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Bacterial Mg^{2+} Homeostasis, Transport, and Virulence

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Abstract

Organisms must maintain physiological levels of Mg^{2+} because this divalent cation is critical for the stabilization of membranes and ribosomes, the neutralization of nucleic acids, and as a cofactor in a variety of enzymatic reactions. In this review, we describe the mechanisms that bacteria utilize to sense the levels of Mg^{2+} both outside and inside the cytoplasm. We examine how bacteria achieve Mg^{2+} homeostasis by adjusting the expression and activity of Mg^{2+} transporters, and by changing the composition of their cell envelope. We discuss the connections that exist between Mg^{2+} sensing, Mg^{2+} transport and bacterial virulence. Additionally, we explore the logic behind the fact that bacterial genomes encode multiple Mg^{2+} transporters and distinct sensing systems for cytoplasmic and extracytoplasmic Mg^{2+}. These analyses may be applicable to the homeostatic control of other cations.

Keywords

ATP; CorA; gene regulation; lipopolysaccharide; MgtA; MgtB; MgtE; PhoP/PhoQ

INTRODUCTION

Mg^{2+} is the most abundant divalent cation in living cells and the second most abundant cation after K^+ (102). Mg^{2+} plays several essential roles, including stabilizing

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macromolecular complexes and membranes, neutralizing nucleic acids and nucleotides in the cytoplasm and phospholipid head groups and surface molecules outside the cytoplasm, and acting as a cofactor in a variety of enzymatic reactions. Certain cations can replace Mg^{2+} for some of these functions, but other activities strictly depend on Mg^{2+}, indicating that cells must have mechanisms to maintain physiological levels of Mg^{2+}.

The gram-negative bacterium *Salmonella enterica* serovar Typhimurium (hereafter referred to as *Salmonella*) is the best-understood organism in terms of Mg^{2+} homeostasis. This understanding is primarily due to the pioneering research of Michael Maguire and his colleagues, who identified and characterized three Mg^{2+} transporters in *Salmonella* (56, 57, 111). In addition, the realization that a major virulence regulatory system controls transcription of two of *Salmonella*'s three Mg^{2+} transporters led to the discovery of the first signal transduction system that responds to extracytoplasmic Mg^{2+} as its primary signal (34). Furthermore, investigation of one of the Mg^{2+} transporters in *Salmonella* uncovered the first RNA sensor for cytoplasmic Mg^{2+} (19).

Orthologous and nonorthologous Mg^{2+} transporters and Mg^{2+}-responsive signal transduction systems have now been uncovered in a variety of bacterial species. These studies have established that bacteria possess the means to assess the levels of Mg^{2+}, both in their surroundings and inside the cytoplasm, and to mount a response that helps maintain Mg^{2+} at the required levels. Such a response often entails modifying the amounts and/or activities of transporters that move Mg^{2+} from one compartment to another and of enzymes that chemically modify surface molecules harboring negative charges that are normally neutralized by Mg^{2+}. The production of these proteins must be coordinated for a cell to survive and replicate in an environment that is limiting in Mg^{2+}.

In this review, we examine how bacteria achieve Mg^{2+} homeostasis. We explore the signals and mechanisms that govern the expression and activity of Mg^{2+} transporters as well as the roles that Mg^{2+} transporters and Mg^{2+} sensing play in the ability of bacterial pathogens to cause disease. We discuss why organisms harbor distinct systems to sense cytoplasmic and extracytoplasmic Mg^{2+}, and the reasons why a given species has multiple Mg^{2+} transporters.

THE PROPERTIES OF BACTERIAL Mg^{2+} TRANSPORTERS

Three distinct classes of Mg^{2+} transporters have been identified in bacteria: CorA, MgtE, and MgtA (56, 57, 110). Most bacterial genomes encode multiple Mg^{2+} transporters that belong to either the same or different classes. CorA and MgtE, which are considered the primary Mg^{2+} transporters in bacteria, have a wide phylogenetic distribution, and the corresponding genes are reported to be transcribed from constitutive promoters. By contrast, MgtA occurs in only a subset of bacteria and the *mgtA* gene is transcriptionally induced in low Mg^{2+} environments (75, 112). Although all of these transporters can import Mg^{2+}, they differ in the energy requirements for moving Mg^{2+}, their ability to export Mg^{2+}, the conditions under which the proteins are made, and their phylogenetic distribution within bacteria as well as in archaea and eukarya (Table 1). A discussion of these transporters is presented below, and the reader is referred to excellent reviews on Mg^{2+} transporters and their mode of operation for additional information (45, 75, 82).

