AtMGT7: An Arabidopsis Gene Encoding a Low-Affinity Magnesium Transporter

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Abstract

Magnesium (Mg$^{2+}$) is one of the essential cations in all cells. Although Mg$^{2+}$ transport mechanism has been well-documented in bacteria, less is known about Mg$^{2+}$ transporters in eukaryotes. AtMGT gene family encoding putative magnesium transport proteins had been described previously (Schock et al., 2000; Li et al., 2001). We report here that one of the arabidopsis MGT family members, AtMGT7 gene, encodes two mRNAs resulted from alternative splicing variants, designated AtMGT7a and AtMGT7b. Interestingly, the two mRNA variants were expressed with different patterns in that AtMGT7a expressing in all organs, but AtMGT7b appearing only in root and flowers. The AtMGT7a variant functionally complemented a bacterial mutant lacking Mg$^{2+}$ transport capacity whereas AtMGT7b did not. The $^{63}$Ni$^{2+}$ tracer uptake analysis in the bacterial model showed that AtMGT7a mediated low-affinity transport of Mg$^{2+}$. Consistent with the complementation assay result, $^{63}$Ni$^{2+}$ tracer uptake analysis revealed that AtMGT7b did not transport Mg$^{2+}$. This study therefore has identified from a higher plant the first low-affinity Mg$^{2+}$ transporter encoded by a gene with alternatively spliced transcripts that produce proteins with distinct functions.

Keywords: magnesium transporter, AtMGT7, alternative splicing, Arabidopsis

Introduction

Mg$^{2+}$ is the most abundant divalent cation in cells, and is involved in a variety of cellular activities (Grubbs and Maguire, 1987; Walker, 1986; Walker et al., 1983). In higher plants, Mg$^{2+}$ is particularly important for photosynthesis (Walker and Weinstein, 1991; Langmeier et al., 1993; Kupper et al., 1996; Kupper et al., 1998). Intracellular Mg$^{2+}$ concentration is precisely regulated, and there is likely to be a tight control of Mg$^{2+}$ uptake, translocation, and efflux (Shaul, 2002). However, the molecular details of Mg$^{2+}$ transport and homeostasis are still poorly understood.

In earlier studies, two distinct mechanisms of magnesium transport have been characterized thus far at the micromolar level for higher plants. AtMHX is the first magnesium transporter to be cloned from multicellular organism, which is localized in the vacuolar membrane and functions as an electrogenic exchanger of protons with Mg$^{2+}$ and Zn$^{2+}$ ions (Shaul et al., 1999). AtMHX transcript is mainly found in vascular cylinder, and a large proportion of the transcript is localized in close association with the xylem tracheary elements, suggesting that it may control the partitioning of Mg$^{2+}$ and Zn$^{2+}$ between the various plant organs. Interestingly, its homologue in mammalian cells NCX1, is a Na$^+$/Ca$^{2+}$ exchanger and plays a major role in extrusion of Ca$^{2+}$ ions to the extracellular space following exitation (Nicol et al., 1990).

Schock (2000) and Li (2001) independently identified a 10-member Arabidopsis AtMGT gene family encoding Mg$^{2+}$ transport proteins that are homologous to yeast MRS2 gene and bacterial CorA gene. Members of the AtMGT family are expressed in a range of plant tissues.
One family member, AtMGT1, functionally complements a Mg$^{2+}$ transport mutant of *Salmonella typhimurium*, whereas a second member, AtMGT10, complements an equivalent yeast mutant. Both members mediate uptake of cations with similar kinetics to that of CorA, a high-affinity Mg$^{2+}$ transporter in bacteria (Smith et al., 1993; Smith et al., 1998). AtMGT1 was localized in plant plasma membrane (Li et al., 2001) and AtMGT10 (AtMRS2-11) was localized in plant chloroplast envelope membrane system (Drummond et al., 2006), which are consistent with their possible function in Mg$^{2+}$ uptake and translocation. A third member AtMGT2 (AtMRS2-1) complements the yeast deletion mutant mrs2 and restores the intramitochondrial magnesium concentration to nearly wild-type level (Schock et al., 2000).

