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The MgtR regulatory peptide negatively controls expression of the MgtA Mg^{2+} transporter in *Salmonella enterica* serovar Typhimurium

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ABSTRACT

MgtR is a 30 amino acid peptide that is encoded from the *mgtCBR* operon. This peptide has recently been demonstrated to interact with the MgtC virulence protein and lead to MgtC degradation. In the present study, we reveal that the MgtA Mg^{2+} transporter is another protein under the direct control of the MgtR peptide. *Salmonella* expresses the MgtA transporter only in Mg^{2+} depleted conditions. We determined that the MgtR peptide limits levels of the MgtA protein at low Mg^{2+} concentrations. MgtA expression increased in a *Salmonella* strain lacking MgtR but decreased in a strain overexpressing MgtR. Moreover, we found that the MgtR peptide is necessary for the MgtA protein to be induced at the normal timing upon Mg^{2+} starvation. The MgtR peptide did not affect transcription of the *mgtA* gene but specifically bound to the MgtA transporter *in vivo*, resembling the features of MgtR-regulated MgtC expression. MgtR-mediated regulation of MgtA expression was biologically significant because the lack of MgtR enhanced *Salmonella* growth in low Mg^{2+}.

1. Introduction

Mg^{2+} is an abundant and essential metal in living cells [1]. This cation participates in many cellular activities: it functions as a cofactor for various enzymatic reactions requiring ATP and also maintains structures of membranes and ribosomes [2]. Although these facts suggest that cells should carry means to control intracellular concentration of Mg^{2+} at appropriate levels, only a few studies have revealed molecular mechanisms of how cells achieve Mg^{2+} homeostasis.

Many bacterial species utilize multiple membrane transporters to import Mg^{2+} [3]. To date, three different Mg^{2+} transporters, CorA, MgtA, and MgtB proteins, have been identified in *Salmonella enterica* serovar Typhimurium [4]. The CorA protein is constitutively expressed [5]. In contrast, expression of the MgtA and MgtB proteins, encoded from the *mgtA* gene and *mgtCBR* operon, respectively, are induced only in cells starved of Mg^{2+} [5,6]. Regulation of the *mgtA* and *mgtB* genes occurs at both the transcriptional and posttranscriptional levels and shares similar mechanisms. First, when *Salmonella* grows at micromolar concentrations of Mg^{2+}, the low Mg^{2+} signal activates the PhoP protein, a response regulator of the PhoP/PhoQ two-component system [7]. The activated PhoP protein then binds to promoters of the *mgtA* gene and *mgtCBR* operon, resulting in their transcription initiation [8,9]. Secondly, the leader regions of *mgtA* and *mgtCBR* act as riboswitches to control expression of the MgtA and MgtB transporters at the posttranscriptional level [10]. When intracellular Mg^{2+} concentrations reach certain levels, Mg^{2+} binds to the leader sequences of *mgtA* and *mgtCBR* to form mRNA structures that inhibit transcriptional elongation [10]. This posttranscriptional control allows *Salmonella* to produce MgtA and MgtB proteins exclusively under Mg^{2+}-limited environments. Therefore, even if transcription of *mgtA* and *mgtCBR* is promoted at millimolar Mg^{2+} concentrations by the acidic pH-activated PhoP [11], both the MgtA and MgtB transporters are not expressed due to the function of the leader sequences [10,11]. Expression of the MgtB protein is also negatively controlled by AmgR RNA that is complementary to the *mgtC* portion of the *mgtCBR* mRNA [12]. In response to low Mg^{2+}, the PhoP protein directly activates transcription of the antisense AmgR RNA from the *mgtC–mgtB* intergenic region [12]. When paired with AmgR, the *mgtCBR* message is degraded in a process requiring RNaseE [12].

MgtR is an *mgtCBR*-encoded 30 amino acid peptide and predicted to be localized in the inner membrane [13]. When co-expressed with the MgtC and MgtB proteins in low Mg^{2+} concentrations, the MgtR peptide directly interacts with the MgtC protein to lead MgtC degradation by the FtsH protease [13]. Consistent with the notion that the MgtC protein is necessary for *Salmonella*'s survival inside macrophages [14], the reduction of MgtC levels resulting from MgtR overexpression impairs *Salmonella*'s replication within

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macrophages [13]. The MgtR peptide displays no effect on expression of the MgtB protein [13] and thus MgtC has been the only protein that is regulated by MgtR.

