2011

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Published in *New Phytologist* 190 (2011), pp. 510–513.

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Abstract
Magnesium (Mg) is among the most abundant mineral elements in plants, yet the knowledge of which genes control its accumulation in specific tissues and organelles lags behind that of many other mineral elements. Only in recent years has identification of important molecular players begun to take shape. In this issue of *New Phytologist*, Conn et al. (pp. 583–594) shed additional light on two Mg transporters that play important roles in accumulation of Mg in leaf cell vacuoles. Using subcellular-level ion measurements on leaves, gene expression measurements after single-cell sampling, a genetic approach, and clever use of calcium (Ca) and Mg supply to plants or detached leaves, Conn et al. have demonstrated that vacuoles of mesophyll rather than epidermal or bundle sheath cell types of *Arabidopsis* leaves are the main sites of Mg accumulation. They have also shown the effects of mutations in *MRS2-1* and *MRS2-5* genes on this accumulation and a role for these genes in plant adaptation to adverse soil environments.
Keywords: magnesium, MGT, MRS2, serpentine, transporters

Biological roles for magnesium

Total Mg concentrations in cells range from 15 to 25 mM (Moomaw & Maguire, 2008). However, most Mg ions are bound or incorporated into cellular components, which leaves free cytosolic Mg^{2+} in the range of 0.4 to 0.5 mM (Karley & White, 2009; Maathuis, 2009). Typically 15–20% of total leaf Mg is in chlorophyll (White & Broadley, 2009), although this percentage can be higher or lower depending on plant Mg status (Marschner, 1995). The largest pool of Mg is devoted to protein synthesis by bridging ribosome subunits. Magnesium also coordinates with nucleotides and nucleic acids, and bridges enzyme and substrate for many types of reactions including phosphorylation and dephosphorylation (Marschner, 1995; Maathuis, 2009). Phloem loading of carbohydrates depends on adequate Mg levels, as Mg deficient leaves accumulate starch and sugars (Marschner, 1995; Karley & White, 2009). Free Mg is primarily stored in leaf cell vacuoles, organelles that are a driving force for cell expansion by providing an osmotic potential. Certain adverse soil environments, known as serpentine soils, have low Ca and high Mg concentrations. Plants species tolerant to these environments typically have lower leaf Mg concentrations, and polymorphisms in some Mg transporters have been associated with increased tolerance to serpentine soils (Turner et al., 2010).

Microbial magnesium transporters

Magnesium transporters were first identified in bacteria in a screen for cobalt (Co) resistance, and were thus named *CorA* (Moomaw & Maguire, 2008). The *CorA* protein acts as an ion channel that transports Mg, Co, and nickel across membranes with their concentration gradients. The functional *CorA* protein is a homopentamer, in which each subunit has two transmembrane domains, with most of the protein oriented to the inside of the cytosol (Moomaw & Maguire, 2008). *CorA* proteins form a superfamily with members also present in eukaryotes.

Fungal homologues of *CorA* were identified in *Saccharomyces cerevisiae* in screens that were not specifically designed to find Mg transporters. Nonetheless, there are five *CorA* family genes in total that mediate uptake across the plasma membrane, uptake into mitochondria, and efflux from vacuoles. The first yeast Mg transporters were discovered because they conferred aluminum resistance when overexpressed, and were named *ALR1* and *ALR2* (MacDiarmid & Gardner, 1998). These genes function for Mg uptake across the plasma membrane into the cells. Shortly thereafter, a mitochondrial Mg transporter, *MRS2* (Bui et al., 1999), was identified for its ability to correct an RNA-splicing defect, demonstrating another important role for Mg in cellular biochemistry. Based on sequence similarity, a second mitochondrial Mg transporter, *LPE10* (Gregan et al., 2001), was also characterized. More recently, the fifth yeast *CorA* homologue, *MNR2*, has been shown to be necessary for cells to access Mg stored in the vacuole (Pisat et al., 2009). However, the genes that load Mg into the yeast vacuole have not been identified, and are apparently not *CorA* family members.

