A Mg^{2+} -responding RNA That Controls the Expression of a Mg^{2+} Transporter

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Mg^{2+} is the most abundant divalent cation in biological systems. It is required for ATP-mediated enzymatic reactions and as a stabilizer of ribosomes and membranes. The enteric bacterium *Salmonella enterica* serovar Typhimurium harbors three Mg^{2+} transporters and a regulatory system—termed PhoP/PhoQ—whose activity is regulated by the extracytoplasmic levels of Mg^{2+} . We have determined that expression of the PhoP-activated Mg^{2+} transporter MgtA is also controlled by its 5'-untranslated region (5'UTR). The 5'UTR of the *mgtA* gene can adopt different stem-loop structures depending on the Mg^{2+} levels, which determine whether transcription reads through into the *mgtA*-coding region or stops within the 5'UTR. This makes the *mgtA* 5'UTR the first example of a cation-responding riboswitch. The initiation of *mgtA* transcription responds to extracytoplasmic Mg^{2+} , and its elongation into the coding region to cytoplasmic Mg^{2+} , which provides a singular example where the same ligand is sensed in different cellular compartments to regulate disparate steps in gene transcription. The PhoP-activated Mg^{2+} transporter MgtB is also regulated by Mg^{2+} in a strain lacking the Mg^{2+} sensor PhoQ, suggesting the presence of additional Mg^{2+} -responding devices.

All living organisms need Mg^{2+} . This is because Mg^{2+} is an essential cofactor for all ATP-mediated enzymatic reactions and because Mg^{2+} helps maintain the stability of membranes as well as of certain macromolecular complexes such as the ribosome (Reinhart 1988). Therefore, cells must preserve physiological Mg^{2+} concentrations, which might not be the same in different subcellular compartments. To achieve this task, cells need to have the means to sense the levels of Mg^{2+} as well as the ability to modify the movement of this essential cation across biological membranes and/or from storage locations.

The gram-negative bacterium *S. enterica* serovar Typhimurium harbors a Mg^{2+} -responding regulatory system (i.e., PhoP/PhoQ) (Groisman 2001), three Mg^{2+} transporters (i.e., CorA, MgtA, and MgtB) (for review, see Smith and Maguire 1998; Maguire 2006), and an integral membrane protein that is required for optimal growth in low Mg^{2+} (i.e., MgtC). The PhoP/PhoQ system consists of the PhoQ protein, a sensor for extracytoplasmic Mg^{2+} , and the transcriptional regulator PhoP (Groisman 2001). When *Salmonella* experiences low Mg^{2+} , the PhoQ protein promotes phosphorylation of the PhoP protein, which can then bind to its target promoters to modify gene transcription: PhoP-activated genes are turned on and PhoP-repressed genes are turned off. And when *Salmonella* faces high Mg^{2+} , PhoQ promotes the unphosphorylated state of the PhoP protein, which is unable to bind to its target promoters and results in no transcription of PhoP-activated genes and derepression of PhoP-repressed genes (Soncini et al. 1996; Castelli et al. 2000;

Chamngongpol and Groisman 2000; Montagne et al. 2001; Chamngongpol et al. 2003).

The CorA protein can mediate both influx and efflux of Mg^{2+} and does not exhibit sequence similarity to the MgtA or MgtB proteins, which are 50% identical to one another and solely mediate Mg^{2+} influx (for review, see Smith and Maguire 1998; Maguire 2006). MgtA and MgtB also differ from CorA in that transcription of the *mgtA* and *mgtB* genes is directly regulated by the PhoP protein in response to extracytoplasmic Mg^{2+} sensed by the PhoQ protein (Soncini et al. 1996; Yamamoto et al. 2002; Zwir et al. 2005), whereas expression of CorA responds neither to Mg^{2+} nor to the PhoP/PhoQ system (Chamngongpol and Groisman 2002). Yet, the activity of the CorA transporter is enhanced in a *phoP* mutant by an unknown mechanism (Chamngongpol and Groisman 2002).