In the present study, we report that the MgtR peptide also directly targets the MgtA Mg^{2+} transporter. This regulatory peptide binds to the MgtA protein *in vivo* and negatively controls the levels and timing of MgtA expression. We also found that the lack of MgtR enhances MgtA-mediated Mg^{2+} uptake.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

Bacterial strains and plasmids used in this study are shown in Table 1. *S. enterica* serovar Typhimurium strains were derived from strain 14028s. Phage P22-mediated transductions were conducted as described [15]. Bacteria were grown at 37 °C in N-minimal medium [6] adjusted to pH 7.7 and supplemented with 0.1% casamino acids, 38 mM glycerol, and different concentrations of $MgCl_2$. Ampicillin (Ap) and kanamycin (Km) were used at 50 μg/ml. Isopropyl-β-D-thiogalactopyranoside (IPTG) was used at 0.1 or 0.5 mM.

2.2. Construction of bacterial strains

S. Typhimurium strains carrying gene deletion were constructed by using the one-step gene inactivation method [16]. For deletion of the *mgtR*, *mgtCB*, *mgtCBR* and *corA* genes, the Km^R cassette from plasmid pKD4 [16] was amplified using primer pairs DEL-mgtR-F/DEL-mgtR-R, DEL-mgtC-F/DEL-mgtB-R, DEL-mgtC-F/DEL-mgtR-R, and DEL-corA-F/DEL-corA-R, respectively. The resulting PCR products were integrated into the chromosome of strain 14028s as described [16]. The Km^R cassette was removed using plasmid pCP20 [16]. Deletion of the corresponding genes was verified by colony PCR. The strain EN336 expresses the MgtA protein with a HA tag at the C-terminus in the normal *mgtA* chromosomal location. For its construction, the Km^R cassette was amplified using the primer pairs mgtA-HA-F/mgtA-HA-R and integrated into the 3′end of the *mgtA* gene. The Km^R cassette was removed from the resulting strain using pCP20, and the presence of a HA-tag at the C-terminus of MgtA was confirmed by nucleotide sequencing. The sequences of primers used are indicated in Table S1.

2.3. Plasmid construction

Plasmid pMgtR expressing the MgtR peptide from the *lac* promoter was constructed. The *mgtR* gene was amplified using primer pairs EX-mgtR-F/EX-mgtR-R and chromosomal DNA from the 14028s strain. The PCR products were purified and introduced between BamHI and PstI restriction sites of pUHE21-2*lacI*^{q} [17]. For the bacterial two-hybrid assay, plasmids pT18-MgtA, pT18-MgtC, pT25-MgtR and pT25-FeoA were constructed. The *mgtA*, *mgtC*, *mgtR* and *feoA* genes were amplified by PCR using primer pairs TH-mgtA-F/TH-mgtA-R, TH-mgtC-F/TH-mgtC-R, TH-mgtR-F/TH-mgtR-R and TH-feoA-F/TH-feoA-R, respectively. After purification, the PCR products were introduced between PstI and BamHI sites of the plasmid vector pKT25 [18] or between the BamHI and EcoRI sites of the pUT18 [18]. Sequences of particular genes on the recombinant plasmids were confirmed by nucleotide sequencing. The sequences of primers used are indicated in Table S1.

2.4. Western blot analysis

Salmonella strains expressing the MgtA-HA protein were grown in 20 ml of N-minimal medium. When OD_{600} values of the cultures reached ~0.5, bacterial cells were washed with phosphate-buffered saline (PBS), suspended in 0.5 ml of PBS and opened by sonication. Total protein concentrations were determined by the BCA method. Whole-cell lysates containing 25 μg of total protein were resolved on 12% SDS polyacrylamide gels, transferred to nitrocellulose membranes and analyzed by Western blot using monoclonal anti-HA (Sigma) or anti-DnaK antibodies (Stressgen). Blots were developed by using anti-mouse IgG horseradish peroxidase-linked antibody (GE Healthcare) and the ECL detection system (GE Healthcare).