AtMHX and the above described AtMGT members are shown to function in high affinity magnesium uptake. Here we present evidence that another AtMGT member, AtMGT7a, functions as a low-affinity Mg$^{2+}$ transporter.

**Materials and Methods**

**Strains, Plasmids, and Media**

*Escherichia coli* strains were cultivated at 37°C in LB medium supplemented with ampicillin (100µg/mL). *S. typhimurium* MM281 was grown at 37°C in N-minimal medium (Nelson and Kennedy, 1971) containing 0.1% casamino acids and 0.2% glucose, supplemented with MgSO$_4$(10mM) and chloramphenicol (34µg/mL). pMD18-T vector and pTrc99A were used as cloning and expression vector respectively.

**Cloning of AtMGT7**

The oligo nucleotide primers used for cDNA cloning of the *AtMGT7* gene is as follows:

5’-GGCATTGGGTTTAAGCAGTGA-3’ (forward primer) and 5’-AAATGCTGGCGAGAAGAGACC-3’ (reverse primer). Another pair of primers were used for subcloning into pTrc99A: 5’-TAGAGCTCATGTCACCTGACGGAG-3’ (forward primer) and 5’-GCGGTACCCTAATGATCCGATGAGT-3’ (reverse primer) with SacI and KpnI restriction site (underlined). PCR cycle for *AtMGT7* consisted of the following steps: an initial hold at 96°C for 4min, 28 cycles of 96°C denaturation for 40s, 54°C annealing 40s, and 72°C elongation for 1min 30s, and a final extension of 10min at 72°C. Using the primers listed above, cDNAs corresponding to *AtMGT7* gene were amplified from the ecotype Columbia.

**Expression Patterns of the *AtMGT7a/7b* Transcripts**

Comparing with *AtMGT7b*, *AtMGT7a* has a 45bp-long specific sequence. We designed the forward and reverse primers flanking the two ends of this sequence region for RT-PCR analysis of *AtMGT7a* and *AtMGT7b*. The predicted fragments are 400bp long for *AtMGT7a*, and 355bp for *AtMGT7b*. The mixtures of the PCR fragments of the truncated *AtMGT7a* and *AtMGT7b* can be set apart on 2% agarose gel electrophoresis. Samples of root, stem, leaf, flower and silique were prepared from 7 weeks old Arabidopisis plants as described previously (Li et al., 2001). Total RNA samples from different organs were extracted and converted to cDNAs as described above. The
primers for RT-PCR are as follows: 5’-GAAGAGCTTGAATGGTTGCTT-3’ (forward primer)
and 5’-AAATGCTGCGAGAAGAGACC-3’ (reverse primer). Four gradient PCR cycles were
performed (20, 24, 28, 32 cycle separately). PCR cycle parameters were set as follows: an initial hold
at 96°C for 4 min. different cycles of 94°C denaturation for 40 s, 56°C annealing 40 s, and 72°C
elongation for 1 min, and a final extension of 10 min at 72°C. The normalization of the cDNA
contents of different reverse transcription mixtures (by amplifying the transcripts of AtActin3)
and the evaluation of the transcript levels of AtMGT7a and AtMGT7b gene were conducted as described
previously (Li et al., 2001).