The PhoP/PhoQ system controls expression of about 3% of the genome. This includes not only the genes encoding the Mg^{2+} transporters MgtA and MgtB, but also the genes coding for products that modify the negatively charged residues in the lipopolysaccharide (i.e., LPS), which are neutralized primarily by Mg^{2+} (Groisman et al. 1997; Groisman 2001). The LPS is the dominant molecule in the outer leaflet of the outer membrane of *Escherichia coli* and *Salmonella*, and it has been estimated that more than one-third of the total Mg^{2+} content is located in the LPS (Groisman et al. 1997). The PhoP/PhoQ system and the MgtA and CorA transporters are widely distributed within the family Enterobacteriaceae (Smith and Maguire 1995; Blanc-Potard and Groisman 1997). This is in contrast to the sporadic distribution of the MgtB and MgtC proteins. This paper discusses the discovery, characterization, and function of the first example of an Mg^{2+} -responding riboswitch, and how such RNA regulates cytoplasmic Mg^{2+} levels.

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SECTION THEMES

The 5'UTR of the *mgtA* mRNA Harbors a Mg^{2+} -responding Regulatory Element

PhoP* is a variant of the PhoP protein that can promote gene transcription in the absence of the Mg^{2+} sensor PhoQ (Chamnongpol and Groisman 2000). As expected, transcription of the PhoP-activated genes *pcgL* and *pmrC* did not respond to Mg^{2+} in a *phoP** Δ *phoQ* strain. In contrast, Mg^{2+} could still modulate *mgtA* transcription in this strain. This suggested that an Mg^{2+} -sensing system can regulate *mgtA* expression independently of the PhoP/PhoQ system. Consistent with this notion, Maguire's laboratory demonstrated that luciferase activity originating from a plasmid-borne *mgtA-lux* fusion was still up-regulated in media of very low Mg^{2+} in *phoP* and *phoQ* mutants (Tao et al. 1998).

The 5'UTR of the *mgtA* gene is 264 nucleotides long. Such a long 5'UTR is unusual, raising the possibility that it might be involved in the regulation of *mgtA* expression. Consistent with this notion, replacement of 100 nucleotides of the 5'UTR by an 84-bp "scar" sequence abolished Mg^{2+} regulation of *mgtA* expression in the *phoP** Δ *phoQ* strain (Cromie et al. 2006). Moreover, Mg^{2+} regulated the production of β -galactosidase in a wild-type *Salmonella* strain harboring a plasmid containing the DNA sequence corresponding to the 264-bp *mgtA* 5'UTR region cloned between a derivative of the *lac* promoter and a promoterless *lacZ* gene (Cromie et al. 2006). These results demonstrated that the 5'UTR of *mgtA* is necessary and sufficient for Mg^{2+} regulation.

Analysis of the Potential Structures That May Be Adopted by the Phylogenetically Conserved *mgtA* 5'UTR Suggests a Possible Mechanism by Which *mgtA* Expression Might Be Regulated

The M-fold program predicted that the *mgtA* 5'UTR could form several stem-loop structures including two, designated A and B, with predicted energies of -21.4 and -13.2 kcal/mole, respectively. Interestingly, an alternative stem-loop structure—termed C—could be formed by pairing of sequences originating from the right arm of stem A and the region that separates stem-loops A and B with sequences in the left arm of stem-loop B. The resulting stem has a predicted energy of -12.8 kcal/mole, which is very similar to that predicted for stem-loop B (Fig. 1A).

Examination of the microbial genome databases revealed that in several enteric species, the *mgtA* open reading frame is preceded by sequences that upon transcription could adopt stem-loop structures similar to those described above for the *Salmonella mgtA* 5'UTR. The high degree of primary and secondary structure conservation suggests that the possibility of forming the alternative stem-loop structures A plus B versus C is conserved in the *mgtA* 5'UTR of *E. coli*, *Citrobacter rodentium*, *Klebsiella pneumoniae*, *Erwinia chrysanthemi*, *Serratia marcescens*, and *Yersinia enterocolitica* (Fig. 1).

