

Moving magnesium

Magnesium is required for multiple processes that are critical for plant growth and development. It is an integral component of the chlorophyll molecule, is required for CO₂ assimilation, acts as a cofactor for numerous enzymes, and is even a structural component of the ribosome (Chaudhry et al., 2021). Magnesium levels vary widely in natural soils leading to a heterogeneous and often dynamic Mg²⁺ environment around the root. Magnesium deficiency leads to an array of effects ranging from degradation of chlorophyll and accompanying chlorosis to a complex cascade of molecular changes including the production of reactive oxygen species and large-scale transcriptional reprogramming (Chaudhry et al., 2021). Indeed, approximately 10% of the entire *Arabidopsis* genome responds to reduced Mg²⁺ availability (Hermans et al., 2010). Not surprisingly then, plants have a series of transporters that take up Mg²⁺ from the soil, redistribute it throughout the plant, and then sequester it at a cellular level to establish a closely controlled magnesium concentration. Meng et al. (2022) have made an important advance in our understanding of these systems by identifying key transporters that help export Mg²⁺ from the root to the rest of the plant.

Roots have a series of Mg²⁺ uptake transporters known as the magnesium transporters (MGTs)/mitochondrial RNA splicing 2 (MRS2) proteins. For simplicity, we will use the MGT naming convention. MGTs belong to the highly conserved CorA family of proteins that are known to play roles in Mg²⁺ transport in all organisms from bacteria and fungi to animals and plants. In *Arabidopsis* there are nine MGT proteins, and MGT6 and MGT7 appear to play major roles in Mg²⁺ uptake into the root symplast, with OsMGT1 playing a similar role in rice (Yan et al., 2018). MGT6 is a plasma membrane protein highly expressed in root hairs. However, MGT7 is reported to be localized to the ER, highlighting how the Mg²⁺ uptake and homeostasis systems likely reflect the coordinated activities of transporters at many cellular locales.

Once taken up into the root, Mg²⁺ must then be redistributed to the aerial tissues. This is largely accomplished by loading Mg²⁺ into the transpiration stream. However, the efflux transporters key to moving Mg²⁺ from root symplast into the xylem vessels have up until now remained largely undefined. Meng et al. (2022) took a homology-based approach to solve this conundrum. In other organisms a family of related ancient conserved domain proteins (ACDPs) have been associated with Mg²⁺ transport activity. For example, mutations in the human ACDP homologs, the family cyclin M proteins or CNNMs, are linked to Mg²⁺ deficiency pathologies such as retinal or kidney dysfunction. The CNNMs have a conserved structure of a transmembrane region flanked by an extracellular N-terminal domain and a cytosolic tail. The tail contains two characteristic cystathionine-β-synthase domains, motifs that are implicated in Mg²⁺ transport in, for example, bacteria. The conserved nature

of these structures lends itself to searching for homologs in other species.

Meng et al. (2022) examined the *Arabidopsis* genome and found nine putative ACDPs split into three clades. These proteins are named magnesium release transporters, or MGRs. MGR8 and MGR9 form clade III and are localized to the chloroplast (Tang et al., 2022). The clade I MGRs (MGR1, MGR2, and MGR3) are tonoplast proteins, where they are likely playing important roles in vacuolar Mg²⁺ sequestration as part of the cellular Mg²⁺ homeostasis machinery. However, the clade II MGRs (MGR4, MGR5, MGR6, and MGR7) are localized to the plasma membrane and are expressed primarily in the vasculature of the mature root, most likely in xylem parenchyma cells. This is precisely where transporters loading Mg²⁺ into the transpiration stream would be predicted to be active.

Of these clade II transporters, MGR4 and MGR6 in particular appear key to supporting Mg²⁺ delivery from the root to the shoot. Thus, loss-of-function mutants in either of these genes were found to be hypersensitive to low Mg²⁺ stress (Meng et al., 2022). Conversely, they grew more vigorously than wild type plants at high Mg²⁺ levels. In contrast, mutants in MGR5 and MGR7 behaved much as wild type in these assays. The *mgr4* and *mgr6* mutants also showed reduced levels of Mg²⁺ in their shoots and elevated amounts in their roots consistent with a role in root-to-shoot translocation of Mg²⁺. Direct measurement of Mg²⁺ levels in the xylem coupled with some clever reciprocal grafting between mutant and wild type roots and shoots confirmed that MGR4 and MGR6 are likely the elusive transporters that load Mg²⁺ into the transpiration stream in the root. Both MGR4 and MGR6 are upregulated in low-Mg²⁺ environments, suggesting that this transport system can tailor its activities to the changing Mg²⁺ levels of the soil.

