Activated by different signals, the PhoP/PhoQ two-component system differentially regulates metal uptake

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Activated by Different Signals, the PhoP/PhoQ Two-Component System Differentially Regulates Metal Uptake

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The PhoP/PhoQ two-component system controls several physiological and virulence functions in Salmonella enterica. This system is activated by low Mg2+, acidic pH, and antimicrobial peptides, but the biological consequences resulting from sensing multiple signals are presently unclear. Here, we report that the PhoP/PhoQ system regulates different Salmonella genes depending on whether the inducing signal is acidic pH or low Mg2+. When Salmonella experiences acidic pH, the PhoP/PhoQ system promotes Fe2+ uptake in a process that requires the response regulator RstA, activating transcription of the Fe2+ transporter gene feoB. In contrast, the PhoP-induced RstA protein did not promote feoB expression at neutral pH with low Mg2+. The PhoP/PhoQ system promotes the expression of the Mg2+ transporter mgtA gene only when activated in bacteria starved for Mg2+. This is because mgtA transcription promoted at high Mg2+ concentrations by the acidic-pH-activated PhoP protein failed to reach the mgtA coding region due to the mgtA leader region functioning as a Mg2+ sensor. Our results show that a single two-component regulatory system can regulate distinct sets of genes in response to different input signals.

Signal transduction mediated by two-component regulatory systems enables bacterial cells to rapidly adapt to and survive various stressful conditions. The PhoP/PhoQ two-component system is comprised of the response regulator PhoP and the sensor PhoQ. The PhoP protein has been demonstrated to sense Mg2+ concentrations as a specific signal (14). When the environmental Mg2+ concentration is lowered to micromolar levels, PhoQ promotes the phosphorylated state of the PhoP protein (14, 32). In Salmonella enterica, the low-Mg2+-activated PhoP protein directly regulates the transcription of many genes that are necessary for virulence in mammalian hosts, as well as other physiological processes (15). In particular, consistent with the environment where the system is activated, PhoP/PhoQ allows Salmonella to grow at limited concentrations of Mg2+ (14), which results from PhoP-activated expression of the mgtA and mgtB genes, encoding Mg2+ transporters (34). The PhoP-mediated PhoP phosphorylation also occurs at acidic pH (31), and transcription levels of the PhoP-activated genes pagA, phoN, and pmrD increase in Salmonella experiencing acidic conditions (3, 28, 31). Taking into account that certain antimicrobial peptides promote the expression of a subset of the PhoP-regulated genes through the PhoP and PhoQ proteins (2), the activity of the PhoP/PhoQ system appears to respond to at least three different signals.

The PhoP/PhoQ system also regulates gene expression by controlling the levels and/or activity of other regulators (22). The RstA/RstB two-component system, which consists of a response regulator RstA and its partner sensor RstB, is the one whose expression is regulated by the PhoP/PhoQ system. In Escherichia coli, the PhoP protein binds to the rstA promoter, and transcription of the rstA gene is repressed by the PhoP/PhoQ system in cells grown at a high concentration of Mg2+ (26). The RstA protein promotes transcription of the asr gene, coding for a product necessary for adaptation to acidic stress (27). Consequently, at acidic pH, transcription of the asr gene is not fully activated in a strain lacking the phoP gene (27), due to the reduced levels of the RstA protein. In addition, the rstA gene has been identified as a multicopy suppressor of the essential genes yjeE, yeaZ, and ygdD (5, 16).

The Salmonella PhoP protein also directly binds to and activates the rstA promoter at low Mg2+ (Fig. 1) (I. Zwir et al., unpublished data). We have recently demonstrated that when overexpressed from a plasmid, the RstA protein specifically binds to the feoA promoter and promotes transcription of the feoAB operon encoding the ferrous iron (Fe2+) transporter FeoB (Fig. 1) (20).

