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Magnesium transport and function in plants: the tip of the iceberg

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Abstract

The maintenance of Mg^{2+} homeostasis in the plant is essential for viability. This review describes Mg^{2+} functions and balancing in plants, with special focus on the existing knowledge of the involved transport mechanisms. Mg^{2+} is essential for the function of many cellular enzymes and for the aggregation of ribosomes. Mg^{2+} concentrations also modulate ionic currents across the chloroplast and the vacuolar membranes, and might thus regulate ion balance in the cell and stomatal opening. The significance of Mg^{2+} homeostasis has been particularly established with regard to Mg^{2+}'s role in photosynthesis. Mg^{2+} is the central atom of the chlorophyll molecule, and fluctuations in its levels in the chloroplast regulate the activity of key photosynthetic enzymes. Relatively little is known of the proteins mediating Mg^{2+} uptake and transport in plants. The plant vacuole seem to play a key role in Mg^{2+} homeostasis in plant cells. Physiological and molecular evidence indicate that Mg^{2+} entry to the vacuole is mediated by Mg^{2+}/H^+ exchangers. The *Arabidopsis* vacuolar Mg^{2+}/H^+ exchanger, AtMHX, is highly transcribed at the vascular tissue, apparently most abundantly at the xylem parenchyma. Inclusion of Mg^{2+} ions into the vacuoles of this tissue may determine their partitioning between the various plant organs. Impacts of Mg^{2+} imbalance are described with respect for both plant physiology and for its nutritional value to animal and human.

Introduction

Mg^{2+} is the most abundant free divalent cation in the plant cytosol. The functions of Mg^{2+} in plants (as well as in other organisms) are mainly related to its capacity to interact with nucleophilic ligands. It is the central atom of the chlorophyll molecule and a bridging element for the aggregation of ribosomes. Mg^{2+} is essential for the function of many enzymes, including RNA polymerases, ATPases, protein kinases, phosphatases, glutathione synthase, and carboxylases. Key chloroplast enzymes are strongly affected by small variations in Mg^{2+} levels at the cytosol and the chloroplast, exemplifying the significance of maintaining Mg^{2+} homeostasis in plants. This review describes Mg^{2+} function in plants with special focus on the existing knowledge of plant Mg^{2+} transport mechanisms. For recent general reviews of ion transport in plants see Chrispeels *et al.* 1999; Maathuis & Sanders 1999; Martinoia *et al.* 2000; Williams *et al.* 2000; Zimmermann & Sentenac 1999.

As membranes provide a barrier to ion movements, ions are transported through specialized proteins – channels, transporters or ATPases. In plants, proton pumps are located at both the plasma membrane and the vacuolar membrane (the *tonoplast*), driving H^+ from the cytoplasm to either the extracellular space (the *apoplasm*) or to the vacuole, respectively. These pumps generate a proton-motive force, comprising gradients of both pH (ΔpH) and membrane potential ($\Delta\Psi$). The cytoplasm is less acidic and is negatively charged compared to both the apoplasm and the vacuole. In general, the pH of the plant cytosol is 7.3–7.6, that of the vacuole is 4.5–5.9, and that of the apoplasm about 5.5 (Kurdjian & Guern 1989). Besides proton gradients, $\Delta\Psi$ across the cellular membranes is also determined by the equilibrium of other ions. $\Delta\Psi$ across the plasma membrane is in general -120 to -180 mV negative in the cytosol compared to the

apoplasm (Marschner 1995). Different values have been obtained for $\Delta\Psi$ across the tonoplast. A value of -20 mV (indicating that the lumen of the vacuole is positively charged compared to the cytosol) is generally assumed to be reasonable reflection of the physiological steady-state situation (Allen & Sanders 1997). The driving force generated by ΔpH and $\Delta\Psi$ energizes secondary transport processes. Transport driven by ΔpH can occur in the same direction as the H^+ flux (H^+ symport) or in the opposite direction (H^+ antiport or exchange). Secondary ion transport can also be driven by $\Delta\Psi$.

Current knowledge of Mg^{2+} uptake in plants

Mineral uptake into plants occurs in the root. The external cell layer of the root is called *epidermis* or *rhizodermis*, and internal to this layer lies the *cortex*. The innermost cell layer of the cortex is the *endodermis*. The endodermis surrounds the *stele*, composed of a *pericycle* cell layer surrounding the vascular tissue. There are two parallel ways for radial movement of solutes from the soil solution towards the stele: the *apoplasmic* pathway, passing through the cell walls and the extracellular space, and the *symplasmic* pathway, passing from cell to cell in the cytoplasm throughout plasmodesmata (Marschner 1995). Plasmodesmata are fine cytoplasmic strands that connect the protoplasts of adjacent plant cells by passing through their cell walls. Plasmodesmata are lined by the plasma membrane of the two adjacent cells, thus allowing certain molecules to pass directly from the cytosol of one cell to another. Carboxyl groups in the cell wall continuum of root cells act as cation exchangers, to which cations diffuse from the soil solution. Solutes are not restricted by the epidermis but can also penetrate the apoplasm of the cortex. The apoplasmic pathway towards the stele is constrained by the *Casparian band*, which is a hydrophobic barrier in the walls of the endodermal cells (Marschner 1995). This situation is exemplified by the findings of Kuhn *et al.* (2000). These authors have used $^{25}Mg^{2+}$ and $^{44}Ca^{2+}$, and demonstrated the existence of a free apoplasmic path for divalent cations in the cortex of mycorrhizal roots of Norway spruce. $^{25}Mg^{2+}$ in the labeling solution exchanged rapidly with Mg^{2+} in the cortical apoplasm. In contrast, exchange through the plasma membrane with Mg^{2+} present in the cytoplasm of the same cortical cells was almost two orders of magnitude slower. The results of this study showed that in these roots, entry of Mg^{2+} and Ca^{2+} up to the endodermis is faster through the apoplasmic compared to the symplasmic pathway.

Such data also demonstrate that the endodermis is a major barrier to the apoplasmic movement of Mg^{2+} and Ca^{2+}. Further radial transport should thus occur in the symplasmic pathway and requires entry through the plasma membrane into the cytosol of the root cells. After reaching the stele, minerals have to be released into the stelar apoplasm, as transport from root to shoot takes place in the apoplasm of the nonliving *xylem* vessels. This release, which is usually referred to as *xylem loading*, can be mediated by *xylem parenchyma* cells bordering the xylem vessels, and requires a second step of membrane transport. To date, almost nothing is known on the proteins mediating these two essential membrane transport steps, i.e., Mg^{2+} uptake into the root symplasm, and its subsequent release to the xylem or stelar apoplasm.