When wild-type *Salmonella* harbored a plasmid with the *mgtA* 5'UTR region from *E. coli mgtA* behind a derivative of the *lac* promoter and in front of a promoterless *lacZ*

gene, β -galactosidase activity was 15-fold higher in bacteria grown in low Mg^{2+} than in those grown in high Mg^{2+} (Cromie et al. 2006). Although this is lower than the 23-fold ratio exhibited by the isogenic strain with the plasmid harboring the *Salmonella mgtA* 5'UTR, these results demonstrate that the *E. coli mgtA* 5'UTR can also confer Mg^{2+} regulation.

Taken together with the fact that the mRNA levels for the 5'UTR and coding region of the *Salmonella mgtA* gene are differentially regulated by Mg^{2+} (Cromie et al. 2006), the analyses described above suggest a model by which *mgtA* expression might be controlled. According to this model, the *mgtA* 5'UTR may adopt different stem-loop structures, which would be determined by the cytoplasmic levels of Mg^{2+} , and depending on which structure is formed (stem-loops A plus B vs. stem-loop C; Fig. 1A), transcription would stop within the 5'UTR or continue into the *mgtA*-coding region. Genetic analysis suggested that formation of stem-loops A plus B is associated with *mgtA* transcription not reaching the coding region and could be the structure favored in high Mg^{2+} . In contrast, formation of stem-loop C would result in readthrough into the *mgtA*-coding region, which would be expected for a structure forming in low Mg^{2+} . In sum, the 5'UTR of the *mgtA* gene appears to use an attenuation-like mechanism (Landick et al. 1996; Henkin and Yanofsky 2002) to control *mgtA* expression.

 Mg^{2+} Can Modify the Structure of the *mgtA* 5'UTR

During the last few years, research carried out primarily by the Breaker, Nudler, and Henkin laboratories has demonstrated that the 5'UTR of certain bacterial transcripts can bind specific metabolites including amino acids, nucleotides, and sugars (for reviews, see Winkler 2005; Winkler and Breaker 2005). Binding of such a metabolite alters the expression of the open reading frames that follow the 5'UTR, which are typically involved in the biosynthesis or transport of the metabolite. Thus, when the levels of the metabolite build up in the cytoplasm of the bacterial cell, the 5'UTRs function as "riboswitches" that upon binding the metabolite, turn off expression of the biosynthetic or transport proteins promoting accumulation of the metabolite (Winkler 2005; Winkler and Breaker 2005).

If the *mgtA* 5'UTR functions as an Mg^{2+} -responding riboswitch, one would expect that (1) it should bind Mg^{2+} , (2) Mg^{2+} binding should promote the formation of particular structures in the 5'UTR thereby affecting *mgtA* expression, and (3) favoring the formation of particular structures by mutation should be associated with expression/lack of expression independently of the Mg^{2+} concentration experienced by the bacterial cell.

Enzymatic and chemical probing of the *mgtA* 5'UTR demonstrated that Mg^{2+} can alter the structure of this RNA at several places and that the 5'UTR can adopt secondary structures which are consistent with the model predicted by the M-fold program (Fig. 1A). Mg^{2+} binding to the *mgtA* 5'UTR was entirely expected given that Mg^{2+} has been known to bind to different classes of RNA molecules in different capacities (e.g., Mg^{2+} has a critical role in the

Figure 1. Predicted secondary structure of the phylogenetically conserved *mgtA* 5'UTR. (A) Schematic representation of the secondary structure of the 264-nucleotide *mgtA* 5'UTR as predicted by the M-fold program. (Left) Stem-loops A and B, which are postulated to form in high Mg^{2+} ; (right) stem-loop C, which is predicted to form in low Mg^{2+} . Sequences in color represent regions involved in stem structures. The predicted energy for stem-loops A, B, and C is presented underneath. (B–G), Predicted stem-loop structures A, B, and C corresponding to the potential 5'UTRs for the *mgtA* gene from *E. coli*, *C. rodentium*, *K. pneumoniae*, *E. chrysanthemi*, *S. marcescens*, and *Y. enterocolitica*. Sequences in color represent regions involved in stem-loop structures. The predicted energy for the three stem-loops A, B, and C is presented underneath. Note conservation of AUGG sequence of loop A in all species but *Y. enterocolitica* and of the short stem-loop within loop A in *S. enterica*, *E. coli*, *C. rodentium*, and *K. pneumoniae*. Other conserved regions are shown in Cromie et al. (2006). (Reprinted, with permission, from Cromie et al. 2006 [© Elsevier].)

