or intracellular perfusion results in strong stimulation of Cl influx (Sanders, 1980a). The uptake of Cl in other plant species is inhibited by NO_3 (Glass and Siddiqi, 1984), implying that there is competition between the two anions for the same anion transporter.

Efflux of Cl occurs through channels on the plasma membrane that are activated by hyperpolarization (Ryan *et al.*, 1997), suggesting that Cl may be released as a means of preventing the membrane PD from becoming so negative that it would cause the plasma membrane H^+-ATPases to stall. An inability to pump protons from the cell would limit the ability of H^+-ATPases to regulate pH and so anion channels may play an indirect role in internal pH regulation. The rapid efflux of Cl associated with cytosolic acidification by weak acids is consistent with this view (Smith and Reid, 1991). Depolarization-activated anion channels have been described in wheat root cells and these channels may function in the uptake of NO_3 and Cl at high concentrations (Skerrett and Tyerman, 1994). Little is known about the molecular structure of plant anion channels. Genes with strong sequence homology to the CLC family of transporters that act as anion channels in animal cells have been detected in plants but, so far, demonstrating a functional role for these genes in plants has proven elusive (Barbier-Brygoo *et al.*, 2000).

C. Molybdenum

Plant requirement for Mo is related to its role as a component of nitrate reductase, and for nitrogenase in rhizobial symbioses. The requirement is thus related to the nature and supply of N; plants fed adequate reduced N (e.g., as NH_4^+) have a very low Mo requirement.

Mo is believed to be absorbed into the plant as the MoO_4^- or MoO_4^{2-} anions and there is evidence for interaction between MoO_4 uptake and the uptake of P_i and sulfate (Heuwinkel *et al.*, 1992; Macleod *et al.*, 1997; Llamas

et al., 2000). In *Chlamydymonas*, uptake of MoO_4 is small and blocked by sulfate when grown on NH_4 medium but much higher and relatively insensitive to sulfate when grown on NO_3 (Llamas *et al.*, 2000). This suggests that MoO_4 may enter via the same transporter as sulfate but that there also exists a selective MoO_4 transporter that is induced by NO_3. Mo has received very little attention from plant molecular researchers. It is known that MoO_4 transport in bacteria is mediated by an ABC transporter (Neubauer *et al.*, 1999) but in view of its interactions with the transport of other anions, it seems more likely that MoO_4 uptake in plants is driven by proton symport.

D. Boron

Boron differs from all other essential nutrients in that under physiological conditions, it moves into and out of cells in an uncharged form. Boron occurs as boric acid, a small lipophilic molecule and a weak acid ($pK_a = 9.25$). In this form B is able to diffuse across lipid bilayers and has a large permeability coefficient. The theoretical membrane permeability of plant membranes to boric acid is 8×10^{-6} cm s^{-1} (Raven, 1980), whereas permeabilities of 4×10^{-7} cm s^{-1} (Dordas and Brown, 2000; Stangoulis *et al.*, 2001) have been determined experimentally. This compares to a permeability to water of 3×10^{-3} cm s^{-1} (Dordas and Brown, 2000).

The kinetics of boron uptake were determined in giant algal cells of *Chara* (Stangoulis *et al.*, 2001). Whereas the *Chara* study showed conclusively that boric acid moves freely and rapidly through the plasma membrane, influx was biphasic and suggested the existence of a component of facilitated transport of B in addition to membrane permeation. Investigations using foreign gene expression in *Xenopus* oocytes also indicated that B uptake into cells could be stimulated by the expression of particular major intrinsic proteins (MIPs, Dordas *et al.*, 2000). These proteins are types of aquaporins—proteinaceous channels found in membranes that most commonly are implicated in the transport of water, but also of other small, neutral solutes such as urea.

Recently, a gene encoding a putative boron transporter protein was isolated from *Arabidopsis* (Takano *et al.*, 2002). The *bor1-1 Arabidopsis* mutant shows symptoms of B deficiency when grown with a low supply of B, but could not be distinguished from wild-type plants at higher external concentrations (Noguchi *et al.*, 1997). Complementation of the mutant with the *BOR1* gene lowered the B requirement for normal growth. Thus, although boric acid has a high membrane permeability, a component of channel-mediated transport is implicated in the uptake of B, which is likely to be particularly important when the external supply of B is limited.