The PhoP/PhoQ system promotes the expression of the RstA protein when activated at either low Mg2+ or acidic pH (Fig. 1). We now report that the RstA protein promotes transcription of the feoB gene exclusively when activated at acidic pH, thereby enhancing the growth of Salmonella in environments with limited iron. We show that, in contrast to the PhoP-controlled Fe2+ uptake, the expression of the Mg2+ transporter MgtA occurs only when the PhoP/PhoQ system is activated at low Mg2+. Thus, depending on the input signals, a single signal transduction system can differentially regulate its target genes.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Salmonella enterica serovar

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† Supplemental material for this article may be found at http://jb.asm.org/.
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FIG. 1. Model illustrating Fe$^{2+}$ and Mg$^{2+}$ uptake regulated by the PhoP/PhoQ system and RstA protein in Salmonella. (A) When activated at millimolar Mg$^{2+}$ concentrations by the acidic-pH signal, the phosphorylated PhoP protein promotes transcription of the rstA and mgtA genes. The induced RstA protein is activated, possibly by phosphorylation. The increase in feoB expression resulting from RstA binding to the feoA promoter enhances the FeoB-mediated Fe$^{2+}$ uptake. In contrast, mgtA transcription fails to proceed due to Mg$^{2+}$ binding to the mgtA leader. (B) The low-Mg$^{2+}$ signal activates the PhoP/PhoQ system at neutral pH. Activation of mgtA transcription by phosphorylated PhoP increases production of the full length of the mgtA mRNA, leading to expression of the MgtA protein, which promotes Mg$^{2+}$ uptake. In contrast, the RstA protein induced at neutral pH lacks the activity to promote transcription of the feoB gene.

Typhimurium strains are derived from strain 14028s. Phage P22-mediated transductions were performed as described previously (11). Bacteria were grown at 37°C in N-minimal medium (33), pH 7.7 or pH 5.7, supplemented with 0.1% Casamino Acids, 38 mM glycerol, and different concentrations of MgCl$_2$. Ampicillin, chloramphenicol, and kanamycin were used at 50 µg/ml, 25 µg/ml, and 50 µg/ml, respectively.

**Construction of bacterial strains.** The one-step gene inactivation method (10) was used for chromosomal gene deletion and epitope tagging. The sequences of primers used are indicated in Table S1 in the supplemental material. For construction of the rstB deletion strain, DS603, the Cmr cassette from plasmid pKD4 (10) was amplified using primers DE-rstB-R and pKD4 as DNA template; the Cmr cassette was obtained by PCR amplification of pKD3 with primers DE-mntH-R and DE-mntH-H. The purified PCR products were introduced into strain 14028s, resulting in the Delta(stABCD::Kmr or Delta(stABCD::Cmr) strain, respectively. Second, the Delta(stABCD::Kmr strain was transferred into the Delta(stABCD::Cmr) strain by phage P22-mediated transduction. Finally, both the Km$^r$ and Cmr cassettes were removed from the Delta(stABCD::Kmr or Delta(stABCD::Cmr) strain using pCP20. Deletion of the corresponding genes was verified by colony PCR. The Salmonella Typhimurium strain DS604 encoding the RstA protein with a FLAG tag at the C terminus in the normal rstA chromosomal location was constructed as follows: the Km$^r$ cassette was amplified by PCR using primers rstA-FLAG-F and rstA-FLAG-R and pKD4 as template and integrated at the 3’ end of the rstA gene. The Km$^r$ cassette was removed from the resulting strain by using pCP20, and the presence of a FLAG tag at the C terminus of RstA was confirmed by nucleotide sequencing.

**TABLE 1. Bacterial strains and plasmids used in this study**

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<th>Strain or plasmid</th>
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<td>* UTR, untranslated region; FRT, FLP recombination target.</td>
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quences of the rstA-FLAG gene and PhoP*-encoding regions on the recombinant plasmids were confirmed by nucleotide sequencing. The sequences of the primers used are indicated in Table S1 in the supplemental material.