CorA was the first divalent cation transporter to be crystallized (28, 71, 91). It is a funnel-shaped homopentamer in which each monomer consists of a large N-terminal cytoplasmic domain, with no significant sequence similarity to other protein families, and two C-terminal transmembrane domains (TM1 and TM2) connected by a short periplasmic loop (Figure 1*a*). The five TM1 helices comprise the pore, which, unexpectedly, contains no charged residues (28). Inhibition of CorA by cation hexaammines suggests that CorA initially interacts with a fully hydrated Mg^{2+} ion, which is thought to become partially dehydrated as it traverses the membrane (46, 64, 71, 94). The narrowest constriction in the pore is formed at the membrane-cytoplasm interface by a bulky side chain and likely represents a hydrophobic gate (71). A ring of positively charged residues surrounds this constriction and may further impede cation passage in the closed state. Putative Mg^{2+} binding sites located at monomer-monomer interfaces in the cytoplasmic domain are thought to regulate channel opening and closing in response to intracellular Mg^{2+} levels (71). It has been proposed that loss of Mg^{2+} at these sites facilitates opening of the hydrophobic gate and import of Mg^{2+} (46, 94). However, the fact that CorA can mediate export of Mg^{2+} (111) suggests that the gate could also be open when bacteria experience high cytoplasmic Mg^{2+} levels.

MgtE is unrelated to CorA in both sequence and structure. It is a homodimer in which each monomer contains five transmembrane helices, resulting, like CorA, in a total of ten (Figure 1*b*) (52). A connecting helix links the transmembrane domain to the cytoplasmic portion of each monomer, which contains two domains, an N domain with structural similarity to the soluble portion of the flagellar motor protein FliG, and a tandemly repeated cystathionine-β-synthase (CBS) domain putatively involved in dimerization (82). As with CorA, intracellular Mg^{2+} levels are thought to regulate gating of MgtE via Mg^{2+} binding sites in the cytoplasmic domain (52), albeit by a different mechanism (51, 82). Upon loss of Mg^{2+} at the putative regulatory binding sites, the cytoplasmic domains become more flexible and undergo a conformational change that disrupts the interaction between the connecting helix and the transmembrane domains to facilitate pore opening (51, 82).

CorA and MgtE both utilize the electrochemical gradient across the cytoplasmic membrane to transport their substrates (75). Therefore, they are presumed to be channels rather than transporters (52, 71, 82). Because of the reliance of CorA and MgtE on membrane potential, transport via CorA and MgtE may be influenced by changes in external pH, the activity of electron transport chain components, and/or fluctuations in the concentrations of other ions.

MgtA is a monomer that also has 10 transmembrane helices (Figure 1*c*). Members of the MgtA class of Mg^{2+} transporters are P-type ATPases. This family of proteins derives energy from the hydrolysis of adenosine triphosphate (ATP) to transport a variety of charged molecules. Transfer of the phosphate group from ATP to the protein results in a conformational change in the protein that promotes Mg^{2+} transport (81). The MgtA class can be divided into two groups, MgtA and MgtB, which exhibit ~50% amino acid identity with each other (74, 113, 118). Interestingly, the MgtA and MgtB proteins are more closely related to eukaryotic sarcoplasmic Ca^{2+}-ATPases than to prokaryotic ATPases (74).