2.5. RNA isolation and quantitative real-time RT-PCR (qRT-PCR) analysis

Bacteria were grown in 10 ml of N-minimal medium to OD_{600} = 0.5–0.6. One half milliliters of the culture was removed and mixed with 1 ml of RNAprotect Bacteria Reagent (Qiagen), and RNA was isolated using the RNeasy Mini Kit (Qiagen). The RNA sample was treated further with RNase-free DNase (Ambion). cDNA was synthesized by using Omnitranscript Reverse Transcription reagents (Qiagen), random primers (Invitrogen) and 0.5 μg of

Table 1
Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference or source
S. enterica serovar Typhimurium		
14028s	wild-type	[27]
EN336	*mgtA*-HA	This study
EN341	*ΔmgtR*::Km^R	This study
EN343	*mgtA*-HA *ΔmgtR*::Km^R	This study
EN477	*ΔcorA ΔmgtCBR*::Km^R	This study
EN553	*ΔcorA ΔmgtCB*::Km^R	This study
E. coli		
BTH101	*F- cya-99 araD139 galE15 galK16 rpsL1* (*Strr*) *hsdR2 mcrA1 mcrB1*	[19]
Plasmids		
pUHE21-2*lacI*^{q}	rep_{pMB1} Ap^R *lacI*^{q}	[17]
pMgtR	pUHE21-2*lacI*^{q} *mgtR*	This study
pKD4	$repR_{6K\gamma}$ Ap^R FRT Km^R FRT	[16]
pKD46	$rep_{pSC101}{}^{ts}$ Ap^R P_{araBAD} γ β exo	[16]
pCP20	$rep_{pSC101}{}^{ts}$ Ap^R Cm^R *cI857* λP_R*flp*	[16]
pKT25	P_{lac} p15A *ori* Km^R	[18]
pUT18	P_{lac} ColEI *ori* Ap^R	[18]
pT18-MgtA	pUT18 *mgtA*	This study
pT25-MgtR	pKT25 *mgtR*	This study
pT18-MgtC	pUT18 *mgtC*	This study
pT25-FeoA	pKT25 *feoA*	This study

template RNA. Amounts of cDNA were quantified by real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems) with an ABI7300 Sequence Detection System (Applied Biosystems). The primers used for detection of cDNA corresponding to the *mgtA* mRNA and 16S rRNA were Q-mgtA-F (5′-TAATTGCCACAAA-ACTTATG-3′)/Q-mgtA-R (5′-TCGCGGGAGAGGGGTGGGTT-3′) and Q-rrsH-F (5′-CCAGCAGCCGCGGTAAT-3′)/Q-rrsH-R (5′-TTTACGCCCA GTAATTCCGATT-3′), respectively. Transcription levels of each gene were calculated from a standard curve obtained by PCR with the same primers and serially diluted genomic DNA. mRNA levels of the *mgtA* gene were normalized to 16S rRNA levels.

2.6. Bacterial two-hybrid (BACTH) assay

To assess protein (or peptide)–protein interactions *in vivo*, a BACTH assay was conducted as described [19]. The *Escherichia coli* BTH101 (*cya*$^-$) strain was co-transformed with derivatives of the pUT18 and pKT25 plasmids. The strains were grown overnight at 30 °C in LB supplemented with Ap (50 μg/ml), Km (50 μg/ml) and IPTG (0.5 mM) and β-galactosidase activity was determined in the cultures as described [20].

2.7. Measurement of bacterial growth

Growth of *Salmonella* strains with or without the *mgtR* gene were monitored as follows. Overnight cultures were grown in N-minimal medium containing 1 mM $MgCl_2$. After washing twice with Mg^{2+}-free medium, cells were diluted 1:100 into wells of a 96-well plate containing 150 μl of 1 mM or 10 μM $MgCl_2$ medium and covered with mineral oil to prevent evaporation. By using a Victor^3 plate reader (PerkinElmer), bacterial cells were cultivated at 37 °C with shaking, and their OD_{600} values were measured every 5 min and up to 5 h.