RNA isolation and quantitative real-time reverse transcription-PCR (qRT-PCR) analysis. RNA was isolated from mid-exponential-phase culture (optical density at 600 nm [OD$_{600}$] of 0.5 to 0.6) grown in 20 ml of N-minimal medium. One-half milliliter of the culture was removed and mixed with 1 ml of RNAprotect bacterial reagent (Qiagen), and RNA was isolated using an RNeasy mini kit (Qiagen). The RNA sample was treated further with RNase-free DNase (Ambion). By using Omniscript reverse transcription reagents (Qiagen) and random primers (Invitrogen), cDNA was synthesized from 0.5 μg of RNA. Transcripts were quantified by real-time PCR using SYBR green PCR master mix (Applied Biosystems) on an ABI7300 sequence detection system (Applied Biosystems). The primers used for detection of transcripts of each gene are listed in Table S2 in the supplemental material. The transcription levels of each gene were calculated from a standard curve obtained by PCR with the same primers and serially diluted genomic DNA. The mRNA levels of target genes were normalized to 16S rRNA levels.

We have recently reported that overexpression of the RstA protein from a plasmid activates transcription of the feoAB operon encoding the ferrous iron transporter FeoB by direct RstA binding to the feoA promoter (20). Thus, we explored whether the RstA protein induced at low Mg$^{2+}$ and acidic pH by the PhoP/PhoQ system could promote transcription of the feoB gene.

We determined that, when grown in the presence of 2 mM Mg$^{2+}$, the wild-type strain expressed fourfold higher levels of feoB mRNA at pH 5.7 than at pH 7.7 (Fig. 3A). The feoB activation required the PhoP/PhoQ-dependent RstA protein, because there was no feoB transcription in response to acidic pH in strains with rstA or phoPQ deletion (Fig. 3A). Transcription of the feoB gene was not activated in the wild-type strain grown at pH 7.7 with 50 μM Mg$^{2+}$ (Fig. 3A). This is in spite of the fact that Salmonella bacteria experiencing low Mg$^{2+}$ produced the RstA protein at levels similar to the levels in bacteria grown at acidic pH (Fig. 2B, lanes 2 and 3). When both signals were present (i.e., low Mg$^{2+}$ and acidic pH), feoB transcription took place in an RstA- and PhoPQ-dependent manner (Fig. 3A). This result was not due to differences in the RstA protein levels between Salmonella experiencing pH 5.7 with 50 μM Mg$^{2+}$ versus pH 7.7 with 50 μM Mg$^{2+}$ (Fig. 2B, compare lanes 3 and 4). In sum, our experiments demonstrated that acidic pH can promote feoB transcription in an RstA-dependent fashion, whereas the low-Mg$^{2+}$ signal is unable to elicit feoB transcription.

The RstB sensor is dispensable for activation of feoB transcription at acidic pH. Because the phosphorylation of a response regulator is primarily mediated by its cognate sensor kinase, we next asked whether the RstB sensor is responsible for the acidic-pH-promoted RstA activity. However, the RstB protein does not appear to affect feoB transcription because an rstB deletion mutant grown in medium with high (i.e., 2 mM)
Acidic pH promotes RstA activity, possibly via phosphorylation. The results presented above suggest that the activity of the RstA protein is promoted at acidic pH. To further explore the activation of the RstA protein, we constructed plasmid pEN105, where expression of the RstA-FLAG protein is under the control of the lac promoter. Western blot analysis revealed that the rstA deletion strain harboring pEN105 expressed the RstA-FLAG protein only in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG), regardless of the pH of the medium (Fig. 4A, lanes 2 and 4). (These RstA-FLAG protein levels were similar to those expressed from a strain with a chromosomal rstA-FLAG gene experiencing acidic pH [data not shown].) The feoB mRNA levels were threefold higher in organisms grown at pH 5.7 than in those grown at pH 7.7 (Fig. 4B). Because the rstA gene is expressed from the lac promoter, these data indicate that RstA activity (as opposed to RstA level) is increased in acidic pH.