In contrast to other P-type ATPases, which use energy derived from ATP hydrolysis to move their substrates up an electrochemical gradient, the MgtA class of proteins was

reported to move Mg^{2+} down an electrochemical gradient, making the purpose of coupling this process to ATP hydrolysis unclear (117). Because the Mg^{2+} concentration in the cytoplasm is ~1 mM (107), a transporter operating in an environment in which the Mg^{2+} concentration is <1 mM would be moving Mg^{2+} up a chemical gradient. However, under normal growth conditions, the membrane potential makes the cell negative inside and positive outside of the cytoplasmic membrane, thus favoring Mg^{2+} movement down the electrical gradient. Therefore, the favorable electrical gradient could compensate for the unfavorable chemical gradient and make the requirement for ATP superfluous. Recent findings present an explanation for this paradoxical energy requirement. As discussed below, the Mg^{2+}-responsive PhoP/PhoQ regulatory system, which drives transcription of the *mgtA* and *mgtB* genes, also promotes a reversal in membrane potential (i.e., making it positive inside and negative outside of the cytoplasmic membrane) (1). This means that the MgtA and MgtB proteins are made and operate under conditions in which both the chemical and electrical gradients are unfavorable for Mg^{2+} movement through a channel, thereby providing a rationale for why ATP hydrolysis is needed to bring Mg^{2+} into the cytoplasm.

BACTERIAL REQUIREMENTS FOR GROWTH IN LOW Mg^{2+}

Many bacterial species harbor sensor proteins that respond to changes in extracytoplasmic Mg^{2+} by modifying the activity of cognate DNA-binding regulatory proteins. These DNA-binding proteins, in turn, elicit a transcriptional response that helps the organism cope with the new Mg^{2+} condition. The PhoP/PhoQ system from *Salmonella* provided the first example of a biological system that responds to Mg^{2+} as its primary signal (34). PhoP and PhoQ constitute a two-component regulatory system in which PhoQ is a sensor of extracytoplasmic Mg^{2+} and PhoP is its cognate DNA-binding transcriptional regulator. When PhoQ detects low Mg^{2+}, it promotes the phosphorylated state of PhoP (PhoP-P), and when Mg^{2+} levels are high, PhoQ favors the unphosphorylated state of PhoP (106). PhoP-P binds with higher affinity to its target promoters than does PhoP (105). Thus, low Mg^{2+} promotes transcription of PhoP-activated genes, including the Mg^{2+} transporter genes *mgtA* and *mgtB* (114), and decreases expression of PhoP-repressed genes (Figure 2*a*).

The PhoP/PhoQ system is essential for *Salmonella* to adapt to low Mg^{2+} conditions because *phoP* and *phoQ* mutants cannot form colonies on low (i.e., ≤ 40 μM) Mg^{2+} solid media but grow like the wild-type strain at high (i.e., ≥ 500 μM) Mg^{2+} (34). Paradoxically, the inability of the *phoP* and *phoQ* mutants to grow on low Mg^{2+} media is not simply due to the requirement for PhoP/PhoQ to promote transcription of the *mgtA* and *mgtB* genes because an *mgtA mgtB* double mutant forms colonies on low Mg^{2+} media like wild-type *Salmonella* (114). This suggests that growth in low Mg^{2+} requires functions in addition to those directly mediating Mg^{2+} uptake into the bacterial cytoplasm. Moreover, it indicates that CorA is unable to support bacterial growth on low Mg^{2+} media when the PhoP/PhoQ system is absent.

The PhoP/PhoQ regulatory system is present in several enteric bacteria and some gram-negative species outside the family *Enterobacteriaceae* (92). The PhoP regulons (i.e., the collections of genes controlled by PhoP) are widely divergent, possibly because of differences in the ecological niches occupied by bacterial species that harbor PhoP/PhoQ

homologs. Nonetheless, these regulons share certain features: First, PhoP regulates expression of at least one Mg^{2+} transporter gene (92), e.g., *mgtA* in *Escherichia coli* and *mgtE* in *Serratia marscecens* (5, 61). Second, the PhoP/PhoQ system positively regulates its own transcription in *Salmonella* (115), *E. coli* (61), and *Yersinia pestis* (92), and this seems to be conserved in other enteric species. This establishes a positive feedback loop, which is required to generate sufficient levels of PhoP-P to activate genes that promote normal growth in low Mg^{2+} (92). Third, PhoP activates expression of the small lipoprotein SlyB. This protein acts as a negative regulator of the PhoP/PhoQ system because deletion of *slyB* results in hyperactivation of PhoP-activated genes, whereas *slyB* overexpression lowers the transcription of PhoP-activated genes and hinders growth in low Mg^{2+} (92). Together, PhoP autoregulation and SlyB regulate the transcriptional output of the PhoP/PhoQ regulon in the *Enterobacteriaceae* (92).