structure and/or enzymatic activity of several ribozymes; Hanna and Doudna 2000). Thus, we focused our analysis on those regions of the *mgfA* 5'UTR that were anticipated to be differentially affected by Mg^{2+} in ways that would affect gene expression. For example, G146 and G147 are predicted to be part of the loop in stem-loop B, which is anticipated to form in high Mg^{2+} but to be base-paired as part of stem C in low Mg^{2+} (Fig. 1A). By using RNase T1, which cleaves unpaired G residues, we detected more cleavage at both G146 and G147 at high Mg^{2+} than at low Mg^{2+} , in agreement with the prediction. Likewise, G151 is predicted to be base-paired in high Mg^{2+} but unpaired in low Mg^{2+} (Fig. 1A), and that is what the T1 cleavage treatment showed (Cromie et al. 2006). On the other hand, G149 was cleaved to a similar extent in low and high Mg^{2+} as suggested by the model (Fig. 1A). Importantly, the low and high Mg^{2+} concentrations used in this experiment correspond to those around the physiological range (Froschauer et al. 2004).

Formation of Stem-loop C Results in *mgfA* Expression

The genetic and biochemical data discussed above suggest that when stem-loop C is formed, the *mgfA*-coding region is transcribed. To test this model, we investigated the behavior of strains harboring mutant *mgfA* 5'UTRs in which the ability to form stem-loop C had been compromised. G120 is predicted to base-pair with C145 in the middle of stem C (Fig. 1A). Mutants with either the G120C or C145G single-nucleotide substitutions failed to express a reporter gene that was cloned behind the 5'UTR region (Cromie et al. 2006). This was true regardless of the Mg^{2+} concentration in which the organism was grown. On the other hand, the G120C C145G double mutant exhibited Mg^{2+} -regulated expression of the reporter gene. This supports the notion that G120 can pair with C145 and that the ability to form stem C is necessary for *mgfA* expression. Although C61 is predicted to base-pair with G120 in stem A, a strain with the C61G substitution still expressed the reporter gene in a Mg^{2+} -regulated fashion. However, instead of the tenfold ratio in transcription levels in low versus high Mg^{2+} exhibited by organisms with the wild-type *mgfA* 5'UTR, the ratio was less than fivefold. Thus, it would appear that the ability to form stem A is partially compromised in the C61G mutant, which results in more than twofold higher levels of expression in high Mg^{2+} , but not eliminated because base-stacking of G residues may still favor formation of stem A. Finally, the RNAs corresponding to the G120C and C145G single mutants exhibited aberrant T1 cleavage patterns at positions away from the mutated nucleotides, indicative that these mutations can affect the formation of structures in other regions of the 5'UTR (Cromie et al. 2006).

Looking for the Mg^{2+} -sensing Domain of the *mgfA* Riboswitch

In their most straightforward form, riboswitches can be divided into two domains: an aptamer domain that is responsible for binding a specific ligand and an expression

platform domain that is responsible for altering gene expression in response to ligand binding (Winkler and Breaker 2005). In fact, RNAs can be engineered to respond to particular ligands by mixing and matching RNAs of different origins (Kim et al. 2005; Muller et al. 2006). Then, which are the regions of the 5'UTR responsible for Mg^{2+} sensing and for modulating *mgfA* expression upon Mg^{2+} binding?