Arabidopsis magnesium transporters

Compared to single-celled organisms, plants have additional challenges in distributing Mg to required locations. In addition to uptake into root cells, plants will have various other uptake and efflux steps as vascular tissues are loaded and unloaded, and Mg is distributed away from or into xylem and phloem and associated parenchyma. Also, plant cells have plastids, and Mg must be imported into this organelle for chlorophyll synthesis. *Arabidopsis thaliana* has several *CorA/MRS2* homologues, with nine genes (table 1) and two pseudogenes. The first *Arabidopsis CorA* homologues were identified in expressed sequence tag (EST) databases by homology to yeast *MRS2*. *Arabidopsis MRS2-1* functionally complemented the yeast *mrs2* mutant (Schock et al., 2000). A complementation screen of the yeast *alr1/alr2* Mg uptake mutant strain identified *MGT10* (also called *MRS2-11*) as a high affinity Mg transporter (Li et al., 2001), which was later localized to the chloroplast (Drummond et al., 2006). A second family member, *MGT1* (*MRS2-10*), was capable of complementing a bacterial *CorA* mutant and was localized to the plasma membrane. Both of these research groups described the remaining family members (Schock et al., 2000; Li et al., 2001). It has now been demonstrated that all the family members can complement yeast *mrs2* mutants if the genes are fused to yeast native mitochondrial targeting sequences (Gebert et al., 2009).

Most of the *AtMRS2* genes are found on the Affymetrix ATH1 microarray, and the transcript expression levels for *MRS2* genes have been quantified in major tissues over plant development (Schmid et al., 2005). These studies have given clues to physiological functions of the individual genes of the family. Curiously, unlike transporters for most other mineral elements, short (28 h) term (Hermans et al., 2010a) and long (1 wk) term (Hermans et al., 2010b) Mg deficiency did not induce expression of *MRS2* family genes. This lack of upregulation of Mg transport has contributed to the difficulty in identifying root Mg uptake genes. Recently, it was shown that a mutation in *MRS2-7* resulted in a growth defect on low Mg nutrient solution (Gebert et al., 2009), implicating this gene in uptake. Consistent with this, developmental expression studies indicated highest expression of *MRS2-7* in roots, and lowest in leaves (table 1). An *MRS2-7*-GFP fusion indicated localization in the endomembrane system (Gebert et al., 2009). The authors also tested knockouts in *MRS2-1*, *MRS2-5*, and *MRS2-10*, and constructed double mutants of *mrs2-5/2-1* and *mrs2-5/2-10*, none of which showed a growth defect phenotype on low Mg. Knockouts of two genes, *MRS2-6* (Li et al., 2008) and *MRS2-2* (Chen et al., 2009), were shown to have defects in pollen development, and consistent with this, both have higher than average expression levels in floral parts or pollen. MRS2-6 protein was localized to the mitochondria. *MRS2-3* also has high expression in flowers and in developing seeds, and this gene co-localized with a Quantitative Trait Locus (QTL) for seed Mg concentration (Waters & Grusak, 2008a). On the contrary, *MRS2-10* and *MRS2-11* have quite low expression in pollen. *MRS2-11* also has low expression in root tissue, but high expression levels in rosette leaves, which fits well with its chloroplast localization (Drummond et al., 2006). Expression of two family members, *MRS2-4* and *MRS2-10*, are increased in the oldest flower petals, in cauline leaves, and in senescing rosette leaves, which are tissues that supply certain minerals to developing seeds through remobilization (Waters & Grusak, 2008b).

New insights into vacuolar magnesium accumulation and fitness

Conn et al. continue to advance our knowledge of roles of *MRS2* genes in Mg localization within leaves of *Arabidopsis*. Using careful and detailed measurements of Mg in specific cell types in leaves, Conn et al. demonstrated that Mg was at its highest levels in the mesophyll rather than epidermal or bundle sheath cells, and that after increasing supply of Mg to leaves, Mg concentration increases primarily in the vacuoles of palisade and spongy mesophyll cells. This was followed by cell-type specific expression analysis, both by microarray and real-time reverse transcription-polymerase chain reaction (RT-PCR), which established expression levels of specific *MRS2* genes in palisade mesophyll and epidermal cells. Two of these genes, *MRS2-1* and *MRS2-5*, were the subject of further study. Both genes were localized to the vacuole, which corresponded with previous proteomic data (Alexandersson et al., 2004; Carter et al., 2004; Whiteman et al., 2008). By using a genetic approach coupled with manipulation of the environment, that is, Ca and Mg supply to cells, Conn et al. were able to demonstrate a subtle phenotype showing that *MRS2-1* and *MRS2-5* are important for Mg accumulation in vacuoles of mesophyll cells. This becomes more important in serpentine soil environments, when soil Ca levels are greatly reduced relative to Mg. In these conditions, the authors hypothesize that Mg can substitute for Ca as an osmoticum in leaf vacuoles to drive expansion of leaf cells for growth. Indeed, in low Ca solution, vacuole osmolality in *mrs2-1* and *mrs2-5* was altered and shoot growth rate was reduced, conditions that did not occur under normal Ca supply. Interestingly, when *Arabidopsis* was grown in low Ca solution, several *MRS2* genes had increased expression, a phenomenon not observed under Mg deficiency (Hermans et al., 2010a,b), suggesting that plant cells may compensate for low Ca by increasing Mg transport. The increased *MRS2* expression was even more pronounced in the *cax1/cax3* Ca transporter double mutant. Mutations in *CAX1* were previously shown to confer