Cell Surface Modifications May Aid Adaptation to Low Mg^{2+} Environments

The envelope of gram-negative bacteria consists of two distinct membranes, a cytoplasmic membrane and an outer membrane, separated by a hydrophilic compartment termed the periplasm (Figure 2*a*). A significant fraction of the total Mg^{2+} content of gram-negative bacteria is present in the cell envelope (39), highlighting the need to maintain Mg^{2+} homeostasis in subcellular compartments outside the cytoplasm. Here, we consider gene products other than Mg^{2+} transporters that participate in Mg^{2+} homeostasis and how these proteins may make Mg^{2+} available to be imported into the cytoplasm.

The outer membrane of gram-negative bacteria is an asymmetric bilayer containing lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner one (85). The LPS consists of three distinct regions: the innermost lipid A, the core region, and the outermost O antigen (99, 100). The LPS is negatively charged because of the phosphate residues present both in the lipid A and core regions. Mg^{2+}, and to a lesser extent other divalent cations, normally neutralizes these negative charges, thereby preventing electrostatic repulsion between adjacent LPS molecules (48).

When *Salmonella* experiences low Mg^{2+}, the PhoP/PhoQ system promotes expression of proteins that covalently modify phosphate groups in the LPS (12, 27, 37) (for details, see Reference 12). By reducing the net negative charge of the LPS molecules, these modifications aid neutralization of the LPS when Mg^{2+} is limiting. Thus, mutants unable to carry out some of these LPS modifications are defective for growth on low Mg^{2+} solid media (39). Moreover, modifications of the LPS free up Mg^{2+} for other cellular functions that exhibit a strict dependence on Mg^{2+}.

We propose that the LPS might function as a Mg^{2+} reservoir for the cell. In agreement with this idea, it has been estimated that a third of the total Mg^{2+} content of *E. coli* is present in the LPS (18, 39). Therefore, when bacteria experience a low Mg^{2+} environment, the Mg^{2+} that is imported by the Mg^{2+} transporters may be Mg^{2+} that was originally neutralizing negative charges in the LPS.

HOW BACTERIA SENSE Mg^{2+}

Sensing Extracytoplasmic Mg^{2+}

The Mg^{2+} sensor PhoQ from *Salmonella* harbors two transmembrane helices that delineate a periplasmic domain of 146 amino acids. This domain is required for PhoQ to respond to Mg^{2+} because a *Salmonella* strain harboring a chimeric PhoQ protein, containing the transmembrane and periplasmic domains from the sensor EnvZ fused to the cytoplasmic domain of PhoQ, expresses PhoP-activated genes in an Mg^{2+}-independent fashion (10). Furthermore, as few as two amino acid substitutions in the periplasmic domain of PhoQ are sufficient to render this protein constitutively active (i.e., unresponsive to repression by Mg^{2+}) (10, 34). A model for Mg^{2+} sensing based on the crystal structure of PhoQ from *Salmonella* has been proposed. According to this model, Mg^{2+} ions form metal bridges between a patch of negatively charged amino acid residues on PhoQ's periplasmic domain and phospholipid head groups of the cytoplasmic membrane. This stabilizes PhoQ in a rigid conformation that promotes the unphosphorylated form of PhoP. Disruption of these metal bridges by removal of Mg^{2+} ions results in destabilization of this conformation, thereby furthering the phosphorylated form of PhoP (13). Mn^{2+} and Ca^{2+} can also silence the PhoP/PhoQ system during growth in defined media in vitro (13, 34). However, it is unclear whether the effect of these two cations is physiologically significant given that *Salmonella* is unlikely to experience high enough concentrations of Mn^{2+} and/or Ca^{2+} in its natural environment to silence the PhoP/PhoQ system.