Although there is large inter- and intraspecific variation in plants in both requirement for B and the capacity for B uptake (Paull *et al*., 1988; Bellaloui and Brown, 1998), there appears to be no capacity for feedback-based regulation of uptake of B within a given plant. To date, the question of regulation of B uptake has not been directly addressed. However, toxicity studies indicate that cultivars or species that are tolerant to excessive B accumulate less B in shoots and leaves than sensitive varieties, across a wide range of external B concentrations extending from deficient to toxic levels (Nable *et al*., 1990). Some reports suggest that crude control of B uptake can be achieved by alteration of membrane lipid composition (Dordas and Brown, 2000) or by accumulation of complexing solutes such as sorbitol in cells (Bellaloui *et al*., 1999), but these are not general phenomena or mechanisms by which plants could finely control B uptake. Physiological investigations using the cloned *BOR1* gene may help to establish the basis, degree, and flexibility of control of B uptake into plants.

V. Functional Mineral Nutrient Transporters on the Plasma Membrane

Molecular studies are revealing more and more genes with potential transport functions, but many of these will undoubtedly turn out to be redundant. More functional studies are needed to sort those that are important from those that have no useful role in modern plants. In the meantime, it is difficult to be precise about how any of the nutrients is taken up. Nevertheless it is possible to make some informed guesses as to the likely routes taken by different nutrients. The summary presented in Fig. 4 lists the main groups of transporters for which some functional information is available from empirical studies. The transporters are numbered from 1 to 21 and a brief description is provided below.

Notation used in Fig. 4: M^{2+} = unspecified divalent metal cation; A^- = unspecified monovalent anion; elements in parentheses indicate that they are not the primary substrate; *n* before H^+ is used to indicate that the number of protons involved in the cotransport is either unknown or variable.

1. Potassium inward rectifying channel (KIRC) is likely to be the dominant pathway for K uptake.
2. Potassium outward rectifying channel (KORC) is activated by depolarization and would allow efflux of K from the cell.
3. Depolarization-activated cation channel (DACC) may play a role in allowing rapid entry of Ca into the cytosol for signaling purposes, but also appears to allow entry of a range of other divalent cations including many trace metals.

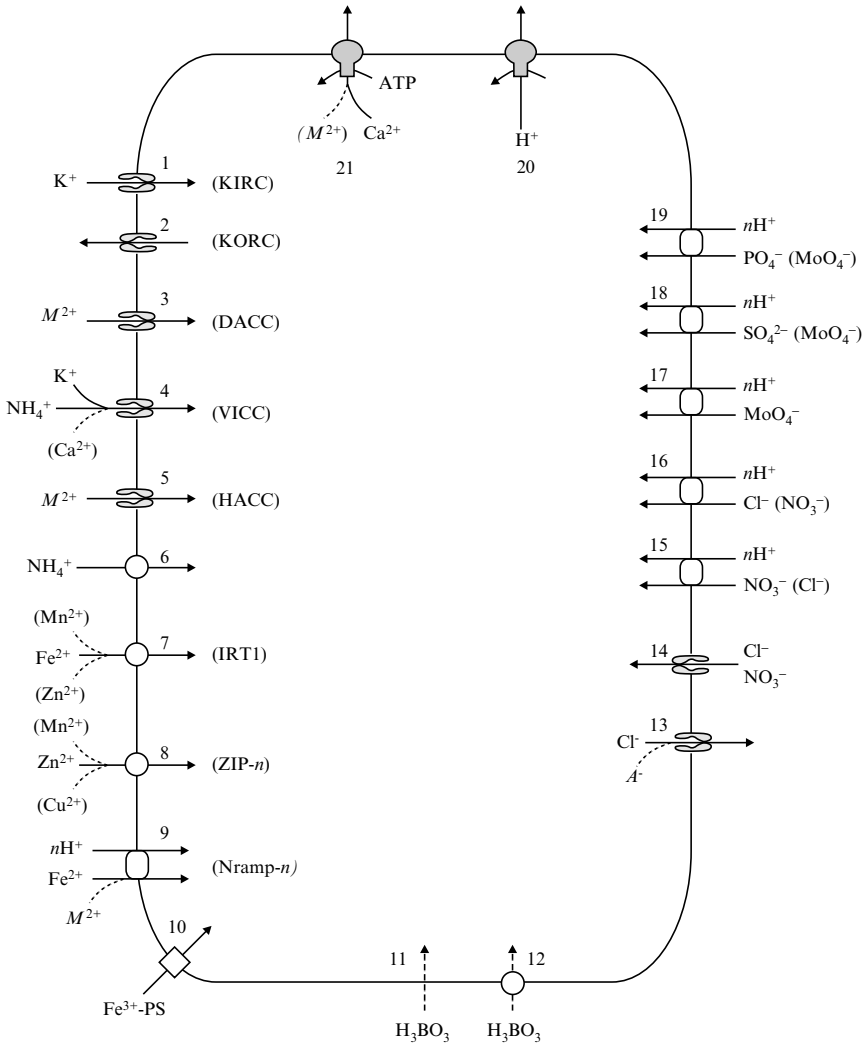

FIG. 4 Summary of the most likely mechanisms for uptake of nutrients in plants. Brief descriptions of each transporter are given in Section V. More detailed descriptions can be found in the relevant section for each nutrient.