Table 1. Some characteristics of the *MRS2/MGT* gene family of *Arabidopsis thaliana*

Locus	Gene name	mrs2 mutant compl.	Reference[a]	*corA* mutant compl.	Reference	Localization	Reference	Mutant phenotype	Reference	Affymetrix array element	Expression (average signal intensity (SE))	Notable tissue expression	Expression (signal intensity)	Sample_Id[b]
At1g80900	MGT1, MRS2-10	Y	4, 8	Y	2	PM	2			261894_at	74.4 (0.7)	Senescing leaves	160	ATGE_25
												Stage 15 petals	183	ATGE_42
												Mature pollen	18	ATGE_73
At1g16010	MGT2, MRS2-1	Y	1, 4, 8			Vacuole	9, 10			261795_at	354.6 (2.7)	Senescing leaves	1011	ATGE_25
At2g03620	MGT3, MRS2-5	Y	8			Vacuole PM	9, 11 3			np[c]				
At3g19640	MGT4, MRS2-3	Y	8							257019_at	298.8 (4.2)	Flower, stage 9	726	ATGE_31
												Seeds, stage 6	744	ATGE_79
At4g28580	MGT5, MRS2-6	Y	8	Y	5	Mitochondria	5	Pollen development defect	5	253781_at	18.2 (0.5)	Mature pollen	57	ATGE_73
												Flower, stage 10/11	38	ATGE_32
At3g58970	MGT6, MRS2-4	Y	8							251508_at	147.2 (1.8)	Senescing leaves	407	ATGE_25
												Cauline leaves	451	ATGE_26
At5g09690	MGT7, MRS2-7	Y	8	Y	7	Endomembrane system	8	Growth defect on low Mg	8	250487_at	55.5 (1.1)	Root	137	ATGE_99
												Rosette leaf	24	ATGE17
At5g64560	MGT9, MRS2-2	Y	8	Y	6	defect		Pollen development	6	247248_at	132.6 (1.6)	Stamen, stage 15	604	ATGE_43
												Root	162	ATGE_99
At5g22830	MGT10, MRS2-11	Y	2, 4, 8	Y	5	Chloroplast	4			249864_at	395.8 (6.0)	Mature pollen	93	ATGE_73
												Rosette leaf	841	ATGE17

a. *References:* 1, Schock et al. (2000); 2, Li et al. (2001); 3, Alexandersson et al. (2004); 4, Drummond et al. (2006); 5, Li et al. (2008); 6, Chen et al. (2009); 7, Mao et al. (2008); 8, Gebert et al. (2009); 9, Conn et al. (this issue of *New Phytologist* pp. 583–594); 10, Carter et al. (2004); 11, Whiteman et al. (2008).
b. Refers to Schmid et al. (2005).
c. np = not present on microarray

tolerance to serpentine conditions (Bradshaw, 2005) and result in a higher Mg requirement for optimal growth, and lower leaf Mg concentrations.

A greater understanding of the roles of Mg transporters in plant physiology and development, as well as adaptation to specific environments, could have widespread applications in agriculture. For example, breeding crop varieties that could better grow in adverse environments, such as low-Ca soils, could raise yields or allow food to be produced in areas that are not currently usable. In addition, production of new varieties biofortified with minerals such as Mg would have beneficial effects on human health (White & Broadley, 2009).

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