The activation of response regulators usually results in phosphorylation at a conserved aspartic acid residue (38). Thus, we reasoned that RstA phosphorylation might be responsible for the acidic-pH-promoted RstA activity. To test this idea, we constructed plasmid pEN106, expressing a mutant form of RstA-FLAG with a single amino acid substitution in the predicted phosphorylation site (i.e., a D52A substitution). We determined that when the rstA deletion strain carrying pEN106 was grown in the presence of IPTG, the feoB mRNA levels were slightly higher at pH 5.7 than at pH 7.7 but failed to reach the threefold difference exhibited by the isogenic strain with the original RstA-FLAG protein (Fig. 4B). The levels of the mutant RstA-FLAG protein were similar to those of the wild-type RstA-FLAG protein (Fig. 4A, compare lanes 4 and 8), indicating that the differences in feoB mRNA levels were not due to altered RstA-FLAG amounts. Cumulatively, these results suggest that the activity of the RstA protein is promoted under acidic pH conditions, possibly via phosphorylation of the conserved aspartic acid which, in turn, activates transcription of the feoB gene.

The PhoQ protein does not affect the activity of the RstA protein at acidic pH. Though it is rare, some response regulators can be phosphorylated by noncognate sensors (24). As RstB was not required for the RstA-promoted transcription of feoB, we wondered whether the PhoQ protein, whose activity is promoted at acidic pH (31), might be responsible for activation of the RstA protein at acidic pH. To test this idea, we compared feoB transcription between isogenic phoPQ+ and phoPQ− strains that had the chromosomal copy of the rstA gene deleted and expressed the rstA gene from the lac promoter. This allowed us to explore the contribution of the PhoP/PhoQ system to RstA activity independently from its role in RstA expression. We determined that, when RstA protein was induced by IPTG, both the ΔrstA and ΔrstA ΔphoPQ strains displayed ~2.8-fold-higher levels of feoB transcription at pH 5.7 than at pH 7.7 (Fig. 5A), indicating that the PhoP and PhoQ proteins are not required for the acidic-pH-promoted RstA activity.

PhoP+ is a variant of the PhoP protein that can promote gene transcription independently of the PhoQ protein (7). Thus, to further assess the participation of the PhoQ protein in RstA activity, we constructed a strain with the phoPQ operon deleted and carrying the pDS303 plasmid expressing the PhoP+ protein from the lac promoter. We determined that the
Acidic pH promotes *feoB* transcription in a strain lacking acetyl phosphate production. We hypothesized that acetyl phosphate might be necessary for RstA activity at acidic pH because this small molecule serves as a phosphodonor for many response regulators (41). To test this, we determined the *feoB* transcription levels in a strain with both the *ackA* and *pta* genes, encoding the enzymes that are required for acetyl phosphate production, deleted (41). The ΔackA-pta strain still produced threefold-higher levels of *feoB* mRNA at pH 5.7 than at pH 7.7, though the lack of *ackA* and *pta* genes slightly reduced the wild-type levels of *feoB* transcription at acidic pH (see Fig. S1 in the supplemental material). Thus, this result suggests that the RstA protein can be activated at acidic pH in the absence of acetyl phosphate.