Salmonella typhimurium Mutant Complementation

The S. typhimurium mutant MM281 lacks CorA, MgtA, MgtB genes and has losen capability of
Mg²⁺ uptake and translocation (Hmiel et al., 1989). For the complementation experiment, MM281 cells were transformed with pTrc99A vector, pTrc99A-AtMGT7a and pTrc99A-AtMGT7b plasmid by electroporation. Cells were plated onto Luria-Bertani medium supplemented with 100 mM Mg²⁺ and appropriate antibiotics (50 µg/mL ampicillin, 34 µg/mL chloramphenicol), and incubated at 37°C overnight. The transformants were confirmed by PCR amplification of both the vector and AtMGT7 coding sequence. Individual transformants were grown in Lennox Base liquid medium containing the same concentrations of Mg²⁺ and antibiotics. AtMGT1 was used as a positive control. The cultures were adjusted to 1.0 OD₆₀₀, diluted in a 10-fold series, and spotted (2 µL) onto N-minimal medium supplemented with different concentrations of MgSO₄ and the antibiotics. Growth of different strains was scored after incubation at 37°C for 2 days.

Growth in Liquid Minimal Medium

MM281 mutant and MM281 cells overexpressing AtMGT7a, AtMGT7b, AtMGT1 were grown
in LB medium to log phase (OD₆₀₀ = 0.6 to 0.8). The cells were harvested by centrifugation, washed
twice with distilled water to remove traces of Mg²⁺, and resuspended in distilled water. Aliquots of
20 mL of N-minimal medium were prepared containing increasing concentrations of MgSO₄
(100 µM, 500 µM, 1 mM, 10 mM), cells were then added to give a final OD₆₀₀ of 0.001-0.002. The
growth of the cultures was monitored over 24 h by following OD₆₀₀. Results displayed are averages
for four cultures grown on different days.

Measurement of Mg²⁺ by Atomic Absorption Spectroscopy

MM281, AtMGT7a- and AtMGT7b-transformed MM281 cells were grown to log phase
(OD₆₀₀ = 0.6 to 0.8) in LB medium containing 100 mM MgCl₂. The cell cultures were washed twice
with milli-Q water and incubated at the same density in LB medium without MgCl₂ for 24 hr. After
incubation, the cultures were centrifuged at 500 g for 2 min, resuspended in 5 mM Tris-succinate
buffer, pH 4, supplemented with 1% glucose and incubated for 30 min at 25°C. The bacterial
cultures were centrifuged at 500 g, and the pellet was resuspended in 5 mM Tris-succinate buffer,
pH 4, supplemented with 4 mM MgCl₂. Samples of 10 mL were drawn at indicated time points,
washed three times in 10 mM of the following solutions (milli-Q water, 1 mM EDTA, milli-Q water),
and then resuspended in 2 mL of milli-Q water. A 0.5-mL sample was taken to measure the final OD$_{600}$, and the remaining 1.5 mL was mixed with an equal volume of concentrated HNO$_3$, incubated at 100°C for 1 hr, and diluted with 2 volumes of LaCl$_2$ buffer (240 mM HCl and 10 mM LaCl$_3$). The Mg content was measured in a Varian 1275 atomic absorption spectrophotometer (Palo Alto, CA) using an air-acetylene flame at a wavelength of 285.2 nm.

**Tracer Uptake Assays**

Uptake of $^{63}$Ni$^{2+}$ was performed as described previously (Snavely et al., 1989; Smith et al., 1998). $^{63}$Ni$^{2+}$ uptake was assayed using strain MM281-AtMGT1 for AtMGT1, MM281-AtMGT7a for AtMGT7a, and MM281-AtMGT7b for AtMGT7b. MM281 was used as a negative control. Briefly, cells were grown overnight in LB with 100 mM MgSO$_4$ and appropriate antibiotics. The MgSO$_4$ concentration was 100 mM because the background strain MM281 requires high concentrations of Mg$^{2+}$ for growth. When the concentration of the cells reached OD$_{600}$=0.8, 0.05 mM IPTG was added to the culture medium and the culture was grown for 4-6 hrs. Cells were washed twice in N-minimal medium (without Mg$^{2+}$) and then diluted 1:5 with the same medium containing 1 mM Mg$^{2+}$ and appropriate antibiotics. After a 5-hr subculture, cells were collected by centrifugation at 1000 g for 15 min and washed twice with ice-cold Mg$^{2+}$-free N-minimal medium. The washed cells were resuspended in the same medium to 2.0 OD$_{600}$. For a standard assay, uptake was initiated by adding 0.2 mL of cells to 0.8 mL N-minimal medium containing 100 µM of NiCl$_2$ and 0.5 µCi of $^{63}$Ni$^{2+}$ to a final volume of 1 mL. For the ionic selectivity assay, various concentrations of divalent cations were added in the tracer uptake buffer. Typically, uptake was conducted by incubating at 37 °C for 5 min, then uptake was stopped by adding 5 mL of ice-cold wash solution (N-minimal medium without glucose and casamino acids and with 10 mM MgSO$_4$ and 0.5 mM EDTA). Cells were washed four times with 1.5 mL of ice-cold washing buffer and resuspended in 1.5 mL wash solution. The samples were placed in a 4-mL Biosafe II scintillation mixture (Research Products International Corp., Mount Prospect, IL) and the $^{63}$Ni$^{2+}$ content of the bacterial cells was determined by using a Packard A210001 scintillation counter. The relative tracer uptake was standardized against the maximal value and presented as % of maximal uptake.