3. Results

3.1. The MgtR peptide negatively regulates levels of the MgtA protein

In *S.* Typhimurium, the MgtR peptide that is encoded from the *mgtCBR* operon has been demonstrated to negatively control expression of the MgtC virulence protein [13]. We initially wanted to explore whether the MgtR peptide could affect expression of the MgtA Mg^{2+} transporter. For this purpose, we constructed a strain expressing the MgtA protein with a HA epitope at its C-terminus from the normal chromosomal location. Western blot analysis determined that when bacterial cells were grown with 10 μM Mg^{2+}, which is a condition allowing MgtA expression [5,21], levels of the MgtA protein were higher in the *mgtR*$^-$ strain than in the *mgtR*$^+$ strain (Fig. 1A, compare lanes 2 and 4). Consistent with the notion that MgtA expression is repressed by milimolar concentrations of Mg^{2+} [21], we were unable to detect the MgtA protein in both the *mgtR*$^+$ and *mgtR*$^-$ strains grown with 1 mM Mg^{2+} (Fig. 1A, lanes 1 and 3).

As these results showed the possibility of MgtR as a factor repressing MgtA expression, we reasoned that overexpression of the MgtR peptide might reduce the MgtA protein levels. To test this idea, we constructed an *mgtR* deletion strain carrying a pMgtR plasmid in which expression of the *mgtR* gene is under control of the *lac* promoter. Western blot analysis revealed that the IPTG-induced MgtR peptide reduces levels of the MgtA protein expressed at 10 μM Mg^{2+} (Fig. 1B, compare lanes 3 and 5). Cumulatively, these results indicate that the MgtR peptide acts as a negative regulator for MgtA expression.

3.2. The MgtR peptide controls the timing of MgtA expression

To further elucidate the role of MgtR in expression of the MgtA protein, we examined expression kinetics of MgtA in *mgtR*$^+$ and *mgtR*$^-$ strains. We initially grew bacteria in medium supplemented with 1 mM Mg^{2+} to suppress MgtA production and then transferred them into medium with 10 μM Mg^{2+} to induce MgtA expression. Western blot analysis revealed that deletion of the *mgtR* gene enabled *Salmonella* to express the MgtA protein in low Mg^{2+} concentrations at an earlier time: when transferred to 10 μM Mg^{2+}, the *mgtR*$^+$ strain began to accumulate the MgtA protein after 45 min, whereas the *mgtR*$^-$ strain did so after 30 min (Fig. 1C). We also found that the MgtA levels increased at all time points after induction in the absence of MgtR (Fig. 1C). Thus, these results indicate that MgtR can control not only the levels but also the timing of MgtA expression.

3.3. MgtR does not affect transcription of the mgtA gene

We wondered whether the MgtR peptide inhibits transcription of the *mgtA* gene so that it reduces MgtA protein levels. qRT-PCR revealed that the wild-type strain highly expresses the *mgtA* mRNA at 10 μM Mg^{2+}, levels of which were hardly changed by deletion of