As the cytoplasm is negatively charged compared to the apoplasm, divalent cations may enter the root symplasm down their electrochemical potential gradient. This uptake should occur through channel or transporter proteins in the plasma membrane of the root cells located before the endodermal barrier. The pathway(s) of Mg^{2+} entry through the plasma membrane of root cells have not been clearly determined. One possible way of Mg^{2+} entry may be through putative homologs of the *rca* channel observed in roots of wheat. The *rca* channel is defined as a calcium channel, but is permeable to a wide variety of monovalent and divalent cations, including Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+} K^+, and Na^+ (Pineros & Tester 1997; Pineros & Tester 1995; White *et al.* 2000). This channel opens upon plasma membrane depolarization, which may occur under many biotic and abiotic stimuli (Pineros & Tester 1997). Differences in permeation between Ca^{2+} and Mg^{2+} through this channel were attributed to lower affinity and higher free-energy barriers for Mg^{2+} (White *et al.* 2000). The role of the *rca* channel (and its putative homologs) in Mg^{2+} uptake from the soil still have to be determined.

Besides channels, it is evident that cation uptake into roots also occurs through transporters. However, transporters engaged in Mg^{2+} uptake from the soil have not been identified to date. In comparison, much more is known about K^+ uptake into roots by both channels and transporters (for a recent review on K^+, NO_3^-, SO_4^{2-}, and phosphate uptake see Chrispeels *et al.* 1999). Current knowledge on root transporters of divalent metal cations is detailed below. Fe^{2+} defi-

ciency induces the expression in plant roots of multispecific metal transporters, such as those encoded by the *Arabidopsis IRT1* gene (Eide *et al.* 1996) and members of the *AtNramp* gene family (Thomine *et al.* 2000). *IRT1* complements yeast strains deficient in the uptake not only of Fe^{2+}, but also of Mn^{2+} and Zn^{2+} (Korshunova *et al.* 1999). The *AtNramp* gene family complements yeast strains deficient in the uptake of Fe^{2+} and Mn^{2+} and increases the accumulation of Cd^{2+} (Thomine *et al.* 2000). The *AtNramp* gene family plays a role not only in Fe^{2+}-deficiency-induced but also in constitutive metal transport.

A gene family of Zn^{2+} transporters, which are induced by Zn^{2+} deficiency and are probably involved in Zn^{2+} uptake, have been identified in *Arabidopsis* (Grotz *et al.* 1998). A related transporter, ZNT1, mediates a high affinity Zn^{2+} uptake and a low affinity Cd^{2+} influx in *Thlaspi caerulescens* (Lasat *et al.* 2000). The difference in Zn^{2+} content between Zn^{2+} hyperaccumulating and non-hyperaccumulating species of *Thlaspi caerulescens* is partly explained by the constitutive high expression of ZNT1 in roots and leaves of the former. The product of the wheat *LCT1* gene mediates the uptake of Ca^{2+} and Cd^{2+} in yeast (Clemens *et al.* 1998).

Information on transport proteins participating in xylem loading of mineral nutrients is also limited. A gene encoding the SKOR outward rectifying K^+ channel has been cloned from *Arabidopsis*. This channel is expressed at both the pericycle and the xylem parenchyma cells, and mediates K^+ release to the stelar and xylem apoplasm (Gaymard *et al.* 1998). Three channels for anion secretion to the xylem have been physiologically characterized in the xylem parenchyma of barley roots (Kohler & Raschke 2000). Transport proteins involved in xylem loading (and unloading) of divalent cations, including Mg^{2+}, have not been identified to date.

Transport of water and solutes in the xylem vessels is driven by transpiration. Xylem parenchyma cells (and stem tissue in general) play a buffering role in ion absorbance and release along the pathway to the shoot, and also in *xylem unloading* at the shoot (Marschner 1995). Movement in the living *phloem* sieves proceeds from sites of assimilate production to sites of utilization. Xylem to phloem transport is of particular importance for the mineral nutrition of plants, because xylem transport is directed mainly to the sites of highest transpiration, which are usually not the sites of highest demand for mineral nutrients. This transport can take place all along the pathway from root to shoot and is mediated by specialized xylem parenchyma transfer cells (Marschner 1995).

The role of the vacuole in Mg^{2+} homeostasis

Part of the cellular Mg^{2+} is bound to the cell wall or sequestered in vacuoles. The concentration of Mg^{2+} in the metabolic pool (the cytoplasm and the chloroplast) of leaf cells is assumed to be in the range of 2–10 mM (Leigh & Wyn Jones 1986). Free Mg^{2+} concentration in the cytosol might be lower because Mg^{2+} is complexed with various molecules such as ATP. *In vivo* ^{31}P NMR spectroscopy showed that in Mg^{2+}-sufficient mung bean root tips about 90% of the cytoplasmic ATP is complexed to Mg^{2+}, and the concentration of free Mg^{2+} is only about 0.4 mM as compared with total Mg^{2+} concentrations of 3.9 mM in this tissue (Yazaki *et al.* 1988). The free levels of Mg^{2+} in the cytosol have to be strictly regulated due to Mg^{2+} effect on photosynthesis and on membranal ionic currents (see below).

The vacuole is the main organelle involved in cellular Mg^{2+} balance

The vacuole, which occupies 80–90% of the whole plant cell volume, has a major role in the regulation of ion homeostasis in the cell and in detoxification of the cytosol. It is involved in the storage of minerals and nutrients (e.g., K^+, NO_3^- , SO_4^{2-}, and phosphate) when these are in ample supply, and releases them when the nutrient supply becomes depleted (reviewed by Allen & Sanders 1997). The vacuole is also the main organelle that determines Mg^{2+} homeostasis in the cytosol and the chloroplast (Marschner 1995).

Vacuolar Mg^{2+} is also important for the cation-anion balance and turgor regulation of cells (Marschner 1995). Stelzer *et al.* (1990) provided experimental evidence supporting the role of the vacuole in Mg^{2+} balancing throughout the year in Norway spruce needles. Ion composition in vacuoles of the needle cells was followed by X-ray microprobe analysis during 16 months. Mg^{2+} was the predominant cation in the vacuoles of the endodermal cells, while K^+ was the predominant cation in the vacuoles of the mesophyll and transfusion parenchyma cells. While Mg^{2+} levels remained almost constant (about 13 mM) in vacuoles of mesophyll and transfusion parenchyma cells throughout the examined period, large variations were observed in Mg^{2+} levels in vacuoles of the endodermal cells (from 20 to more than 120 mM, average

level 64 mM). The changes in the Mg^{2+} content of endodermal vacuoles were accompanied by parallel variations in the major charge-balancing anions, SO_4^{2-} and phosphate. It was suggested that the vacuoles of the endodermal cells serve as a Mg^{2+} and anion storage buffer for the maintenance of constant levels in the mesophyll and transfusion parenchyma cells. In contrast, relatively large changes were observed in K^+ levels in the vacuoles of all three cell types during the examined period. The endodermal cells occupy only 1% of the needle volume, but based on the observed vacuolar Mg^{2+} concentration it was calculated that its storage capacity exceeds 10% of the total needle Mg^{2+}. The high vacuolar Mg^{2+} concentrations found by Stelzer *et al.* (1990) do not necessarily represent free vacuolar concentration, as a large proportion of vacuolar Mg^{2+} is bound to anionic compounds. In comparison, total Mg^{2+} concentration in barley mesophyll vacuoles is 3–7 mM (Dietz *et al.* 1992). No information was found in the current literature about *free* vacuolar Mg^{2+} concentrations in plant cells.