As discussed above, the formation of stem-loops B and C is associated with lack of expression and expression of the *mgfA* gene, respectively. Because the predicted ΔG s for these two stem-loop structures are very similar (Fig. 1A), we would hypothesize that a different part of the 5'UTR determines which of the two structures is formed and that such part of the 5'UTR is likely involved in Mg^{2+} sensing. Stem-loop A is a prime candidate for having this role because (1) this region is transcribed before stem-loops B and C, and thus would be available to bind Mg^{2+} before the rest of the 5'UTR is made, and (2) it is conserved with other enteric species, and some of the conserved nucleotides are differentially cleaved by RNase T1 in the presence of Mg^{2+} . We targeted for mutagenesis a short stem predicted to be present in the loop-A region and found that a mutant in which the ability to form the short stem was abrogated constitutively expressed the *mgfA* gene (i.e., no longer responded to Mg^{2+}). It appears that the short stem and not the particular sequences that make up the stem is the critical element in Mg^{2+} sensing because making the compensatory mutations to restore formation of the short stem brought back Mg^{2+} -regulated expression of the *mgfA* gene. That stem-loop A may be involved in Mg^{2+} sensing is further supported by the Mg^{2+} -promoted changes in the stem-loop A structure when present by itself (Cromie et al. 2006).

The requirement for sequences in loop A for Mg^{2+} sensing does not mean that Mg^{2+} binding to this region is sufficient to trigger the conformational changes that affect *mgfA* expression. Therefore, although it is possible that loop A may constitute a Mg^{2+} -binding pocket analogous to those involved in metabolite-sensing riboswitches (Serganov et al. 2004, 2006; Montange and Batey 2006), we cannot rule out the possibility of Mg^{2+} affecting the formation of the various stem-loop structures by binding to multiple sites in the 5'UTR. In this context, it is important to note that in addition to stem-loops A, B, and C, several regions of the *Salmonella mgfA* 5'UTR show high levels of sequence identity with homologs in other species (Fig. 1A). These regions could also be involved in Mg^{2+} sensing. Alternatively or in addition, they may be conserved due to their role in regulating *mgfA* expression.

How the Mg^{2+} -responding Riboswitch May Control Expression of the *MgfA* Protein

We could recapitulate the Mg^{2+} -regulated transcription elongation beyond the *mgfA* 5'UTR region using an in vitro transcription system consisting of the *E. coli* RNA polymerase σ^{70} holoenzyme, linear DNA templates harboring a derivative of the *lac* promoter (Liu et al. 2004), and different concentrations of Mg^{2+} around the

physiological levels (Froschauer et al. 2004). A template harboring the full-length wild-type 5'UTR of the *mgtA* gene behind the *lac* promoter derivative gave rise to two bands: a large band representing the readthrough transcript, the amount of which decreased as the Mg^{2+} concentration increased, and a small band corresponding to a truncated product of approximately 220 nucleotides. These in vitro experiments demonstrated that the Mg^{2+} -regulated expression mediated by the 5'UTR of the *mgtA* gene does not require proteins other than RNA polymerase, and they are consistent with the notion that the 5'UTR is a Mg^{2+} -responding regulatory element. Yet, it is possible that a protein may participate in the regulation of *mgtA* elongation in vivo.

There are (at least) three possible mechanisms that may explain why the *mgtA* transcript does not go beyond a certain position within the 5'UTR when bacteria experience Mg^{2+} levels that are not too low. On the one hand, the truncated product could result from RNA-mediated cleavage as demonstrated for the glucosamine-6-phosphate-responding riboswitch (Winkler et al. 2004). On the other hand, the truncated product may originate as the result of early transcription termination or due to RNA polymerase pausing at a site and then falling off the DNA template. The *mgtA* 5'UTR does not appear to function as a ribozyme because (1) the truncated product could be labeled using ^{32}P Cp and T4 RNA ligase, (2) ribozymes that yield free 2' and 3' hydroxyl groups are typically larger than the 220-nucleotide *mgtA* 5'UTR (Jacquier 1996), (3) there was no delay in the appearance of the truncated product relative to the readthrough product as one might expect for a maturation reaction, and (4) addition of Mg^{2+} did not promote degradation of the readthrough transcript generated in vitro. Moreover, when the 5'UTR was synthesized in vitro by T7 RNA polymerase, only a single product was of the same size as that corresponding to the readthrough transcript generated with *E. coli* RNA polymerase. (The caveat with the latter result is that T7 RNA polymerase-promoted transcription of the *mgtA* templates may be too fast for the hypothetical structure having the putative ribozyme activity to form.)