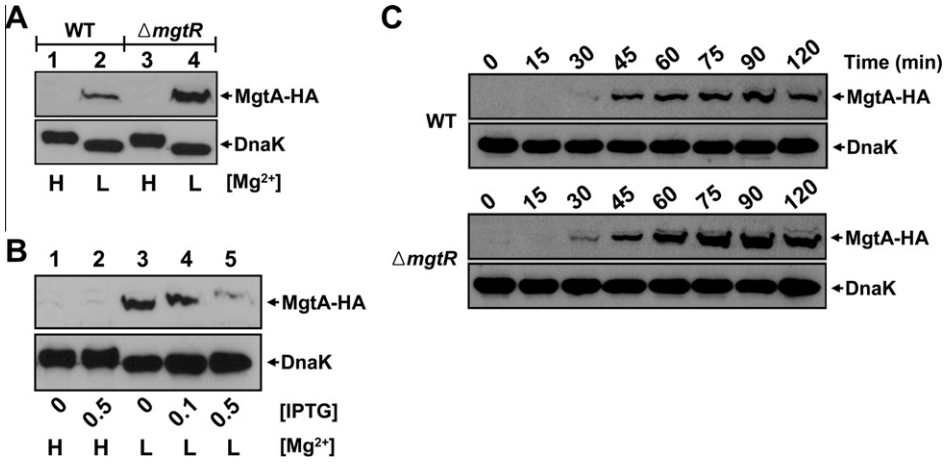

Fig. 1. The MgtR peptide negatively controls the levels and timing of MgtA expression. Western blot analysis was conducted on cell extracts prepared from *Salmonella* strains expressing the MgtA-HA protein from the normal chromosomal location (A and B). Strains were grown in N-minimal medium supplemented with 1 mM (H, high) or 10 μM (L, low) Mg^{2+}. (A) The MgtA-HA protein levels were determined in *mgtR*$^+$ (WT, EN336) and *mgtR*$^-$ (*ΔmgtR*, EN343) strains. (B) The MgtA-HA protein expressed in the EN343 strain carrying the pMgtR plasmid. IPTG (mM) was added into medium as indicated. (C) The levels of MgtA-HA protein were determined using cells extracts from the *mgtR*$^+$ (WT, EN336) and *mgtR*$^-$ (*ΔmgtR*, EN343) strains that were grown in N-minimal medium containing 1 mM Mg^{2+}, transferred into medium with 10 μM Mg^{2+}, and harvested at the designated times. The DnaK protein served as a control.

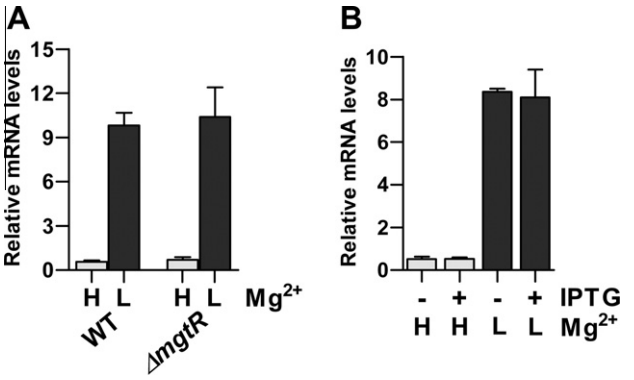

Fig. 2. Neither deletion nor overexpression of *mgtR* affects transcription of the *mgtA* gene. qRT-PCR was performed using RNA isolated from *Salmonella* strains that were grown in N-minimal medium containing 1 mM (H, high) or 10 μM (L, low) Mg^{2+}. (A) The *mgtA* mRNA levels were determined in wild-type (WT, 14028s) and *mgtR* deletion (*ΔmgtR*, EN341) strains. (B) Levels of *mgtA* transcripts expressed in the EN341 strain carrying the pMgtR plasmid in the presence (+) or absence (−) of 0.5 mM IPTG. The relative mRNA levels in the *y*-axis correspond to the *mgtA* mRNA levels divided by 16S rRNA levels. The mean values and standard deviations of three independent experiments are shown.

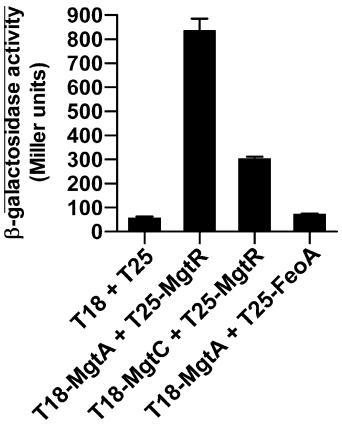

Fig. 3. The MgtR peptide binds to the MgtA protein *in vivo*. Protein (or peptide)–protein interaction was assessed using a bacterial two-hybrid system [19]. β-galactosidase activity (Miller units) was determined in *E. coli* BTH101 strains harboring plasmids co-expressing T18 + T25, T18-MgtA + T25-MgtR, T18-MgtC + T25-MgtR and T18-MgtA + T25-FeoA. The mean values and standard deviations of three independent experiments are shown.

the *mgtR* gene (Fig. 2A). We also found that the overexpressed MgtR peptide does not affect *mgtA* transcription: when grown at 10 μM Mg^{2+}, the *mgtR* deletion strain carrying the pMgtR plasmid expressed the *mgtA* mRNA at similar levels regardless of MgtR-induction (Fig. 2B). Thus, these results suggest that the MgtR peptide controls MgtA expression at the posttranscriptional level.