Mg^{2+} transport into vacuoles is mediated by Mg^{2+}/H^+ exchangers

Physiological and molecular evidence indicate that Mg^{2+} efflux from the cytosol into the vacuole is mediated by Mg^{2+}/H^+ exchangers. The activity of a Mg^{2+}/H^+ exchanger was identified at lutoid membranes from *Hevea brasiliensis* latex (Amalou *et al.* 1992, 1994) and in vacuolar membranes from roots of *Zea mays* L. (Pfeiffer & Hager 1993). Latex is the fluid cytoplasm of the laticiferous vessels containing lutoids, a specialized vacuolar-lysosomal compartment. Mg^{2+} is accumulated 10-fold in lutoids compared to the latex cytosol (d'Auzac *et al.* 1989) and is directly involved in rubber biosynthesis (Kang *et al.* 2000). The K_m of the *Hevea brasiliensis* transporter for Mg^{2+} is 2.6 mM. It was also shown to carry Zn^{2+} and Cd^{2+} ions with a higher affinity than for Mg^{2+}, but its K_m for these ions was not determined. This transporter was indicated to be electroneutral, and thus its stoichiometry was inferred to be $Mg^{2+}/2H^+$. It can be inhibited by amiloride and imipramine, with half-maximal inhibition obtained at 0.3 and 0.12 mM, respectively.

We have cloned from the plant *Arabidopsis thaliana* the *AtMHX* gene encoding a transporter localized at the vacuolar membrane (Shaul *et al.* 1999). Patch-clamp analysis of tobacco cells overexpressing AtMHX demonstrated that it exchanges protons with

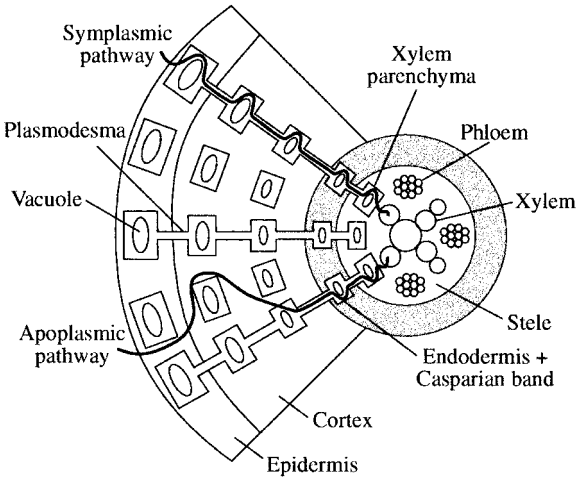

Figure 1. A schematic cross section of a root. The symplasmic and apoplasmic pathways of ion transport across the root are shown in a bold line.

divalent cations, including Mg^{2+} (Shaul *et al.* 1999). In our electrophysiological experiments, currents were activated using either 2 mM Mg^{2+}, 0.2 mM Zn^{2+}, or 0.2 mM Fe^{2+} (Shaul *et al.* 1999). A concentration of 2 mM is within the physiological range of Mg^{2+} levels; the precise K_m of AtMHX for these ions is under investigation at our laboratory. In contrast to Mg^{2+} and Zn^{2+}, under physiological conditions iron is almost exclusively found in complexes in plant cells (Marschner 1995). Thus Fe^{2+} ion is unlikely to be transported in significant amounts by AtMHX *in vivo*. Electrogenic fluxes of Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Na^+ could not be observed. Unlike the *Hevea brasiliensis* transporter, AtMHX is electrogenic, implicating that it exchanges at least 3 H^+ ions per 1 Mg^{2+} ion. None of the Mg^{2+} transporters whose genes have been cloned so far from microorganisms shares sequence homology with AtMHX. AtMHX is the first Mg^{2+} transporter to be cloned from a multicellular organism, and it shares limited sequence homology (36% identity) only with NCX1, a mammalian plasma membrane Na^+/Ca^{2+} exchanger (Nicoll *et al.*, 1990). Besides the plant kingdom, indirect data have led to a recent suggestion that Mg^{2+}/H^+ exchange may occur across rumen epithelia (Leonhard-Marek 1999).

Expression pattern of the Arabidopsis Mg^{2+}/H^+ exchanger and its proposed physiological function

As the vacuole is acidic compared to the cytosol, AtMHX, as well as other tonoplast Mg^{2+}/H^+ ex-

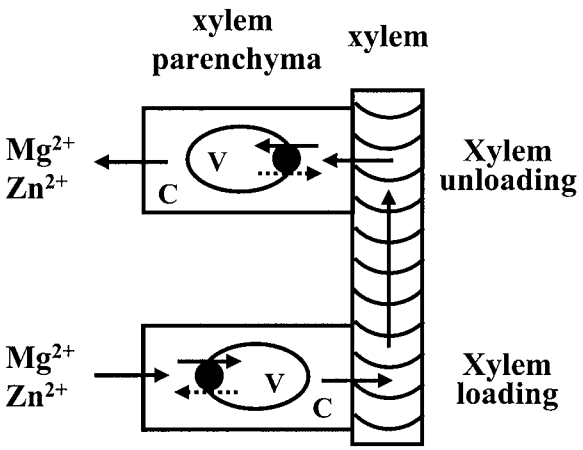

Figure 2. A working hypothesis to explain the physiological role of AtMHX at the xylem parenchyma. The lower and the upper xylem parenchyma cells illustrated here are engaged in xylem loading at the root (bottom) and in xylem unloading at a sink site (top), respectively. AtMHX, represented by the black circle, is hypothesized to determine the proportion of Mg^{2+} and/or Zn^{2+} ions loaded into the vacuoles (V) of the xylem parenchyma cells (solid arrows). The physiological pathway for Mg^{2+} and Zn^{2+} exit from the vacuole (dashed arrows) is not clear at this time. Only ions that reside in the cytosol (C) of the xylem parenchyma cells are available for loading into the xylem (bottom), or, following unloading, for further absorbance into the plant (top). AtMHX and the vacuole could thus serve in a buffering capacity for Mg^{2+} and/or Zn^{2+} ions.

changers, apparently transport cations into the vacuole. AtMHX is encoded by a single gene in *Arabidopsis*. *In-situ* hybridization analyses showed that in all plant organs, a large proportion of AtMHX mRNA is localized at the vascular cylinder, apparently most abundantly close to the xylem tracheary elements (Shaul *et al.* 1999). Expression was also seen in the root epidermis and in meristematic regions (the latter consisting of actively dividing cells). As differentiated xylem tracheary elements are dead cells, the expression observed in this region should reside at the living cell layers that borders the xylem tracheary elements, including the xylem parenchyma. As mentioned above, xylem parenchyma cells may play a key role in ion secretion into the xylem (xylem loading) and in the release of ions from the xylem (unloading) (Marschner 1995). These cells seem also to play a role in resorption of ions from the xylem sap along the pathway to the shoot. It was shown that the xylem parenchyma (and stem tissue in general) of certain species can resorb some minerals (K^+, Na^+, and NO_3^-) from the xylem sap in periods of ample root supply, whereas in periods of insufficient supply these minerals are released into the xylem sap (Marschner 1995). Specialized xylem parenchyma transfer cells also participate in xylem-phloem transfer.