A Mg^{2+}-responsive two-component system, termed CsrR/CsrS (also designated CovR/CovS), regulates virulence functions in Group A *Streptococcus* (GAS), the gram-positive etiologic agent of a variety of clinical syndromes in humans (Figure 2*b*). Like *Salmonella*, GAS responds to elevated levels of extracellular Mg^{2+} by reducing expression of dozens of genes (42, 43). However, the way in which this regulation is implemented differs between the two organisms. First, CsrR functions primarily as a repressor (78). High Mg^{2+} silences CsrR-repressed genes because CsrS responds to high Mg^{2+} by promoting the phosphorylated state of CsrR (CsrR-P), which binds to its target promoters with higher affinity than does unphosphorylated CsrR (Figure 2*b*). Second, unlike PhoQ, whose activity is silenced by Mn^{2+} and Ca^{2+}, these cations have no effect on CsrS (43). Third, CsrS and PhoQ operate in different Mg^{2+} concentration ranges. Maximal repression of CsrR-regulated genes occurs at concentrations ≥10 mM Mg^{2+} (43) whereas significant repression of PhoP-activated genes is already observed at 1 mM Mg^{2+} (34).

A detailed model for the mechanism of Mg^{2+} sensing by CsrS is lacking. However, similar to PhoQ, the ability of this protein to respond to Mg^{2+} requires a patch of negatively charged amino acid residues located on its extracytoplasmic domain (120). It should be noted that although the CsrR/CsrS system responds to Mg^{2+}, it does not appear to regulate genes involved in Mg^{2+} homeostasis, such as Mg^{2+} transporters. Thus, CsrR/CsrS appears to function exclusively in the control of virulence (43).

Sensing Cytoplasmic Mg^{2+}

In addition to the control of transcription initiation by extracytoplasmic Mg^{2+} levels, transcription elongation into the protein-coding regions of Mg^{2+} transporter genes can respond to the concentration of Mg^{2+} in the cytoplasm (19, 22). This enables expression of Mg^{2+} transporters to be shut down when Mg^{2+} needs in the cytoplasm have been met. Whereas bacteria depend on proteins to sense extracytoplasmic Mg^{2+}, they use RNAs to detect Mg^{2+} in the cytoplasm. Specifically, the mRNAs corresponding to the Mg^{2+} transporter genes *mgtA* and *mgtB* in *Salmonella* and *mgtE* in *Bacillus subtilis* include long leader sequences that function as sensing devices for cytoplasmic Mg^{2+} (19, 22).

The leader region of the *Salmonella mgtA* mRNA provided the first example of a metal-sensing riboswitch (19). Riboswitches are segments of mRNAs that bind to specific ligands and respond by undergoing structural changes that modify expression of the associated coding regions (54, 103). The *mgtA* leader RNA has the ability to adopt two mutually exclusive conformations in response to Mg^{2+} (Figure 3*a*): one that is favored in high Mg^{2+} and promotes transcription termination within the leader sequence, and an alternative conformation that is favored in low Mg^{2+} and permits transcription elongation into the *mgtA* coding region (19).

Most riboswitches that control transcription function by affecting formation of an intrinsic transcription terminator, which can be recognized by RNA polymerase in the absence of additional protein factors (54, 103). By contrast, the *mgtA* riboswitch operates by controlling the activity of the transcription termination factor Rho (58). The high Mg^{2+} conformer facilitates interaction of Rho with the nascent RNA, thus promoting transcription termination within the *mgtA* leader region (Figure 3*a*). Because Rho tends to bind to single-stranded RNA sequences that are rich in C nucleotides (93, 98) and such sequences are occluded when the *mgtA* transcript adopts the low Mg^{2+} conformer, Rho-dependent termination is inhibited and transcription continues into the *mgtA* coding region (58). In addition to its effects on Rho-dependent termination, growth in high Mg^{2+} renders the *mgtA* leader mRNA hypersusceptible to degradation by the endonuclease RNase E (116), thereby decreasing the levels of the *mgtA* transcript.