3.4. The MgtR peptide directly interacts with the MgtA protein

The MgtR peptide was demonstrated to control expression of the MgtC protein at the posttranscriptional level. This regulatory peptide directly bound to the MgtC protein *in vivo* and promoted MgtC degradation [13]. Therefore, we reasoned that MgtR might also control MgtA expression via the peptide–protein interaction. To test this idea, we assessed the interaction between the MgtA protein and the MgtR peptide *in vivo* by employing a bacterial two-hybrid system [19]. We constructed a series of plasmids in which proteins were expressed as forms fused to either the T18 or T25 catalytic domain of *Bordetella pertussis* adenylate cyclase. We then introduced these plasmids into the *E. coli* BTH101 strain which lacks the endogenous adenylate cyclase activity. A β-galactosidase assay determined that the BTH101 strain co-expressing the T18-MgtA and T25-MgtR fusion proteins displayed ~14-fold higher levels of the enzyme activity than the strain co-expressing only the T18 and T25 domains (Fig. 3). This result indicated that MgtA interacted with MgtR to complement adenylate cyclase activity, which consequently promoted cAMP-dependent expression of the *lacZ* gene. We further validated the MgtA–MgtR interaction. Co-expression of the T18-MgtC and T25-MgtR fusion proteins in the BTH101 strain increased β-galactosidase activity by ~5-fold over the control (Fig. 3), reproducing the result that MgtC binds to MgtR [13]. Moreover, we observed that MgtA does not interact with FeoA, a cytosolic protein involved in ferrous iron uptake [22]: the BTH101 strain co-expressing the T18-MgtA and T25-FeoA fusion proteins produced β-galactosidase at levels similar to the control (Fig. 3). Cumulatively, our results show that the MgtR peptide specifically binds to the MgtA protein *in vivo*.

3.5. MgtR-regulated MgtA expression affects Salmonella's growth in Mg^{2+}-limited conditions

As deletion of the *mgtR* gene enhanced expression of the MgtA Mg^{2+} transporter in low Mg^{2+} (Fig. 1A), we hypothesized that the *mgtR* deletion mutant might grow better than the wild-type strain under such a condition. However, these two strains grew similarly in medium containing 10 μM Mg^{2+} (data not shown). We noted the fact that *Salmonella* can also import Mg^{2+} via other transporters such as CorA and MgtB [4]. In addition, the MgtC protein contributes to *Salmonella*'s growth in Mg^{2+}-deprived conditions [14], though it is not a Mg^{2+} transporter *per se* [23,24]. We reasoned that these proteins might mask the effect of MgtR-regulated MgtA expression on *Salmonella* growth. Therefore, we reevaluated the role of MgtR using an EN553 strain in which the *corA, mgtB,* and *mgtC* genes were deleted. We found that in 10 μM Mg^{2+}, the EN553 strain can grow to higher levels in the absence of MgtR than its presence (Fig. 4A). In contrast, when expression of the MgtA protein was repressed in 1 mM Mg^{2+} (Fig. 1A), both the $mgtR^{+}$ and $mgtR^{-}$ strains grew similarly (Fig. 4B). Cumulatively, these results suggest that the increase of MgtA expression resulting from *mgtR* deletion enhances Mg^{2+} uptake in Mg^{2+}-limited conditions.

4. Discussion

In *S.* Typhimurium, expression of the MgtA Mg^{2+} transporter is regulated in highly complex fashions. As external Mg^{2+} concentrations decrease to micromolar levels, the PhoP response regulator that is activated by the PhoQ sensor promotes transcription of the *mgtA* gene [7,21]. Consistent with notion that the PhoP/PhoQ system can also be activated by two other signals, acidic pH and antimicrobial peptides [25,26], the acidic pH-activated PhoP protein can promote transcription initiation of *mgtA* even at millimolar Mg^{2+} concentrations [11]. However, under this circumstance, the *mgtA* gene is unable to be fully transcribed [11] because the *mgtA* leader sequences bind to cytoplasmic Mg^{2+} and form a distinct structure that leads to transcriptional termination [10]. In addition to these regulations allowing the MgtA transporter to be expressed only under Mg^{2+}-deplete conditions, our results now reveal that *Salmonella* also possesses the mechanism to limit levels of the MgtA protein that is produced at low Mg^{2+} concentrations.