The localization of AtMHX mRNA at the vascular cylinder region, apparently most abundantly at the xylem parenchyma cells, raises the possibility that AtMHX determines the proportion of absorbed Mg^{2+} and Zn^{2+} ions to be stored in the vacuoles of this cell layer, and, consequently, the amount available in the cytosol for loading into the xylem (Figure 2). Similarly, during unloading of Mg^{2+} and Zn^{2+} from the xylem into the xylem parenchyma, AtMHX may determine the levels of Mg^{2+} and Zn^{2+} that remain available in the cytosol of these cells for further absorption into the plant. According to this suggestion, AtMHX could serve in a buffering capacity by sequestering excess Mg^{2+} or Zn^{2+} amounts, or by creating a vacuolar pool that could be used in periods of deficiency. As shown from the study of Stelzer *et al.* (1990), accumulation and release from vacuoles of specialized cells might be involved in buffering Mg^{2+} content in adjacent cells. This suggestion is also consistent with data indicating that Zn^{2+} sequestration in vacuoles determines the amounts of Zn^{2+} available for loading into the xylem in Zn^{2+} hyperaccumulating and non-hyperaccumulating species of *Thlaspi caerulescens* (Lasat *et al.* 1998; Lasat *et al.* 2000). It was shown that accumulation in the vacuoles of the non-hyperaccumulating species retarded Zn^{2+} translocation to the shoot.

When transgenic tobacco plants overexpressing AtMHX in their tonoplast were grown in the presence of high Mg^{2+} or Zn^{2+} levels, necrotic lesions appeared in their leaves (Shaul *et al.* 1999). Such lesions did not appear in non-transformed plants grown under the same conditions. This suggested that Mg^{2+} and Zn^{2+} are transported by AtMHX *in-planta*. Nevertheless, the total content of Mg^{2+} and Zn^{2+} was similar in shoots of transformed and non-transformed plants grown in either normal or high Mg^{2+} or Zn^{2+} levels (Shaul *et al.* 1999). These observations suggest that, being localized in the internal vacuolar membrane, AtMHX cannot change the total amounts of Mg^{2+} or Zn^{2+} in the cells, but can affect their intracellular distribution. One possible explanation for the necrotic lesions in transgenic plants grown with high Mg^{2+} or Zn^{2+} levels could be that when the cellular levels of these cations are high, their extensive exchange with vacuolar protons may impair the cellular pH or ATP balance (the ATP being needed for re-building the pH balance by H^+-ATPases). It is also possible that excess vacuolar Mg^{2+} impairs cellular ion and charge

balance through its effect on vacuolar ionic currents (see below). It thus appears that even though the total content of Mg^{2+} is similar between transgenic and control plants, impairment of its intrinsic homeostasis is harmful.

Vacuolar channels and a novel Arabidopsis gene family might be also engaged in Mg^{2+} transport

The physiological pathway(s) of Mg^{2+} exit from the vacuole to the cytosol have not been clearly determined. One possible pathway could be through the slow activating vacuolar (SV) ion channel, which is ubiquitous in plant tonoplast membranes. The SV channel is permeable to both mono- and divalent cations, including Mg^{2+} (Allen & Sanders 1996; Pottosin *et al.* 1997; Ward & Schroeder 1994). However, the regulation of the SV channel *in vivo,* and thus its actual contribution to Mg^{2+} release from vacuoles, is still not well understood. The SV channel is regulated by Mg^{2+} (see below), Ca^{2+}, calmodulin, protein kinases, phosphatases, ATP, and redox agents (Allen & Sanders 1995; Bethke & Jones 1994; Carpaneto *et al.* 1999; Weiser *et al.* 1991). In addition, the SV channel is activated *in vitro* by membrane potentials that lie positive of physiological tonoplast potentials. However, it was shown that the activating potential of the SV channel could be strongly shifted, suggesting the existence of additional unknown regulatory factors that are essential for the *in vivo* function of this channel (Pei *et al.* 1999). Moreover, it was suggested that the SV channel may carry both inward and outward currents *in vivo* depending on the conditions (Pei *et al.* 1999). One speculative explanation to the lack of increase in the total Mg^{2+} and Zn^{2+} content of transgenic plants overexpressing AtMHX could be that accumulation is enabled only in cells that have evolved unique mechanisms, e.g., specialized control of the pathway(s) of Mg^{2+} or Zn^{2+} efflux from the vacuole.

Interestingly, it was recently reported that AtMRS2-1, which is encoded by a member of a novel *Arabidopsis* gene family, is a candidate Mg^{2+} transporter (Schock *et al.* 2000). Two *Arabidopsis* genes, *atmrs2-1* and *atmrs2-2*, were cloned during a search for homologs of the *Saccharomyces cerevisiae mrs2* gene. A further eight *mrs2* homologs were identified in the *Arabidopsis* genome. The function of the yeast *mrs2* gene is essential for the splicing of group II intron RNA in mitochondria and for the maintenance of a functional respiratory system (Bui *et al.* 1999). The experimental evidence suggests that the products of the yeast *mrs2* and the *Arabidopsis atmrs2-1* (but not *atmrs2-2*) genes mediate or regulate Mg^{2+} transport (Bui *et al.* 1999; Schock *et al.* 2000). Both MRS2 and AtMRS2-1 are very distantly related to the CorA group of bacterial Mg^{2+} transporters (Smith & Maguire 1998; Kehres *et al.* 1998). The yeast MRS2 protein was localized to the inner mitochondrial membrane, and all its physiological effects were attributed to the maintenance of Mg^{2+} homeostasis in this organelle (Bui *et al.* 1999). Green fluorescent protein fusion analysis of the *Arabidopsis* AtMRS2-1 protein suggested that it is not targeted to the mitochondria. The precise cellular localization and the function of AtMRS2-1, as well as of other members of this novel *Arabidopsis* gene family, remain to be studied.

Mg^{2+} effects on membrane transport

The significance of maintaining an adequate homeostasis of Mg^{2+} in the cell may be partly elucidated by Mg^{2+} effects on cellular ionic fluxes. In fact, Mg^{2+} modulation of ionic membrane fluxes is not restricted to plant cells. For example, intracellular and extracellular Mg^{2+} has been shown to be an important physiological regulator of at least nine different transport pathways for K^+ and Na^+ ions in animal cells (reviewed by Bara *et al.* 1993). Mg^{2+} effects on membrane transport processes can be mediated by screening of negative surface charges on membranes (discussed further below), by blocking or interfering with the passage of cations through channels, and by other effects.