**Results**

**Isolation of AtMGT7 cDNA clones and identification of two alternative splicing variants**

Using the genomic sequence information generated from the AGI project, oligonucleotide primers were synthesized to amplify the cDNA corresponding to the coding sequence of the predicted AtMGT7 gene by RT-PCR. Two AtMGT7 cDNA fragments with different sizes were cloned and sequenced, one contained 1158 base pairs of nucleotides in the open-reading-frame (ORF), encoding a putative protein of 386 amino acid residues, designated AtMGT7a, which is identical to the sequence predicted by AGI, whereas another cDNA clone contained 1113 nucleotides of ORF, encoding a putative truncated protein of 371 amino acid residues, designated AtMGT7b. Comparing with AtMGT7a, AtMGT7b had a 15 amino acid deletion.
(EAYFMQIDSTLNKLT) between L^{278} and E^{292}. The cDNA sequences have been submitted to the GenBank and the accession numbers are DQ408372 for AtMGT7a and DQ408373 for AtMGT7b. AMGT7a has two transmembrane domains (TMs) as predicted at the C-terminal region (Leu^{323} → Pro^{345} for TM1 and Trp^{358}~Phe^{380} for TM2) and possess full transport magnesium structure present in other MGT members and CorA (Figure 1A and Fig1B). In contrast, AtMGT7b retains the transmembrane domains but lacks a coiled coil region that may be important for function of Mg^{2+}-transport (Figure 1A).

**Distinct expression patterns of the splicing variants of AtMGT7**

Because AtMGT7 gene has two transcripts AtMGT7a and AtMGT7b, we examined the expression pattern of each of these two transcripts using RT-PCR. As there is a difference of 45 bp fragment between AtMGT7a and AtMGT7b cDNA, We designed oligo nucleotide primers on the two flanks of the 45bp-specific region of AtMGT7a, to amplify a 400bp fragment for AtMGT7a, and a 380bp fragment for AtMGT7b. The PCR products were run on a 2% agarose gel. The specific bands were recovered and sequenced. Similar expression patterns were obtained when the PCR products were amplified under 20, 24, 28, and 32 cycles respectively (data not shown, results under 28-cycles was showed in Fig 2). There are two different DNA bands set apart on 2% gel, coincident with the predicted sizes of AtMGT7a and AtMGT7b fragments, which were determined by sequencing thereafter. As is shown in Fig 2, AtMGT7a transcripts were expressed in all detected tissues, whereas AtMGT7b transcripts were expressed in root and flower. AtMGT7a mRNA exists mainly in stem, and leaf tissues, and weakly in the root, flower and silique. AtMGT7b transcript is preferentially expressed in flower and root tissues but not in stem, leaf or silique tissues.