Identifying MGR4 and MGR6 as important components of the Mg²⁺ redistribution system in the stele of the root provides key new insight into how Mg²⁺ is moving through the plant. However, there are still many components of the system to be defined. For example, although MGR5 and MGR7 appear less important for xylem loading of Mg²⁺ than MGR4 and MGR6, the phenotypes of *mgr5* and *mgr7* knockout mutants to high and low Mg²⁺ stress indicate they are involved somehow in Mg²⁺ homeostasis. Their precise roles remain to be defined. Similarly, although the work of Meng et al. (2022) reveals MGR4 and MGR6 to be critical to the loading of Mg²⁺ into the xylem, are there other transporters with similar roles still to be discovered? In *Arabidopsis*, MGT9 (Gebert et al., 2009) and the Mg²⁺/H⁺ exchanger MHX1 (Shaul et al., 1999) are highly expressed in the root vasculature, as are OsMGT2, OsMGT6 in rice, and these transporters have also

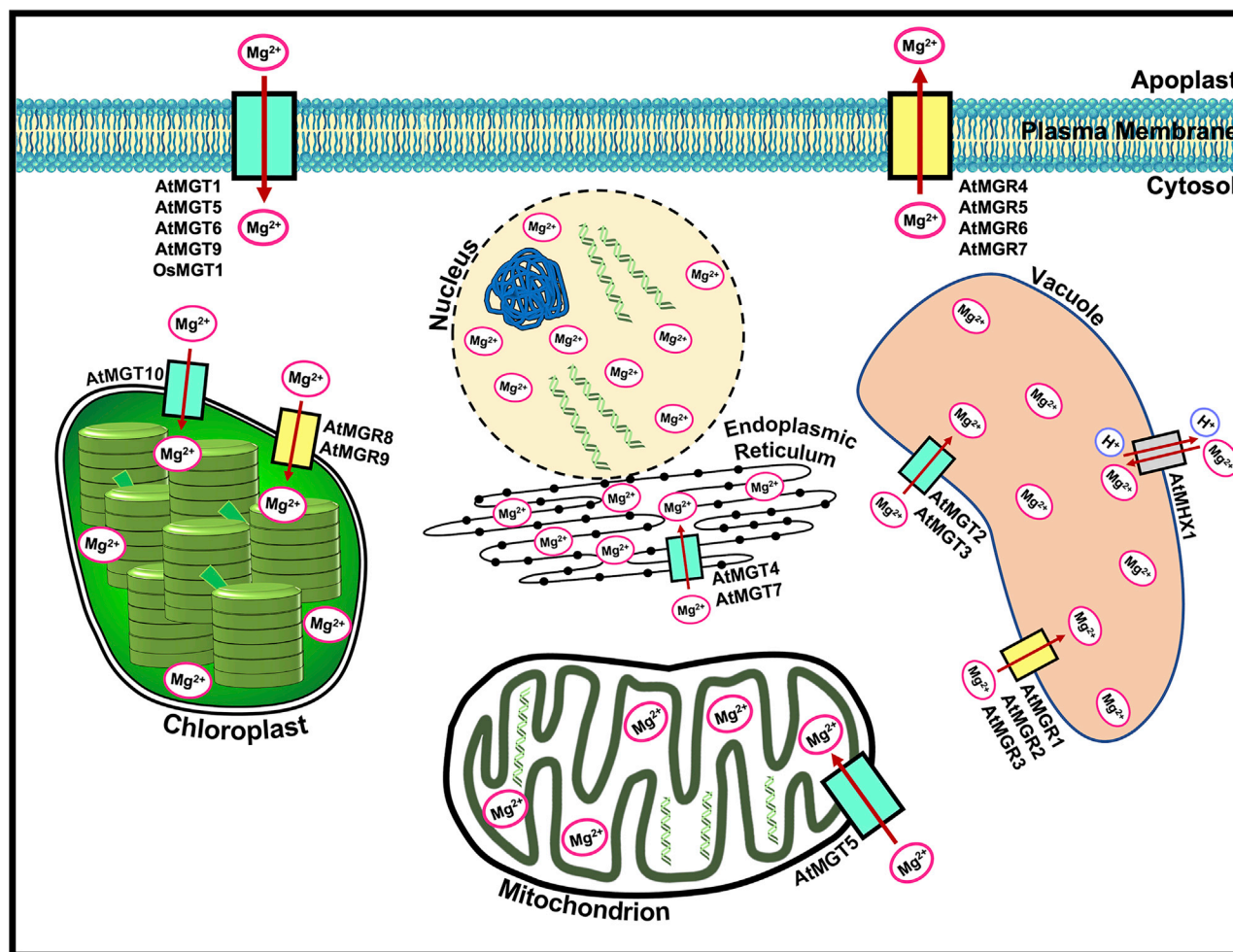


Figure 1. A model for the transport and relocation of Mg^{2+} in plant cells.

Plant roots acquire Mg^{2+} from the soil followed by loading into the xylem and eventually translocating Mg^{2+} to the various parts of the plant body. This process requires the coordinated activity of a variety of Mg^{2+} transporters, localized to different organelles. MGT: magnesium transporter; MGR: magnesium release transporters; At: *Arabidopsis thaliana*; Os: *Oryza sativa*.

been proposed to play roles in xylem loading (Chaudhry et al., 2021). We must await equivalent, careful analysis of mutant phenotypes and Mg^{2+} transport characteristics to those that Meng et al. (2022) applied to the MGRs to answer this question.