In vitro studies have shown that Mg^{2+} can modulate the activity of the vacuolar inorganic pyrophosphatase. Two proton pumps are present in the tonoplast: the vacuolar H^+-ATPase (V-ATPase), and the inorganic pyrophosphatase (V-PP_iase). The activity of these pumps create a proton-motive force across the tonoplast – the lumen of the vacuole is at lower pH and positively charged, compared to the cytosol (see *Introduction*). This provides the driving force for secondary transport events throughout the tonoplast. Both the V-ATPase and the V-PP_iase are dependent on Mg^{2+}, primarily because their substrates are $Mg{\cdot}ATP$ and $Mg{\cdot}PP_i$, respectively. In addition, the activity and the stability of the V-PP_iase is strictly dependent on the concentration of free Mg^{2+} (Maeshima 1991). Free Mg^{2+} acts as an allosteric activator with a Hill coefficient of 2.4, suggesting at least two Mg^{2+} binding sites on the enzyme (Fraichard

et al. 1996). Site-directed mutagenesis pointed to two negatively-charges residues at the cytosolic surface of the V-PP_iase that are important for Mg^{2+} binding and enzyme stability (Nakanishi *et al.* 2000). The concentrations of free Mg^{2+} required for maximal and half-maximal activity of the mung bean V-PP_iase are 150 μM and 42 μM, respectively (Maeshima, 1991). Therefore, the vacuolar V-PP_iase may largely exist as an active complex at physiological Mg^{2+} concentrations.

While it is not clear if Mg^{2+} can regulate the activity of the V-PP_iase under any *in vivo* conditions, physiological levels of Mg^{2+} might play an important regulatory role for two types of ion channels that are ubiquitous in plant tonoplast membranes (Bruggemann *et al.* 1999a; Pei *et al.* 1999). These channels are the previously noted slow activating vacuolar (SV) ion channel and the fast activating vacuolar (FV) ion channel. The SV channel is cation selective, with poor selectivity among divalent cations (Mg^{2+}, Ca^{2+}, and Ba^{2+}) and monovalent cations (K^+, Na^+, and Cs^+) (Allen & Sanders 1996; Pottosin *et al.* 1997; Ward & Schroeder 1994). When the SV channel is open it can mediate the exit of these cations from the vacuole, although it may carry both inward and outward currents *in vivo* depending on the conditions (Pei *et al.* 1999). *In vitro* activation of the SV channel requires cytosolic Ca^{2+} concentrations above the physiological levels of this cation. However, 1–10 mM cytosolic Mg^{2+} was shown to sensitize the SV channel to physiological levels of cytosolic Ca^{2+}. In the absence of Ca^{2+}, Mg^{2+} does not activate the SV channel, indicating a synergistic effect between Ca^{2+} and Mg^{2+} (Pei *et al.* 1999). It was suggested that even if cytosolic Mg^{2+} concentrations do not change, physiological levels of Mg^{2+} ions provide a major mechanism for sensitizing the SV channel to stimulus-induced elevation in cytosolic Ca^{2+} during signal transduction (Pei *et al.* 1999). As mentioned before, SV channels are highly regulated by additional factors.

The FV channel, which is also ubiquitous in plant tonoplast membranes, is selective for monovalent cations (Bruggemann *et al.* 1999b). Its function has not been established, although proposals have been made including mediating K^+ release from guard cell vacuoles during stomatal closing (Allen & Sanders 1996). In contrast to the SV channel, the FV channel is highly active at physiological cytosolic free Ca^{2+} concentrations (< 1 μM) and at physiological tonoplast potentials (Allen & Sanders 1996; Hedrich & Neher 1987; Tikhonova *et al.* 1997). Therefore, it might provide a continuous leak of K^+ ions from the vacuole. Such a leak might interfere with ion homeostasis between the vacuole and the cytosol and in stomatal opening in guard cell. Thus, there should be cellular factors that down regulate the FV channel. Physiological levels of polyamines have been shown to partly down-regulate FV channels (Bruggemann *et al.* 1998; Dobrovinskaya *et al.* 1999). It was recently shown that further down regulation of the FV channel is provided by Mg^{2+} ions (Bruggemann *et al.* 1999a; Pei *et al.* 1999). Free Mg^{2+} concentrations of 1–2 mM are likely to close the FV channel from both the cytosolic and the vacuolar side (Bruggemann *et al.* 1999a). The half inhibitory concentration of cytosolic Mg^{2+} is about 0.23 mM (Pei *et al.* 1999), and changes in the range of free cytosolic Mg^{2+} concentration between 0.1 and 1 mM could effectively regulate the FV channel (Bruggemann *et al.* 1999a). Further discussion of the physiological relevance of Mg^{2+} effect from the vacuolar side of this channel awaits an accurate determination of the free Mg^{2+} concentration in the vacuole.

Besides modulation of fluxes through tonoplast SV and FV channels, Mg^{2+} was also shown to inhibit the transport of positively charged but not of neutral amino acids in isolated barley vacuoles (Dietz *et al.* 1994). Cytosolic Mg^{2+} can block the NH_4^+ permeable channel of the symbiotic membrane of soybean nodules (Whitehead *et al.* 1998). This block is relieved when Mg^{2+} is dislodged by univalent cation flux throughout the pore into the cytoplasm. It is likely that cytosolic Mg^{2+} functions to gate this channel. Mg^{2+} involvement in photosynthesis is also partly due to its effect on ionic fluxes (see below). Effects of Mg^{2+} on other ion fluxes in plant cells still have to be investigated.

The role of Mg^{2+} in photosynthesis and the involved transport mechanisms

Chloroplasts from higher plants are surrounded by a double membrane system consisting of an inner and outer envelope. The chloroplast also contains an internal membrane system, the thylakoid membrane, that encloses an internal space known as the lumen. The stroma is the fluid surrounding the thylakoids. The thylakoid membrane contains the multi-protein photosynthetic complexes photosystems I and II, which include chlorophyll, the molecule capturing light. The most familiar role of Mg^{2+} in photosynthesis is as the

central atom of the chlorophyll molecule. Insertion of Mg^{2+} into the porphyrin structure during chlorophyll formation is catalyzed by Mg^{2+}-chelatase (Walker & Weinstein 1991; Papenbrock *et al.* 2000). Chlorophyll breakdown requires a Mg^{2+}-dechelatase leading to pheophytin (Langmeier *et al.* 1993). The *in vivo* substitution of Mg^{2+} in the chlorophyll by heavy metals (Hg^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}) impairs photosynthesis and is an important damage mechanism in heavy metal stressed plants (Kupper *et al.* 1996, 1998). Mg^{2+} plays other fundamental roles in both the 'light' and 'dark' steps of photosynthesis. In the 'light' step, light energizes proton pumping from the stroma into the thylakoids, and the formed ΔpH facilitates the conversion of ADP and $NADP^+$ into ATP and NADPH, respectively. In the so-called 'dark' step (which actually occurs during the day and for which some of its enzymes require activation by light-dependent processes – see below), enzymes located in the stroma use the high-energy compounds produced in the 'light' step to reduce CO_2 to carbohydrates.