**AtMGT7a but not AtMGT7b complements a bacterial mutant lacking Mg^{2+} transport capability**

A Salmonella mutant strain MM281 lacks Mg^{2+} transport systems CorA, MgtA and MgtB (Maguire, 1992) and requires 10mM Mg^{2+} in the medium for its normal growth. Thus, MM281 has served as a good model system to identify proteins that mediate Mg^{2+} uptake and translocation (Li et al., 2001). We transformed this strain with several plasmids containing either the vector alone or vector plus a MGT member. Complementation analysis showed that the negative control (MM281 and MM281 transformed with empty pTrc99A vector) did not grow in medium containing lower than 10 mM of Mg^{2+}, and the positive control (MM281 transformed with AtMGT1) could grow in medium containing as low as 10µM of Mg^{2+}. MM281 transformed with AtMGT7a could survive in medium containing 500µM of Mg^{2+}, suggesting AtMGT7a is capable of mediating Mg^{2+} uptake in the range of sub-millimolar Mg^{2+}-concentrations, clearly with lower affinity than the AtMGT1. Another splicing variant, AtMGT7b, expressed a protein lacking a 15-amino acid region could not support growth of MM281 on medium containing below 5 mM Mg^{2+}, consistent with the hypothesis that the coiled coil region in the MGT/CorA proteins is critical for Mg^{2+} uptake (Fig.3). Furthermore,
the results from the liquid growth curve analysis were also consistent with the complementation results observed on agar plates (Fig 4).

AtMGT7a is capable of directing Mg\(^{2+}\) uptake into bacterial cells over a short time frame. Bacterial strains were grown at high Mg\(^{2+}\) (100mM) and then starved by growing them for 24hr in minimal media lacking Mg\(^{2+}\). Fig 5A shows that before starvation, the Mg\(^{2+}\) content of AtMGT7a-transformed MM281 cells was 13% higher than that of MM281 strain containing the vector. However, during the 24 hr starvation, Mg\(^{2+}\) content decreased to similar levels in AtMGT7a, AtMGT7b and MM281 strains (Fig 5A). Mg\(^{2+}\) uptake into the starved cells was then compared at 5 mM Mg\(^{2+}\) over a 60-min period. In MM281 expressing AtMGT7a, the total intracellular content doubled after 40 min and increased up to 130% after an hour. In contrast, intracellular Mg\(^{2+}\) content in the AtMGT7b-transformed and MM281 strain increased by only 29% in an hour (Fig 5B). This is consistent with the results of mutant complementation by AtMGT7a and AtMGT7b.

AtMGT7a functions as a low-affinity Mg\(^{2+}\) transporter

The transport properties of AtMGT7a and AtMGT7b were analyzed in uptake experiments using \(^{65}\)Ni\(^{2+}\) as a tracer, based on previously described procedures (Snavely et al., 1991; Li et al., 2001). A number of studies have demonstrated that Mg\(^{2+}\) and Ni\(^{2+}\) can use the same transport system with similar kinetics in the cell (Smith and Maguire, 1998; Szegedy and Maguire, 1999). Inhibition of Ni\(^{2+}\) tracer uptake by non-radioactive Mg\(^{2+}\) or other cations represents the uptake efficiency of Mg\(^{2+}\) or other cations. Studies have proved that AtMGT1 acts as a high-affinity Mg\(^{2+}\) transporter (Li et al., 2001). Figures 6A and 6B show that the uptake kinetics for Ni\(^{2+}\) and Mg\(^{2+}\) displayed distinct patterns for AtMGT1, AtMGT7a and AtMGT7b. In the MM281 mutant strain, Mg\(^{2+}\) and Ni\(^{2+}\) began to inhibit tracer uptake at a concentration of 10 mM and 100mM respectively (for 50% inhibition), which is consistent with the fact that MM281 required 10mM MgSO\(_4\) to grow. This was also the case in AtMGT7b-transformed cells, indicating that AtMGT7b had no measurable transport activity, and this further confirmed that AtMGT7b is not a functional magnesium transporter. In MM281 expressing AtMGT1, tracer uptake inhibition took place in concentrations of 1 to 10µM of Mg\(^{2+}\) or 10-100µM of Ni\(^{2+}\). In MM281 expressing AtMGT7a, tracer uptake inhibition took place in concentrations of 100µM -1mM of Mg\(^{2+}\) or 1mM-10mM of Ni\(^{2+}\), and the uptake kinetics curve is between AtMGT1 and AtMGT7b, suggesting that AtMGT7a functions as a low-affinity Mg\(^{2+}\) transporter.