Transcription attenuation mechanisms have been typically associated with the formation of transcription terminators in one of the alternative structures. However, the sequences preceding nucleotide 220 lack the typical features of Rho-independent terminators (i.e., a GC-rich RNA hairpin followed by a poly(U) sequence [Landick et al. 1996]), and the generation of a truncated product in the in vitro transcription reaction did not require the Rho protein. This raises the possibility that the truncated product may be formed as a consequence of transcription pausing followed by RNA polymerase release from the DNA template. Although more experiments are required to determine how Mg^{2+} affects transcription elongation in the *mgtA* 5'UTR, sequences beyond those corresponding to stem-loops A, B, and C appear to participate in this process because a strain harboring a derivative of the 5'UTR with the sequence corresponding to positions 1–178 (which does not include sequences beyond stem-loop B) exhibited constitutive *mgtA* expression.

A Physiological Rationale for Dual Control of *mgtA* Expression

When *Salmonella* experiences a low Mg^{2+} environment, *mgtA* is the first PhoP-activated gene that is transcribed (our unpublished results), which may reflect the organism's effort to maintain physiological levels of cytoplasmic Mg^{2+} . Once the MgtA protein is produced, it will mediate internalization of Mg^{2+} , which will eventually bind to the *mgtA* 5'UTR to shut off MgtA expression (Fig. 2). This regulatory design allows the rapid uptake of Mg^{2+} and at the same time makes MgtA an arbiter of its own expression.

The *mgtA* gene is controlled at the level of transcription initiation by the PhoP protein, which is activated in response to the extracytoplasmic levels of Mg^{2+} sensed by the PhoQ protein, and at the level of transcription elongation in response to the cytoplasmic levels of Mg^{2+} sensed by the *mgtA* 5'UTR. Thus, Mg^{2+} acts in two different compartments to control different steps in *mgtA* transcription. This dual control of the *mgtA* gene is in contrast to most genes regulated by riboswitches, which are not subjected to regulation at the level of transcription initiation (Winkler and Breaker 2003; Brantl 2004; Nudler and Mironov 2004). The dual regulation of *mgtA* expression is somewhat reminiscent of that governing expression of the *trp* biosynthetic genes of *E. coli*. In this case, the control is exerted both by the tryptophan-responding TrpR protein, a transcriptional repressor that binds to the *trp* promoter-operator region, and by a transcription attenuation mechanism that responds to charged tRNA^{Trp} (Bennett and Yanofsky 1978; Yanofsky 2004). As described for the regulation of *trp*, most of the *mgtA* regulation takes place at the level of transcription initiation (i.e., by TrpR and PhoP), and only a small fraction is controlled at the level of transcription elongation (by the leader sequence and riboswitch, respectively).

What is the purpose of the dual *mgtA* control? It may enable *Salmonella* to exert differential regulation over those determinants directly affecting cytoplasmic Mg^{2+} (i.e., the Mg^{2+} transporters MgtA and MgtB and potentially other proteins) from those mediating modifications in the bacterial cell envelope (i.e., the proteins affecting the negative charges in the LPS) when they are all under transcriptional control of the same regulatory system (i.e., PhoP/PhoQ) (Groisman 2001). Indeed, expression of the PhoP-regulated *mgtCB* operon, which encodes the inner membrane protein MgtC and the Mg^{2+} transporter MgtB, appears to have features in common with those controlling the *mgtA* gene because *mgtCB* transcription also responds to Mg^{2+} in a strain lacking the Mg^{2+} sensor PhoQ protein in a process that requires the 5'UTR preceding the *mgtC* open reading frame (our unpublished results).

Does the *mgtA*-derived 5'UTR Regulate Gene Expression by Affecting Other mRNAs in *trans*?