Mg^{2+} involvement in the process of thylakoid acidification

Proton pumping from the stroma into thylakoids in the light results in acidification of the thylakoid lumen. The accompanied electrical gradient ($\Delta\Psi$) across the thylakoid membrane is compensated by concomitant fluxes of Cl^-, K^+ and Mg^{2+} ions (Dilley & Vernon 1965; Hind *et al.* 1974; Chow *et al.* 1976; Krause 1977). This compensation allows the prolongation of H^+ transport into the thylakoids and the generation of ΔpH of 2–3 pH unit between the lumen and the stroma – in the light, the pH of the stroma is about 8 and that of the thylakoid lumen is less than 6 (Remis *et al.* 1986). In general, the concentrations in the stroma are on the order of 150 mM for K^+ (Demmig & Gimmler 1983), 50 mM for Cl^- (Demmig & Gimmler 1983), and 5 mM for Mg^{2+} (Portis & Heldt 1976). Anions (Cl^- and $NO3^-$) are likely to be transported through the thylakoid membrane via a highly conserved voltage dependent anion channel (Schönknecht *et al.* 1988; Pottosin & Schönknecht 1995b; Pottosin & Schönknecht 1995a). However, anions seem to compensate only about half of the H^+ taken into thylakoids after illumination (Thaler *et al.* 1992), and thus the remaining part of charge balance has to be mediated by efflux of cations from the lumen to the stroma. Light-dependent cation fluxes across the thylakoid membrane are well documented, but the relative contribution of K^+ and Mg^{2+} ions is a matter of debate (Dilley & Vernon 1965; Hind *et al.* 1974; Barber 1976; Vredenberg 1976; Bulychev & Vredenberg 1976; Krause 1977; Fang *et al.* 1995). Cation channels across the thylakoid membrane were mainly investigated in spinach (Tester & Blatt 1989; Enz *et al.* 1993; Fang *et al.* 1995; Pottosin & Schönknecht 1996). Fang and co-workers have identified a 33 kDa polypeptide in the spinach thylakoid membrane as a K^+ channel and showed that K^+ efflux from the thylakoid lumen through this channel contributes to the optimization of photosynthetic capacity (Fang *et al.* 1995). However, blockage of this channel only partly inhibited photosynthesis, indicating the involvement of other transport mechanisms. Pottosin & Schönknecht (1996) have demonstrated that the dominant cation channel in spinach thylakoid membrane is almost equally permeable to K^+, Ca^{2+}, and Mg^{2+} ion. At ionic concentrations mimicking the physiological conditions, the single channel conductance is 60 pS for K^+ and 19 pS for Mg^{2+}. As the total concentrations of K^+ and Mg^{2+} in spinach chloroplasts (stroma plus lumen) are about 80–180 mM and 13–18 mM, respectively (Schroppelmeier & Kaiser 1988), Pottosin and Schönknecht (1996) have suggested that under physiological conditions the current through this channel is mainly carried by K^+ ions. These authors have also calculated that, considering the relative volume of the stroma and the lumen, Mg^{2+} concentration in the lumen of spinach thylakoids should be in the range of 30–50 mM to account for the observed 2 mM increase in stromal Mg^{2+} levels during illumination. Pottosin & Schönknecht (1996) have also calculated that, considering the abundance and the Mg^{2+} conductance of the spinach thylakoid cation channel, Mg^{2+} efflux from the lumen through this channel can be by itself responsible for the observed increase of about 2 mM in the Mg^{2+} concentration of the stroma. The steady state potential across the thylakoid membrane of illuminated chloroplasts is approximately -10 to -15 mV, positive in the lumen, and increases at the onset of illumination to -60 to -80 mV (Bulychev *et al.* 1972; Remis *et al.* 1986). However, the total open probability of the spinach thylakoid membrane cation channel is higher at -10 to -15 mV compared to -60 to -80 mV. Calculations demonstrate that the actual current flux through a single channel is equal throughout this electrical gradient range (Pottosin & Schönknecht 1996).

The physiological significance of the fluctuations in Mg^{2+} and pH levels in the stroma and the lumen

During illumination, the lumen of the thylakoid is acidified and its Mg^{2+} concentration is decreased; reverse alterations occur in the stroma. In general, the light-induced increase in the Mg^{2+} concentration of the stroma was found to be between 1–5 mM, depending on the assay conditions, particularly the pH (Portis & Heldt 1976; Krause 1977). Barber (1976) speculated that this change of stromal Mg^{2+} concentrations affects thylakoid stacking and hence the efficiency of energy transfer between photosystems I and II. Mg^{2+} and pH levels were shown to affect the activity of the thylakoid ATPase complex (ATP synthase, CF_0F_1), which uses the proton gradient to phosphorylate ADP to ATP. This enzyme is activated as a result of the light-induced ΔpH across the thylakoid membrane. The proposed mechanism of thylakoid ATPase activation involves protonation of specific acid-base groups exposed to the lumen, with possible simultaneous deprotonation of groups exposed to the stroma (Mills & Mitchell 1984; Biaudet *et al.* 1988). It was recently shown that the acid-base groups responsible for enzyme reactivation are located within the catalytic part of the thylakoid ATPase, i.e., within the coupling factor CF_1 (Malyan *et al.* 1998). CF_1 was also shown to be reversibly inactivated by Mg^{2+} ions, and the experimental evidence suggested that inactivating Mg^{2+} ions bind to the same groups that undergo protonation in the light (Malyan *et al.* 1998). Competitive interactions between Mg^{2+} and protons were also shown for the mitochondrial coupling factor isolated from animal cells (Bulygin *et al.* 1993).

The fluctuations in Mg^{2+} and pH levels also have an important regulatory role on key stromal enzymes. Illuminated chloroplasts accumulate fixed CO_2 in the form of starch, which is broken down in the dark to supply the energy needs of the cells. Some of the intermediates of starch synthesis and breakdown are identical. Thus it is essential to ensure that the photosynthetic enzymes of CO_2 reduction would be active only after illumination, to avoid redirection of starch breakdown products into starch synthesis (Berkowitz & Wu 1993b). This is accomplished by the tight regulation of key stromal CO_2 reduction enzymes by pH and Mg^{2+} levels in this compartment. Increased Mg^{2+} levels in the light activate the enzymes fructose 1,6-bisphosphatase and sedoheptulose 1,7-bisphosphatase, creating the CO_2 acceptor molecule ribulose 1,5-bisphosphate (Purczeld *et al.* 1978; Gardemann *et al.* 1986). Mg^{2+} also activates the enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) driving carboxylation of ribulose 1,5-bisphosphate resulting in CO_2 fixation (Portis 1992). Activation of the enzymes fructose 1,6-bisphosphatase and Rubisco also requires a high stromal pH of about 8 (Portis 1992; Gardemann *et al.* 1986). As a result of H^+ sequestration to the lumen in the light, the pH of the stroma reaches a level of $\sim$8, while that of the cytosol remains $\sim$7. Artificial lowering of the stromal pH of illuminated chloroplast to that found in the dark eliminates photosynthesis, even when the ΔpH between the stroma and thylakoid is maintained (Enser & Heber 1980; Werdan *et al.* 1975). Thus, this high stromal pH is essential for the activity of CO_2 assimilating enzymes. As described below, the mechanism maintaining this high stromal pH can be affected by the concentration of Mg^{2+} ions in the cytosol. In addition, it was recently suggested that physiological Mg^{2+} concentrations within the chloroplast could be a trans-acting factor mediating differential stability of important chloroplast RNAs, including 16S rRNA, tRNA, the mRNA for rbcL, the large subunit of Rubisco, and the mRNA for psbA, the D1 protein of photosystem II (Horlitz & Klaff 2000).