AtMGT7a is selective for Zn\(^{2+}/Mg^{2+}\) among divalent cations

AtMGT7a functionally complemented a Salmonella mutant lacking Mg\(^{2+}\) uptake capability and effectively transported Mg\(^{2+}\) as indicated by the tracer inhibition assays. To further address the functional identity of AtMGT7a, we performed tracer inhibition assays with a number of other divalent cations to determine the ionic selectivity of AtMGT7a and AtMGT7b. As shown in Figure 7, several divalent cations, including Zn\(^{2+}\), Co\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), significantly
inhibited tracer uptake by AtMGT7a. For example, the concentrations required for 50% inhibition are in the range of 100µM to 1mM for Mg^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+}—more than 1mM for Fe^{2+}, and 10µM or so for Zn^{2+}. These results indicate that AtMGT7a might be capable of transporting both Zn^{2+} and Mg^{2+} under physiological conditions. Although it might be able to transport other divalent cations, the concentrations required for activity went beyond physiological range. Tracer inhibition assay also showed that Zn^{2+}, Co^{2+} and Cd^{2+} significantly inhibited tracer uptake by AtMGT7b, and the concentrations required for 50% inhibition are in the range of 100µM~1mM for Zn^{2+}, Co^{2+} and Cd^{2+}, and more than 1mM for Mn^{2+} and Fe^{2+}. This result suggested that AtMGT7b might be able to transport these divalent cations, but the concentrations required for activity went beyond physiological range.

**Expression of AtMGT7a and AtMGT7b alter cation sensitivity**

Excessive accumulation of divalent cations often asserts a toxic effect on microbial cells. To test whether AtMGT7a actually mediates the uptake of those divalent cations, we assayed the cation sensitivity of the mutant and the mutant transformed with the putative transport gene. As shown in Figure 8, MM281 mutant transformed with AtMGT7a showed significantly higher sensitivity to Co^{2+}, Cu^{2+} and Cd^{2+}, as compared with that of the MM281 mutant. We performed each assay under a series of cation concentrations (0µM, 10µM, 100µM, 1mM, 10mM). The cation sensitivity occurred only when the concentration of Co^{2+} and Cu^{2+} reached 500µM, suggesting that AtMGT7a may take up these cations at a similar concentration, consistent with the tracer inhibition assays shown in Figure 7. We note that AtMGT7b also conferred the cells more sensitivity to Cu^{2+} and Cd^{2+} as compared to the MM281, suggesting that AtMGT7b may function as a transporter for these cations.

These results are consistent with the idea that AtMGT family is capable of transporting other divalent cations in addition to Mg^{2+} and Ni^{2+}. However, the concentrations of metals used in all these experiments are high and rarely encountered in natural conditions. We propose that, like AtMGT1, AtMGT7a is a selective Mg^{2+} transporters under normal physiological conditions. Unlike AtMGT1, AtMGT7a functions at higher extracellular Mg^{2+} concentration, i.e., it serves as a low-affinity transporter.

**Discussion**

In spite of the central role of Mg^{2+} for higher plants, the molecular mechanisms of high- and low-affinity transmembrane transport of Mg^{2+} remain unknown. We report here the characterization of an Arabidopsis transporter, AtMGT7a, the first low affinity Mg^{2+} transporter identified from any plant source. Using both functional complementation and tracer uptake assays, we confirmed that AtMGT7a serves as a Mg^{2+} transporter in the range of sub-millimolar Mg^{2+} concentrations in the medium, placing this transporter in the low-affinity category as compared to the identified high-affinity transporters such as MGT1 and MGT10. Because this Mg^{2+} concentration may exist in some of the soil types, AtMGT7a may function
in Mg\textsuperscript{2+} uptake from the soil. In addition, AtMGT7a may also function in intra-organelle Mg\textsuperscript{2+} translocation if it is located in the endomembrane system.