**Acidic activation of *feoB* transcription enhances *Salmonella* growth under iron-depleted conditions.** We previously reported that RstA-promoted expression of the *feoAB* operon increases Fe^{2+} uptake in *Salmonella* (20). Thus, we hypothesized that FeoB expression at acidic pH might contribute to *Salmonella*’s ability to grow under Fe^{2+}-depleted conditions. However, wild-type *Salmonella* grew equally well in minimal medium that contained sodium ascorbate as an iron-reducing agent and the iron chelator diethylenetriaminepentaacetic acid for restriction of Fe^{2+} and was buffered at pH 7.7 and 5.7 (data not shown). Because *Salmonella* can also import Fe^{2+} using other transporters, such as SitABCD and MntH (4, 23), we reasoned that Fe^{2+} uptake via these routes might mask the effect of FeoB in our experimental conditions. Thus, we re-evaluated bacterial growth using strain JH380, which has both the *sitABCD* and *mntH* genes deleted. Upon Fe^{2+} restriction, strain JH380 grew in the medium at pH 5.7 but not at pH 7.7, regardless of the Mg^{2+} concentration (Fig. 6A). This growth difference is due to FeoB-mediated Fe^{2+} transport, because deletion of the *feoB* gene greatly impaired the growth of the JH380 strain in the Fe^{2+}-depleted medium adjusted to pH 5.7 (Fig. 6B). Moreover, consistent with the regulatory roles of the PhoP/PhoQ and RstA proteins in promoting *feoB* transcription (Fig. 3A), deletion of the *rsta* or *phoPQ* genes prevented the growth of JH380 experiencing acidic pH and Fe^{2+} limitation (Fig. 6B). Cumulatively, these results suggest that activation of the PhoP/PhoQ system and the RstA protein at acidic pH...
increases FeoB-mediated Fe$^{2+}$ uptake under iron-restricted conditions.

**Iron represses feoB transcription at acidic pH.** When bacteria are grown in the presence of iron, the Fur protein associates with Fe$^{2+}$ to repress transcription of genes involved in iron acquisition (1). Because the feoAB operon is one of the Fur-regulated targets (20, 21), we explored how transcriptional activation of the feoB gene at acidic pH is affected by iron. On one hand, iron repressed feoB transcription in both the wild-type and the rsta deletion strain, but the effect was much stronger in the wild-type strain, demonstrating the RstA requirement in feoB transcription (see Fig. S2 in the supplemental material). On the other hand, deletion of the fur gene allowed feoB expression at acidic pH in both the presence and the absence of iron (see Fig. S2 in the supplemental material).

**The mRNA leader sequence allows expression of the mgtA gene only when the PhoP/PhoQ system is activated at low Mg$^{2+}$.** Given that feoB expression was promoted exclusively when the PhoP/PhoQ system was activated at acidic pH (Fig. 3A), we wondered whether there might be a PhoP-dependent gene(s) whose expression occurs only at low Mg$^{2+}$. To explore this possibility, we examined transcription of the Mg$^{2+}$ transporter mgtA gene, which is directly activated by the PhoP protein (25). It has been previously reported that the β-galactosidase activity produced by a strain harboring a lacZ fusion within the mgtA coding region was 12 times higher in organisms grown in N-minimal medium at pH 7.7 with 10 μM Mg$^{2+}$ than in those grown at pH 5.8 with 10 mM Mg$^{2+}$ (35). Why is mgtA not being fully expressed despite the acidic-pH activation of the PhoP protein?

We focused on the recent finding that in response to intracellular Mg$^{2+}$ levels, the expression of the mgtA gene is controlled by a riboswitch on the leader sequences (9). When Mg$^{2+}$ concentration attains certain levels in the cytoplasm, Mg$^{2+}$ binds to the mgtA leader to form a structure that does not favor transcription elongation into the coding region (9). Thus, we hypothesized that mgtA transcription that has been initiated at acidic pH fails to proceed unless cytoplasmic Mg$^{2+}$ levels are sufficiently low. To test this idea, we examined mgtA transcription by conducting qRT-PCR with two primer sets, one specific to the coding region and the other targeting the first 100 nucleotides of the mgtA leader (Fig. 7A). We found that in the wild-type strain, the mgtA leader mRNA levels were 70- and 140-fold higher at pH 5.7 with 10 mM Mg$^{2+}$ and at pH 7.7 without Mg$^{2+}$, respectively, than at pH 7.7 with 10 mM Mg$^{2+}$ (Fig. 7B). In contrast, the mgtA coding region mRNA level was 1,000-fold higher at pH 7.7 without Mg$^{2+}$ but only 6-fold higher at pH 5.7 with 10 mM Mg$^{2+}$ than at pH 7.7 with 10 mM Mg$^{2+}$ (Fig. 7C). As expected, both low-Mg$^{2+}$- and acidic-pH-mediated induction was greatly impaired in the ΔphoPQ mutant (Fig. 7B and C). These results suggest that mgtA transcription initiated in cells experiencing acidic pH fails to reach the coding region.