In response to low Mg^{2+}, the *mgtCBR* operon co-expresses the MgtC virulence protein, MgtB Mg^{2+} transporter and MgtR peptide [13]. Once expressed, the MgtR peptide, which is predicted to be localized in the inner membrane, acts on the MgtC protein to repress MgtC levels [13]. Our data show that the MgtA Mg^{2+} transporter is another protein of which expression is negatively controlled by

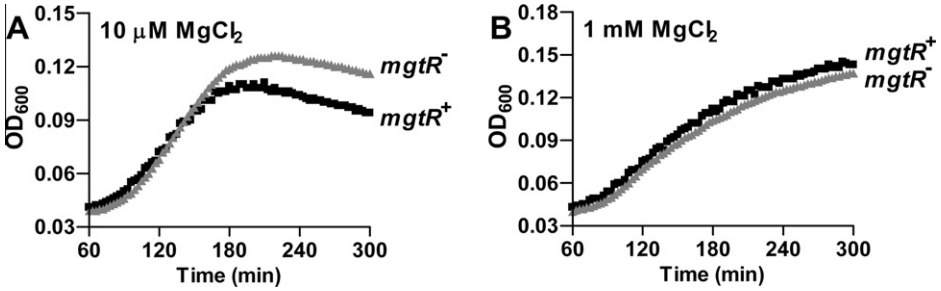

Fig. 4. The lack of MgtR enhances MgtA-mediated Mg^{2+} uptake at low Mg^{2+} concentrations. Growth curves of *Salmonella* strains lacking the *corA* and *mgtCB* genes. EN553 (*mgtR*$^+$) and EN477 (*mgtR*$^-$) strains were grown in N-minimal medium containing 10 μM (A) or 1 mM (B) $MgCl_2$. The OD_{600} values of the cultures were measured every 5 min using a Victor^3 plate reader (PerkinElmer). The results shown are representative of three experiments, which gave similar results.

the MgtR peptide: MgtA expression was enhanced in the *mgtR* deletion mutant but reduced in the strain overexpressing MgtR from a heterologous promoter (Fig. 1). MgtR-regulated MgtA expression seems to be physiologically relevant because deletion of the *mgtR* gene enhanced growth of the *Salmonella* strain lacking the *corA* and *mgtCB* genes in low Mg^{2+} concentrations (Fig. 4), possibly by increasing MgtA-mediated Mg^{2+} uptake. How then could the MgtR peptide repress MgtA expression? The MgtR peptide has been demonstrated to directly interact with the MgtC protein and proposed to unfold MgtC in the inner membrane [13]. Interaction with MgtR leads to MgtC degradation by the membrane-bound FtsH protease [13], though the precise role of MgtR in this event remains unknown. We determined that the MgtR peptide specifically binds to the MgtA protein *in vivo* (Fig. 3) without affecting transcription of the *mgtA* gene (Fig. 2). Therefore, we propose that the MtgA transporter bound by the MgtR peptide might undergo the proteolytic pathway, similar to that of the MgtC protein.

What is the biological significance of MgtR-controlled MgtA expression? *Salmonella* possesses at least three different Mg^{2+} transporters including CorA, MgtA and MgtB [4]. In contrast to the constitutively expressed CorA protein [5], two common mechanisms allow the MgtA and MgtB transporters to be produced only in low Mg^{2+}: the PhoP regulator activates transcription of the *mgtA* and *mgtB* genes [8,9,21], message of which can be fully transcribed by function of the leader sequences [10]. However, as the MgtA and MgtB proteins can import Mg^{2+} with similar efficiency [5], there could be a condition where *Salmonella* needs to express the MgtA and MgtB proteins differentially. The AmgR RNA has recently been identified as a regulator to limit levels of the MgtB protein that is produced in low Mg^{2+} concentrations [12]. We propose that, together with the AmgR RNA, the MgtR peptide could be used for *Salmonella* to achieve differential expression of MgtA and MgtB. When activated by the low Mg^{2+} signal, the PhoP regulator promotes synthesis of both the MgtR peptide [13] and AmgR RNA [12]. However, as PhoP displays much higher affinity for the *mgtCBR* promoter than *amgR* promoter [12], MgtR expression could be earlier than AmgR expression upon PhoP activation. Consequently, the MgtR-mediated MgtA degradation could take place preceding the AmgR-repression of MgtB, lowering the MgtA:MgtB ratio. By balancing levels of the two PhoP-activated Mg^{2+} transporters, this regulatory mechanism could be important for *Salmonella* to maintain Mg^{2+} homeostasis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.11.107.

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