One interesting feature of \textit{AtMGT7} gene is that its transcripts have two splicing variants. While one of these variants, \textit{AtMGT7a}, encodes a functional Mg\textsuperscript{2+} transporter, the other form \textit{AtMGT7b} does not. Concerning the potential transport activity of AtMGT7b, the cation sensitivity assay (Figure 8) provided some clue. Cells transformed with \textit{AtMGT7b} became more sensitive to Cu\textsuperscript{2+} and Cd\textsuperscript{2+} as compared to the control cells, strongly suggesting that AtMGT7b protein in plant cells may function as a divalent transporter selecting for Cu\textsuperscript{2+} and Cd\textsuperscript{2+}. Interestingly, AtMGT7a also conferred cells hypersensitivity to these two cations, implicating proteins expressed from both splicing variants may have a role in transporting Cu\textsuperscript{2+} and Cd\textsuperscript{2+}. In addition, the AtMGT7a, but not AtMGT7b, also rendered cells more sensitive to Co\textsuperscript{2+}. Together with the tracer uptake assays, these results suggested that the two splicing variants both encode functional transporters with AtMGT7a transporting Mg\textsuperscript{2+} and AtMGT7b transporting some of other divalent cations. Comparing the activity of the two forms, it becomes clear that the coiled coil domain in CorA-MGT type transporters is critical for the Mg\textsuperscript{2+}-selectivity.

\textit{AtMGT7} shares some structural features with the bacterial Mg\textsuperscript{2+} transporter CorA. The CorA transport system is constitutively expressed, mediates both influx and efflux of Mg\textsuperscript{2+}, and appears to be the primary Mg\textsuperscript{2+} transport system (Snively et al., 1989). Topological analyses of CorA protein reveal a unique structure that contains a large, acidic, N-terminal domain and two transmembrane domains at the C-terminal region. There is an absolutely conserved GMN motif near the end of one of the hydrophobic regions, and mutagenesis of CorA has shown that these three conserved residues are essential for functional Mg\textsuperscript{2+} transport (Szegedy and Maguire, 1999). Recently, a crystal structure of pentameric cone-shaped CorA Mg\textsuperscript{2+} transporter from \textit{Thermotoga maritima} had been elucidated by several research groups respectively. The CorA monomer has a C-terminal membrane domain containing two transmembrane segments and a large N-terminal cytoplasmic soluble domain. The loop between two transmembrane segments is periplasmic and both N- and C-terminal ends facing the cytosol (Lunin et al., 2006; Eshaghi et al., 2006; Payandeh and Pai, 2006; Maguire, 2006). Just like CorA, the sequence of AtMGT7a has two TM domains, and the second TM contains the conserved GMN motif. AtMGT7b is a splicing variant of AtMGT7a and retains the two transmembrane domains. However, AtMGT7b does not function as a Mg\textsuperscript{2+} transporter, indicating that the functionality of this type of transporter not only needs GMN motif but also the coiled coil region. The fact that AtMGT7b may have transporter activity suggests that the coiled coil region might serve as intracellular Mg\textsuperscript{2+}-selection filter. Further structure-function analysis will test this idea.

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References


Figures and Figure Legends

Figure 1. Topological analysis of AtMGT7a/7b protein sequences

(A). Structure of AtMGT7a, AtMGT7b and AtMGT1.