4. Voltage-insensitive cation channels (VICC) in roots have a high permeability to NH_4 but may also provide basal fluxes of K and Ca. The permeability of these channels to trace metals is unclear but this may also be a route for their low-affinity uptake.

5. Hyperpolarization-activated cation channels (HACC) may function in the uptake of a range of divalent metals in actively growing cells.
6. High-affinity amine uniport has been described in several studies but an exact mechanism has not been identified.
7. IRT1 is induced upon Fe deficiency and may be important when the Fe concentration is low. IRT1 is also permeable to some other trace metals, particularly Mn and Zn.
8. The ZIP*n* genes are both constitutive and induced by Zn deficiency and are primarily active in the uptake of Zn, but also allow entry to other trace metals but not Fe.
9. At least some of the Nramp proteins appear to be involved in Fe transport, and possibly the uptake of other trace metals. Several Nramps are present in roots in nonstarved plants and their expression is increased by Fe deficiency. They may be important at intermediate concentrations of Fe where IRT1 is not present. Nramps have been proposed to act as metal/H^+ symporters.
10. High-affinity uptake of Fe^{3+} as a complex with phytosiderophores.
11. Diffusion of neutral boric acid through the plasma membrane is sufficiently rapid to account for B uptake except at very low external B concentrations.
12. High-affinity transporter for B activated under B deficiency.
13. Cl efflux channel that may also be responsible for efflux of other anions.
14. Anion channels that may function at high external anion concentrations (e.g., Cl^- in saline environments) or in depolarized cells.

15–19. H^+/anion symporters. It is expected that each nutrient will have a different transporter, the control of which is related to the status of that particular nutrient within the plant. The number of protons required to drive the symports is uncertain but probably varies for each nutrient, and the stoichiometry may also vary according to conditions.

20. Proton ATPases with multiple functions including generation of electrical and proton gradients, regulation of cytosolic pH, and excretion of H^+ into the rhizosphere to aid in the mobilization of sparingly soluble nutrients.
21. Ca-ATPase that acts in concert with lower-affinity Ca transporters on the tonoplast to regulate cytosolic Ca concentrations. Low selectivity may allow the export of other divalent metal cations.

VI. Concluding Remarks

Nutrient uptake in plants is a very broad topic and it has been necessary to limit our focus to covering the principal processes involved in accessing and absorbing essential nutrients from their environment. This is but one of

several layers of nutrient uptake that need to be considered in the context of development of the whole plant. Loading into the xylem and the interplay between xylem and phloem in the redistribution of nutrients, particularly under deficiency conditions, require closer scrutiny, as does the role of the vacuole in temporary and long-term storage of nutrients.

Likewise, the regulation of nutrient uptake has mostly been considered only in relation to the factors that directly affect the rate of membrane transport, neglecting the important hormonal signaling that often serves as the communication between nutrient-absorbing structures and nutrient-consuming organs. Gene control of membrane transport has been considered briefly for some transporters where empirical data exist but this is an area of research that will undoubtedly reveal much in the next decade. It is relatively easy to detect the induction or increased expression of transporter genes, but in many cases it may be that morphological changes facilitating better access to nutrients are more important than synthesis of more or higher-affinity transporters. This is particularly true of nutrients with low soil mobility such as P and Fe. The relative importance of this aspect of nutrient uptake has yet to be defined.

The picture of cellular absorption of nutrients portrayed in this review is patchy, or perhaps more correctly, fuzzy. If there is one feature of plant nutrient transport that needs to be highlighted, it is the lack of specificity of most of the membrane transport proteins that mediate influx or efflux. Perhaps this is an evolutionary compromise because design and synthesis of a full spectrum of highly selective transporters would excessively drain resources from developmental processes. The downside of this economy of resources is that plants lack the flexibility to restrict entry to toxic solutes, or when faced with high concentrations of a nutrient, have limited capacity to prevent accumulation to toxic levels. Ultimately the effectiveness of membrane transport in providing or restricting access to substances present in their environment will impact heavily on the ecological success of a plant species.

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