The Storz laboratory has demonstrated that an RNA fragment corresponding to the 5'UTR of the *mgtA* transcript accumulates in *E. coli* grown in LB media and that

Figure 2. Model for regulation of the *Salmonella* MgtA Mg^{2+} transporter. When *Salmonella* experiences a low Mg^{2+} environment, the sensor PhoQ responds to extracytoplasmic Mg^{2+} by promoting phosphorylation of the DNA-binding protein PhoP, which binds to the *mgtA* promoter and initiates *mgtA* transcription. If cytoplasmic Mg^{2+} levels are above a certain threshold, Mg^{2+} binding to the *mgtA* 5'UTR results in the formation of stem-loop structures that promote transcription stopping within the 5'UTR. Once the cytoplasmic Mg^{2+} concentration goes below a certain level, an alternative stem-loop structure is formed, which allows readthrough into the *mgtA*-coding region. Production of the MgtA protein allows internalization of Mg^{2+} , the concentration of which will increase resulting in binding to the *mgtA* 5'UTR and shutting off of MgtA expression. The MgtB Mg^{2+} transporter is also regulated by Mg^{2+} in a process that requires the 5'UTR region preceding the *mgtC* gene. That 5'UTR region could be an Mg^{2+} sensor or it may be the target of the truncated RNA corresponding to the 220-nucleotide fragment originating from the *mgtA* 5'UTR. (Modified, with permission, from Cromie et al. 2006 [© Elsevier].)

the levels of this small RNA are lower in a strain harboring a mutation in the *hfq* gene (Kawano et al. 2005), which encodes a protein shown to bind to bacterial small RNAs exerting regulatory effects *in trans*. When we consider the fact that *mgtA* has the highest affinity of the PhoP-activated promoters (Minagawa et al. 2003) and it is the first and most highly expressed gene when the PhoP/PhoQ system is activated (our unpublished results), this raises the possibility that the *mgtA* 5'UTR might act *in trans* to regulate gene expression. The putative target mRNAs of the *mgtA* 5'UTR should be produced concurrently with the *mgtA* 5'UTR gene, and thus they are likely to be members of the PhoP regulon, but they could also be genes that are constitutively expressed. Therefore, we examined the DNA sequence between the reported transcription start site and the predicted site of translation initiation for several members of the PhoP regulon. We found that in addition to the 5'UTR of the *mgtCB* operon discussed above, several PhoP-activated genes harbor 5'UTRs that are more than 200 nucleotides long (i.e., *pagC*, *nagA*, and *ugtL*; Table 1). Moreover, we could identify regions of complementarity between the *mgtA* 5'UTR and the 5'UTRs of these genes. This suggests that the *mgtA* 5'UTR may have two roles: First, it may act as an Mg^{2+} -responding

riboswitch that determines whether the *mgtA*-coding region is transcribed resulting in the production of the MgtA protein. Second, it may regulate expression of other genes by acting *in trans*. Alternatively, the PhoP-regulated genes with unusually long 5'UTRs may respond to Mg^{2+} or other ligands to alter expression of the corresponding open reading frame (Fig. 2).

CONCLUSIONS

The 5'UTR of the *mgtA* gene constitutes the first example of a cation-responsive riboswitch. However, bioinformatics studies suggest that there are likely to be other cation-responding riboswitches (Barrick et al.

Table 1. PhoP-regulated Genes with Long 5'UTRs

Gene	Size of 5'UTR (in nucleotides)	Function of gene product
<i>mgtCB</i>	281	Mg^{2+} transport, growth in low Mg^{2+}
<i>nagA</i>	417	<i>N</i> -acetylglucosamine deacetylase
<i>pagC</i>	559	outer-membrane protein
<i>ugtL</i>	192	lipid-A phosphatase?

2004; Merino and Yanofsky 2005). This regulation of the Mg^{2+} transporter MgtA protein may enable *Salmonella* to exert differential control over those genes that are involved in cytoplasmic Mg^{2+} homeostasis from those involved in the modification of Mg^{2+} -binding sites in the cell envelope. Moreover, by regulating Mg^{2+} transport, MgtA may impact other riboswitches that respond to other ligands but are affected by Mg^{2+} (Yamauchi et al. 2005). The truncated *mgtA* RNA derived from the 5'UTR when organisms experience high Mg^{2+} might have the additional property of regulating genes *in trans*.

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