Mg^{2+} effect on the mechanisms that maintain a high stromal pH in the light

As the chloroplast membrane is permeable to protons, maintenance of a stromal pH whose level is higher than that of the cytosol requires, in addition to H^+ efflux to the thylakoid lumen, active H^+ pumping from the stroma into the cytosol. As mentioned, plastids are surrounded by two membranes, referred to as the inner and outer envelope. The outer envelope membrane, although recently proved to be less permeable for low molecular mass compounds than had been thought before, apparently does not provide a selectivity barrier for inorganic ions. The inner envelope membrane includes three transport mechanisms that are essential for the regulation of stromal pH (Neuhaus & Wagner 2000): (1) a H^+-ATPase, which utilizes the ATP generated in the 'light' step of photosynthesis to energize proton efflux from the stroma to the cytosol, (2) a K^+ channel mediating K^+ influx into the stroma, which compensates the $\Delta\Psi$ generated by H^+ pumping, and (3) a Cl^- channel mediating Cl^- efflux. Berkowitz and co-workers demonstrated the presence in the chloroplast envelope of both a K^+ stimulated H^+-ATPase (Berkowitz & Peters 1993a;

Wu and Berkowitz 1992a) and a K^+ channel (Wu & Berkowitz 1992b; Wang *et al.* 1993). Patch-clamp studies have also identified a voltage dependent K^+ channel at the inner envelope membrane (Heiber *et al.* 1995). Its voltage dependence indicated that K^+ fluxes across this channel are rectified, with preferential K^+ uptake into the chloroplast. The presence of a low conductance Cl^- channel at the inner envelope was confirmed by electrophysiology (Heiber *et al.* 1995).

Exposure of isolated chloroplasts to high millimolar levels of free external Mg^{2+} inhibits photosynthesis by reducing the stromal pH, thus impairing the function of CO_2 assimilating enzymes (Demmig & Gimmler 1979; Huber 1979; Huber & Maury 1980; Demmig & Gimmler 1983). It was subsequently demonstrated that external Mg^{2+} does not readily penetrate the chloroplast envelope and that the cause of stromal acidification and photosynthesis inhibition is not free stromal Mg^{2+} but Mg^{2+} bound to the negative surface charges of the chloroplast membrane (Gupta & Berkowitz 1989). This binding decreases K^+ conductance across the chloroplast membrane (reviewed by Berkowitz & Wu 1993b). Mg^{2+}-dependent inhibition of K^+ influx to the stroma would consequently inhibit H^+ efflux, preventing stromal alkalization and resulting in photosynthesis inhibition (Berkowitz & Wu 1993b).

The relevance of these experimental observations to the *in vivo* conditions is anticipated by the Gouy–Chapman–Stern equation describing cation effect on the surface potential of biomembranes (Hille *et al.* 1975; McLaughlin *et al.* 1983). According to this equation, the equilibrium constant of cation binding to the negative surface charges is exponentially related to its valence. Therefore divalent cations would have a much stronger effect compared to monovalent cations. Mg^{2+} is the major free divalent cation in the cytosol and is thus the major physiological surface cation at the chloroplast membrane. This predicts that increased cytosolic Mg^{2+} concentrations, which may arise during water loss from the cell as a result of drought or osmotic stress, might inhibit photosynthesis. This inferance is supported by experimental evidence (see below). Ionic analysis of isolated thylakoids and of intact chloroplasts indicate that Mg^{2+} is also the major physiological surface cation at the thylakoid membrane (Nakatani *et al.* 1979).

The physiological implications of Mg^{2+} imbalance

The Mg^{2+} requirement for optimal plant growth is in the range of 0.15–0.35% of the dry weight of the vegetative parts. High Mg^{2+} content in the leaves (e.g., 1.5% of the leaf dry matter) might become critical under drought stress. The content of Mg^{2+} in the leaf influences the photosynthetic response of sunflower to low water potential (Rao *et al.* 1987). Mg^{2+} content was three times higher in leaves of sunflower plants grown in the presence of 10 mM Mg^{2+} in the nutrient solution compared to plants grown with 0.25 mM Mg^{2+} (the leaf content of other minerals remained unchanged). Nevertheless, at high water potential the dry weight and net photosynthesis of plants grown under the different Mg^{2+} nutrition conditions were identical. In contrast, when dehydration was imposed, net photosynthesis (as measured by CO_2 assimilation) was much more inhibited in plants with the high Mg^{2+} content (Rao *et al.* 1987). During dehydration Mg^{2+} concentrations in these plants apparently increased to levels that could impair photosynthesis in several ways, including (1) inhibition of K^+ transport from the cytosol to the stroma (see above), (2) possible interference with Mg^{2+} homeostasis inside the chloroplast and subsequent adverse effects, e.g., inhibition of the chloroplast coupling factor, and (3) dis-regulation of transport events across the tonoplast, which are essential for cellular ion homeostasis and stomatal function, and hence also for photosynthesis.

Thus, excess Mg^{2+} amounts in the plant might inhibit photosynthesis and plant growth, particularly during dehydration. On the other hand, the rate of photosynthesis is also reduced in leaves of Mg^{2+}-deficient plants. The proportion of the total cellular Mg^{2+} bound to chlorophyll depends very much on the Mg^{2+} supply (Michael 1941) and ranges from 6% in leaves of plants with high Mg^{2+} supply to 25–35% in leaves of Mg^{2+} deficient plants. In most instances, growth is depressed and visual symptoms of Mg^{2+} deficiency occur when the proportion of cellular Mg^{2+} bound to chlorophyll exceeds 20–25% (Marschner 1995). Chlorosis of fully expanded leaves is the most obvious visible symptom of Mg^{2+} deficiency. Mg^{2+} deficiency in the plant also impairs the export of carbohydrates from source to sink sites, presumably due to inhibition of the Mg^{2+}-dependent proton-ATPase that provides the driving force for this transport, leading to a decreased starch content of storage tissues (Marschner 1995). Accumulation of carbohydrates in leaves enhances the oxygenase reaction of Rubisco

and thus the formation of superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Mg^{2+}-deficient leaves are therefore highly photosensitive. Mg^{2+} deficiency also impairs root growth and thus the acquisition of mineral nutrients and of water (Marschner 1995). In addition, Mg^{2+} has an essential function as a bridging element for the aggregation of ribosome subunits, which is necessary for protein synthesis. When the level of free Mg^{2+} ions is deficient or in the presence of excessive levels of K^+, the subunits dissociate and protein synthesis ceases (Sperrazza & Spremulli 1983). Mg^{2+} deficiency might also interfere with the proper regulation of membrane transport events that are modulated by this cation.