Clade II MGRs and MGTs 1, 5, 6, and 9 are plasma membrane proteins, but Mg^{2+} transporters are known to exist elsewhere in the cell. For example, on the tonoplast, there is MHX1 as well as MGRs 1, 2, and 3 (Tang et al., 2022) along with MGT2 and MGT3 (Conn et al., 2011). MGT10 and MGR 8 and 9 operate in the chloroplast (Sun et al., 2017), whereas MGT4 and 7 are reported on the ER. Other channel families may also participate in Mg^{2+} fluxes (Figure 1). For example the cyclic nucleotide-gated channel AtCNGC10 has been shown to mediate root uptake of Mg^{2+} , although it is important to note here that this channel shows relatively poor selectivity for Mg^{2+} over other cations (Guo et al., 2010). These other transporters are likely playing roles in the cellular Mg^{2+} homeostasis system, but how they interact to sustain Mg^{2+} levels and how this network then coordinates Mg^{2+} fluxes through the plant has yet to be defined.

Regulation of cellular Mg^{2+} levels also appear closely linked to Ca^{2+} -dependent signaling. For example, a calcineurin B-like (CBL)/CBL-interacting protein kinase (CIPK) regulatory system acts at the tonoplast to modulate Mg^{2+} homeostasis. Thus, CBL2 and CBL3 pair with CIPK3, -9, -23, and -26 to regulate Mg^{2+} levels, with, for example, the *cbl2/cbl3* mutant showing reduced Mg^{2+} uptake (Tang et al., 2015). How this regulatory system is integrated with controls on Mg^{2+} transporters throughout the cell and how this further plays into the regulation of other cation transport systems is yet another area to be explored. For example, similar CBLs, CIPKs, and other Ca^{2+} -dependent protein kinases of the CPK family are known to also control Mn^{2+} transport (Zhang et al., 2021; Ju et al., 2022).

Meng et al. (2022) have made an important advance in our understanding of Mg^{2+} partitioning within the plant by placing MGR4 and MGR6 in the pathway to xylem loading. They have also set a new challenge to the field for further understanding how coordinating activities of multiple Mg^{2+} transporters control the fluxes of this essential macronutrient through the plant.

FUNDING

This work was supported by NSF MCB2016177 and NASA 80NSSC21K0577.

ACKNOWLEDGMENTS

No conflict of interest declared.