To evaluate the role of the mgtA leader region, we constructed a strain where the sequence corresponding to positions 148 to 247 of the mgtA leader was replaced with the 84-bp “scar” sequence (10) (Fig. 7A). This mutation has been previously shown to abolish the Mg$^{2+}$-sensing ability of the mgtA leader (9). We determined that the mgtA leader mutant strain expressed the mgtA coding region even at pH 5.7 with 10 mM Mg$^{2+}$ at levels that were 53% of those expressed in organisms grown at pH 7.7 without Mg$^{2+}$ (Fig. 7C). This was contrary to the behavior of the wild-type strain grown at pH 5.7 with 10 mM Mg$^{2+}$, where the mgtA coding region mRNA level was only 0.5% of the level produced following growth at pH 7.7 without Mg$^{2+}$ (Fig. 7C). The mgtCB operon encodes another Mg$^{2+}$ transporter, MgtB, whose expression is also determined at the transcription level by the PhoP protein and at the posttranscription level by the leader preceding the first gene in the operon (9, 37). To examine whether the differential pH and Mg$^{2+}$ regulation displayed by the mgtA gene also applies to mgtB, we determined the mRNA levels for the mgtB coding region in the wild-type
and ΔphoPQ strains experiencing different growth conditions. The PhoP-dependent expression of the mgtB gene took place at low Mg^{2+} but not at acidic pH with high Mg^{2+} (see Fig. S3 in the supplemental material), indicating that mgtB transcription can reach the coding region only when intracellular Mg^{2+} levels are sufficiently low. Our results suggest that the leader regions allow the MgtA and MgtB proteins to be produced only when Salmonella faces limiting Mg^{2+} levels, dramatically decreasing the expression of these Mg^{2+} transporters when the PhoP/PhoQ system is activated by the acidic-pH signal at high Mg^{2+} concentrations.

**DISCUSSION**

Certain two-component regulatory systems are activated by multiple signals. For example, the PhoP/PhoQ system is activated when Salmonella is grown in low Mg^{2+} (14), in an acidic pH (31), or with certain antimicrobial peptides (2). In the case of the PmrA/PmrB system, both ferric iron (42) and acidic pH (28) promote PmrB activity. These facts raise the question of whether a single regulatory system controls the expression of distinct sets of genes when activated by different signals. We have now demonstrated that the acidic-pH activation of the PhoP/PhoQ system promotes transcription of the Fe^{2+}-transporter-encoding gene feoB, whereas its activation by low Mg^{2+} promotes the expression of the Mg^{2+}-transporter-encoding mgtA gene (Fig. 1).

**Regulation of feoB expression by the PhoP/PhoQ system and the RstA protein.** We demonstrated that the PhoP/PhoQ system promotes the expression of the FeoB Fe^{2+} transporter when activated by acidic pH but fails to do so when activated at neutral pH by the low-Mg^{2+} signal (Fig. 3A). This control requires a second response regulator, RstA, which functions as the direct activator of feoB transcription (20). PhoP binds to the rstA promoter region (I. Zwir et al., unpublished) and activates rstA transcription (29, 30). Even though the low-Mg^{2+} and acidic-pH signals can promote the expression of the RstA protein equally well (Fig. 2), transcription of the RstA-dependent feoB gene took place only when bacteria experienced an acidic pH (Fig. 3A).