Similar to other cations, deficiency of Mg^{2+} in the plant may be induced not only by its absolute limitation in the soil, but also by other cations that compete with Mg^{2+} for binding to the negative charges of the root apoplasm (Marschner 1995). Mg^{2+} deficiency induced by competing cations is a fairly widespread phenomenon, e.g., in forest ecosystems in Central Europe (Liu & Huettl 1991). Induced Mg^{2+} deficiency is extended by certain ecological factors such as soil acidification (high H^+ levels), and from high levels of other cations, especially Al^{3+} (aluminum), Mn^{2+}, Ca^{2+} (calcareous soil), and Na^+ (saline soil). The use of magnesium fertilizers is reviewed by Draycott & Allison (1998).

The impact of Mg^{2+} limitation on the nutritional value of plants

In most instances, elevated Mg^{2+} content improves the nutritional quality of plants for animal and human diet. In animals, unlike some other biometals, there does not appear to be a significant body reserve of available Mg^{2+} that might be mobilized to maintain serum concentrations during periods of deficit. Thus, the major source of supply to extracellular fluids is by direct absorption from the gut. Any reduction of Mg^{2+} supply or sustained increase in Mg^{2+} excretion will rapidly lower serum Mg^{2+} levels (see also articles by Meij *et al.* and Satoh & Romero in this volume). An adequate supply of Mg^{2+} in the diet is therefore important for the productivity of animals. Hypomagnesemia (grass tetany) is a serious disorder of ruminants caused by low Mg^{2+} content of plant feed (Grunes *et al.* 1970). In grass tetany, the animals generally are grazing on cool season forages in which Mg^{2+} concentration or bioavailability is low. Grass tetany occurs in the spring, when heavy lactation by ruminants demands substantial Mg^{2+}. In addition, as temperature increases and plants grow rapidly in the spring, their relative content of Mg^{2+} is decreased. The rapid onset of hypomagnesemia makes it particularly difficult to control in grazing animals. Although many prophylactic measures are available, they vary in cost and efficacy. None provide complete protection against the disease. Losses attributed to grass tetany are an important source of economic loss (Harris *et al.* 1983).

There is a significant genetic variation in Mg^{2+} content in all C3 forage grasses evaluated to date (Sleper *et al.* 1989). The largest portion of this genetic variation is additive, and plant breeders should be able to increase the Mg^{2+} concentration by breeding and selection. Moseley & Baker (1991) studied the efficacy of a cultivar of Italian ryegrass, which was specifically bred for a high Mg^{2+} content, in alleviating the incidence of hypomagnesemia in lactating ewes. Pasture and animal management were regulated to maximize the potential for the development of hypomagnesemia. The incidence of clinical hypomagnesemia in ewes grazing the control pasture was 21% within the first 10 days, and this was accompanied by a significant fall of 35% in serum Mg^{2+} concentration. The incidence in ewes grazing the high Mg^{2+} cultivar was only 2–5%, and there was no significant change in the serum Mg^{2+} concentration. It was concluded that the high Mg^{2+} grass provided an effective means of controlling hypomagnesemia under grazing, despite the extreme predisposing conditions imposed in this trial.

Over the past 40 years, human Mg^{2+} deficiency, resulting from insufficient Mg^{2+} intake in the diet, has become recognized as a world-wide clinical problem [(Flink 1990) and the article by Meij *et al.* in this volume]. Patterns of Mg^{2+} intake in the USA suggest that about 50% of the intake is from foods of plant origin. About half of this intake is from vegetables. While direct supplementation with Mg^{2+} appears more efficacious in the prevention of Mg^{2+} deficiency in humans, research with susceptible ruminants indicate that complete protection can only be assured by adequate daily intake of bioavailable Mg^{2+} in their consumed food. A similar situation would be expected to prevail in humans (Wilkinson *et al.* 1987). Survey of agricultural raw products indicates that genetic factors (species and cultivars) have more effect on plant Mg^{2+} composition than do soil and environmental factors (Wilkinson *et al.* 1987). Consequently, plant breed-

ers aim to produce cultivars with an increased Mg^{2+} content.

Concluding remarks

Considering the physiological significance of Mg^{2+} homeostasis in plants, it is surprising that so little is known on the molecular mechanisms mediating this balance. It is currently not known through which transport proteins Mg^{2+} enters the root symplasm, is loaded and unloaded from the xylem and the phloem, exits the vacuole, and enters and exits through other organellar membranes. The *Arabidopsis* vacuolar Mg^{2+}/H^{+} exchanger AtMHX might be engaged in determining Mg^{2+} partitioning between the cytosol and the vacuole of *Arabidopsis* root epidermal, xylem parenchyma (and possibly other stelar cells), and meristematic cells. However, maintenance of cellular Mg^{2+} homeostasis is essential in all cells. Our *in situ* hybridization analyses did not allow us to exclude the possibility that AtMHX is expressed at low levels or under specific physiological conditions in other cell types. This question is under investigation in our laboratory. Perhaps some of the 'missing' transport mechanisms are related to the AtMRS2 proteins, the candidate Mg^{2+} transporters with distant homology to the bacterial CorA Mg^{2+} transporter recently identified in *Arabidopsis*. In addition, our knowledge of Mg^{2+} effects on cellular transport events have been recently expanded with the identification of its influence on vacuolar SV and FV channels. It would be interesting to find if, analogous to the situation in animal cells (Bara *et al.* 1993), Mg^{2+} can affect more cellular ionic fluxes in plant cells than currently known.

Note added in proof

While this article was in proof, a publication in the December issue of *Plant Cell* shed more light on the function of the *Arabidopsis* MRS2 homologs with distant homology to bacterial CorA transporters (Li L, Tutone AF, Drummond RS, Gardner RC, Luan S. 2001 A novel family of magnesium transport genes in *Arabidopsis. Plant Cell* **13**, 2761–2775). These 10 proteins, previously annotated AtMRS2-1 to AtMRS2-10, are now nominated AtMGT1 to AtMGT10. AtMGT1 and AtMGT10 functionally complemented bacterial and yeast strains, respectively, defective in Mg^{2+} uptake. AtMGT10 expression increased the Mg^{2+} content of starved mutant yeast cells. Most members of this family are expressed in the majority of *Arabidopsis* tissues. AtMGT1 was localized to the plasma membrane. These data are consistent with the possibility that the AtMGT family of transporters is involved in Mg^{2+} acquisition from the soil and/or in Mg^{2+} transport in the plant. The sensitivity of AtMGT1 and AtMGT10 to Al^{3+} might provide an explanation to Al^{3+}-induced Mg^{2+} deficiency in plants.

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