*Arkadipta Bakshi and Simon Gilroy**

Department of Botany, University of Wisconsin-Madison, Birge Hall, 430 Lincoln Drive, Madison, WI 53076, USA

*Correspondence: Simon Gilroy (sgilroy@wisc.edu)
<https://doi.org/10.1016/j.molp.2022.04.005>

REFERENCES

- Chaudhry, A.H., Nayab, S., Hussain, S.B., Ali, M., and Pan, Z.** (2021). Current understandings on magnesium deficiency and future outlooks for sustainable agriculture. *Int. J. Mol. Sci.* **22**:1819.
- Conn, S.J., Conn, V., Tyerman, S.D., Kaiser, B.N., Leigh, R.A., and Gilliam, M.** (2011). Magnesium transporters, MGT2/MRS2-1 and MGT3/MRS2-5, are important for magnesium partitioning within *Arabidopsis thaliana* mesophyll vacuoles. *New Phytol.* **190**:583–594.
- Gebert, M., Meschenmoser, K., Svidová, S., Weghuber, J., Schweyen, R., Eifler, K., Lenz, H., Weyand, K., and Knoop, V.** (2009). A root-expressed magnesium transporter of the MRS2/MGT gene family in *Arabidopsis thaliana* allows for growth in low-Mg²⁺ environments. *Plant Cell* **21**:4018–4030.
- Guo, K.M., Babourina, O., Christopher, D.A., Borsic, T., and Rengel, Z.** (2010). The cyclic nucleotide-gated channel AtCNGC10 transports Ca²⁺ and Mg²⁺ in *Arabidopsis*. *Physiol. Plant* **139**:303–312.
- Hermans, C., Vuylsteke, M., Coppens, F., et al.** (2010). Systems analysis of the responses to long-term magnesium deficiency and restoration in *Arabidopsis thaliana*. *New Phytol.* **187**:132–144.
- Ju, C., Zhang, Z., Deng, J., et al.** (2022). Ca²⁺-dependent successive phosphorylation of vacuolar transporter MTP8 by CBL2/3-CIPK3/9/26 and CPK5 is critical for manganese homeostasis in *Arabidopsis*. *Mol. Plant* **15**:419–437.
- Meng, S.-F., Zhang, B., Tang, R.-J., et al.** (2022). Four plasma membrane-localized MGR transporters mediate xylem Mg²⁺ loading for root-to-shoot Mg²⁺ translocation in *Arabidopsis*. *Mol. Plant* <https://doi.org/10.1016/j.molp.2022.01.011>.
- Shaul, O., Hilgemann, D.W., de-Almeida-Engler, J., Van Montagu, M., Inz, D., and Galili, G.** (1999). Cloning and characterization of a novel Mg(2+)/H(+) exchanger. *EMBO J.* **18**:3973–3980.
- Sun, Y., Yang, R., Li, L., and Huang, J.** (2017). The magnesium transporter MGT10 is essential for chloroplast development and photosynthesis in *Arabidopsis thaliana*. *Mol. Plant* **10**:1584–1587.
- Tang, R.-J., Zhao, F.-G., Garcia, V.J., Kleist, T.J., Yang, L., Zhang, H.-X., and Luan, S.** (2015). Tonoplast CBL–CIPK calcium signaling network regulates magnesium homeostasis in *Arabidopsis*. *Proc. Natl. Acad. Sci. U S A* **112**:3134–3139.
- Tang, R.-J., Meng, S.-F., Zheng, X.-J., Zhang, B., Yang, Y., Wang, C., Fu, A.-G., Zhao, F.-G., Lan, W.-Z., and Luan, S.** (2022). Conserved mechanism for vacuolar magnesium sequestration in yeast and plant cells. *Nat. Plants* **8**:181–190.
- Yan, Y.-W., Mao, D.-D., Yang, L., Qi, J.-L., Zhang, X.-X., Tang, Q.-L., Li, Y.-P., Tang, R.-J., and Luan, S.** (2018). Magnesium transporter MGT6 plays an essential role in maintaining magnesium homeostasis and regulating high magnesium tolerance in *Arabidopsis*. *Front. Plant Sci.* **9**:274.
- Zhang, Z., Fu, D., Sun, Z., et al.** (2021). Tonoplast-associated calcium signaling regulates manganese homeostasis in *Arabidopsis*. *Mol. Plant* **14**:805–819.