(B). HMHMM posterior probabilities for AtMGT7a/7b. Transmembrane domains were predicted by TMHMM2 software at the website www.cbs.dtu.dk/cgi-bin/nph-webface?jobid=TMHMM2.
Figure 2. Expression patterns of *AtMGT7a* and *AtMGT7b* transcripts

Lane M, DNA MW marker. Lanes 1-5 showed transcripts in different organs including roots(1), stems(2), leaves(3), flowers(4) and siliques(5). The PCR cycling number is 28. Primers for the *AtActin3* gene were used as standard control to monitor the cDNA yield and integrity in the samples.
Figure 3. Complementation of MM281 Mutant by AtMGT7a and AtMGT7b

Growth of different bacterial strains on the N-minimal medium containing 10 μM, 100 μM, 500 μM, 1 mM, 2 mM, 10 mM MgSO4. Rows 1-5 of each plate showed growth status of MM281 or MM281-transformed cells: row 1, MM281; row 2, MM281 transformed with pTrc99A vector as negative control; row 3, MM281 transformed with AtMGT1 cDNA in pTrc99A vector as positive control; row 4, MM281 transformed with AtMGT7a cDNA in pTrc99A vector; row 5, MM281 transformed with AtMGT7b cDNA in pTrc99A vector. The horizontal spots of each row stand for the bacteria grown from cultures diluted in a 10-fold series from left to right.
Figure 4. Bacterial growth curves when cultured in liquid medium

The following concentrations of MgSO₄ were used: 100µM (solid circle), 500µM (open circle), 1mM (solid triangle), 10mM (open triangle).
Figure 5. Uptake of Mg\(^{2+}\) in MM281 by AtMGT7a and AtMGT7b

(A) Time course of Mg\(^{2+}\) starvation. The Mg\(^{2+}\) content is shown for MM281-transformed cells pre-grown in minimal (SD) medium containing 150mM Mg\(^{2+}\) and then cultured for 24 hr in media without Mg\(^{2+}\). Diamonds, pTrc99A-AtMGT7a/MM281; cycles, pTrc99A-AtMGT7b/MM281; triangles, pTrc99A/MM281. Results are averages(±SE) of duplicate measurements made for three cultures grown on different days.

(B) AtMGT7a mediate Mg\(^{2+}\) uptake. Starved cells were pre-incubated with glucose, and then Mg\(^{2+}\) uptake was measured in succinate buffer, pH4, with 4mM Mg\(^{2+}\) for 1 hr. Symbols and measurements are as given in (A).
Figure 6. Ni²⁺ and Mg²⁺ Transport Measured by Inhibition of ⁶³Ni²⁺ Uptake.

(A). Ni²⁺ inhibition of ⁶³Ni²⁺ uptake.

(B). Mg²⁺ inhibition of ⁶³Ni²⁺ uptake.

Transport assay was performed using four Salmonella strains as described in Methods. Tracer uptake is presented as the percentage of the maximal uptake by each strain. Data are average values of three independent experiments and are presented as mean ±SE. Uptake was measured for 3 min with triplicates at each cation concentration.
Fig 7. Cation Selectivity of AtMGT7a and AtMGT7b.

Inhibition of AtMGT7a-, AtMGT7b-mediated tracer uptake by divalent cations. The data shown are normalized to the percentage of control ($^{63}$Ni$^{2+}$ uptake with 100µM Ni$^{2+}$ in the solution without other divalent cations.)

Data are average values of three independent experiments and are presented as mean ±SE. Uptake was measured for 3min with triplicates at each cation concentration.
Figure 8. Cation Sensitivity

Growth of MM281 cells or MM281 transformed with different plasmids on the N-minimal medium supplemented with divalent cations. Row 1, MM281; row 2, MM281 transformed with pTrc99A vector; row 3, MM281 transformed with AtMGT1 cDNA in pTrc99A vector;
row 4, MM281 transformed with AtMGT7 cDNA in pTrc99A vector; row 5, MM281 transformed with AtMGT7-1 cDNA in pTrc99A vector. The horizontal spots stand for the bacteria grown from cultures diluted in a 10-fold series from left to right.