We propose that acidic pH controls RstA activity via phosphorylation, because the RstA protein expressed from a heterologous promoter activated feoB transcription at pH 5.7 but not at pH 7.7 and because the activation required RstA’s predicted phosphorylation site (i.e., D52) (Fig. 4). Although phosphorylation of a response regulator is generally mediated by its cognate sensor kinase, intriguingly, the acidic-pH activation of feoB transcription still took place in a mutant with a deletion of the rstB gene, which codes for the cognate sensor for RstA (Fig. 3B). That the RstA protein promoted feoB transcription at acidic pH in the absence of PhoQ (Fig. 5) argues against the possibility of cross-phosphorylation of RstA by the noncognate sensor PhoQ. In addition, the finding that acidic pH still activates feoB transcription in a strain that does not synthesize acetyl phosphate rules out the possibility of RstA phosphorylation by this small-molecule phosphodonor (see Fig. S1 in the supplemental material). Therefore, it is likely that a sensor kinase other than RstB and PhoQ might phosphorylate the RstA protein, because some response regulators are phosphorylated by noncognate sensors (24).

**PhoP/PhoQ-mediated iron homeostasis.** Under aerobic conditions at neutral pH, iron is present in an oxidized ferric form. Consistent with the notion that iron is reduced to a ferrous form under anaerobic conditions, transcription of the feoAB operon increases when *E. coli* is grown without oxygen (21). What, then, is the biological significance of the pH-regulated feoB expression? It has been found that *Salmonella* possesses extracellular enzyme activities to reduce iron (40) and that an acidic pH keeps ferrous iron stable in the presence of oxygen (6). Thus, FeoB induction resulting from activation of the PhoP/PhoQ system and RstA protein could enhance Fe^{2+} uptake in *Salmonella* growing in acidic environments with limited iron.

In bacterial cells grown with oxygen, cytoplasmic Fe^{2+} participates in the Fenton reaction and catalyzes the formation of hydroxyl radicals, which causes DNA damage (39). The Fur protein is a primary regulator that senses intracellular Fe^{2+} levels (1). When associated with Fe^{2+}, the Fur protein represses the expression of genes for iron acquisition, which minimizes the accumulation of free Fe^{2+} in the cytoplasm (1, 12). Not surprisingly, the Fur protein repressed *feoB* transcription even at acidic pH when iron was plentiful (see Fig. S2 in the supplemental material).

The PhoP/PhoQ system is also necessary for the survival of *Salmonella* under Fe^{2+}-dependent oxidative stress (8). Although the CorA protein is a Mg^{2+} transporter, it has been reported to import Fe^{2+} as well (17). When aerobically grown at low Mg^{2+}, a *Salmonella* phoP mutant displayed increased Fe^{2+} accumulation in a process dependent on the CorA protein (8). The finding that neither the expression level of the CorA protein nor its membrane location is affected by the PhoP protein implies the presence of a PhoP-regulated gene product(s) regulating CorA activity (8). Indeed, the *phoP* mutant was hypersensitive to Fe^{2+}-dependent oxidative-stress-mediated killing, which was rescued by inactivation of the *corA* gene (8). Taking these findings together, the PhoP/PhoQ system is likely to play dual roles in iron homeostasis: PhoP enhances Fe^{2+} uptake when iron is scarce at acidic pH, whereas downregulation of CorA activity at low Mg^{2+} protects *Salmonella* from Fe^{2+}-mediated killing.

**PhoP/PhoQ-controlled Mg^{2+} uptake.** *Salmonella* imports Mg^{2+} via three transporters: CorA, MgtA, and MgtB (18, 19, 33). The *corA* gene is constitutively expressed (8), whereas transcription of the *mgtA* and *mgtB* genes is directly activated by the PhoP protein (25, 43). As opposed to the PhoP-controlled Fe^{2+} uptake at acidic pH, we determined that MgtA expression takes place only at low Mg^{2+} (Fig. 7). This is because the *mgtA* leader functions as an RNA sensor (9) such that when the cytoplasmic Mg^{2+} concentration reaches a certain high level, Mg^{2+} binds to the *mgtA* leader to form a structure that prevents RNA polymerase from proceeding to the *mgtA* coding region (9). The full-length transcript, including the *mgtA* coding region, is produced when intracellular Mg^{2+} is low enough to promote the formation of a different structure in the leader region (9).

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REFERENCES