Analogous to the *mgtA* leader, the leader region of the polycistronic *mgtCBR* transcript, which encodes the *Salmonella* Mg^{2+} transporter MgtB, controls transcription elongation in response to cytoplasmic Mg^{2+}: Low Mg^{2+} favors expression of the downstream coding region and high Mg^{2+} hinders it (19, 116). The structure of the *mgtCBR* leader RNA is affected by the presence of Mg^{2+} in vitro (67), but the mechanism by which this conformational change regulates expression of the *mgtCBR* operon is presently unclear.

Expression of the *B. subtilis mgtE* coding region is also regulated by a Mg^{2+}-sensing riboswitch (22). Similar to the *mgtA* leader, Mg^{2+} affects the formation of particular structures in the *mgtE* leader RNA, which, in turn, govern transcription elongation into the *mgtE* coding region (22) (Figure 3*b*). However, the *mgtE* riboswitch is unrelated to the *mgtA* riboswitch in either sequence or structure, and the Rho protein is not required for transcription termination in the *mgtE* leader. Instead, high Mg^{2+} promotes folding of the

mgtE leader RNA into a compact tertiary structure that allows formation of an intrinsic transcription terminator (22).

The *mgtE* Mg^{2+} riboswitch, often referred to as the M-box, is also found upstream of the coding region of genes other than *mgtE*. These include genes encoding Mg^{2+} transporters that belong to families other than MgtE, genes that participate in Mg^{2+} homeostasis functions other than Mg^{2+} uptake, and even genes specifying products with no obvious role in Mg^{2+} homeostasis (22, 101). Genes harboring an M-box include a *corA* homolog in *Clostridium thermocellum*, genes in *Bacillus cereus* and *Bacillus halodurans* that are homologous to the *Salmonella mgtC* gene, which does not specify a Mg^{2+} transporter (47) but is necessary for normal growth in low Mg^{2+} (7), and putative transporters of other substrates (22, 101). By contrast, the *mgtA* riboswitch appears to be present exclusively upstream of *mgtA* coding regions.

Both the *Salmonella mgtA* riboswitch and the *B. subtilis mgtE* riboswitch are specific sensors of Mg^{2+}. Only Mg^{2+} appears to act on the *mgtA* leader RNA in vivo even though other cations can silence the extracytoplasmic Mg^{2+} sensor PhoQ (34), be transported by the MgtA protein, and/or inhibit Mg^{2+} uptake by MgtA (111). Similarly, other divalent cations can modify the structure of the *mgtE* leader mRNA in vitro but are unable to stimulate transcription termination in vitro (22). The existence of two distinct, specific RNA sensors for Mg^{2+} highlights the importance of RNA- as well as protein-based mechanisms for maintaining Mg^{2+} homeostasis (19, 22).

Why Expression of Mg^{2+} Transporters is Controlled by Both Cytoplasmic and Extracytoplasmic Mg^{2+}

Expression of the *mgtA* gene responds both to extracytoplasmic Mg^{2+} at the level of transcription initiation and to cytoplasmic Mg^{2+} at the level of transcription elongation into the coding region. This raises the question: Why might *mgtA* transcription be regulated by Mg^{2+} levels both outside and inside the cytoplasm? The riboswitch-mediated control of *mgtA* transcription imposes a second layer of Mg^{2+}-dependent regulation that allows *Salmonella* to exert differential control over those PhoP-regulated genes that impact cytoplasmic Mg^{2+} levels directly (i.e., Mg^{2+} transporters) versus those that play other roles. Whereas transcription initiates at the promoters of all PhoP-activated genes when extracytoplasmic Mg^{2+} is low, the *mgtA* coding region is transcribed only when cytoplasmic Mg^{2+} levels drop below a particular threshold that demands Mg^{2+} uptake by MgtA.

As with the *mgtA* gene, transcription initiation from the *mgtCBR* promoter is induced in response to low extracytoplasmic Mg^{2+} via the PhoP/PhoQ system, whereas transcription elongation into the *mgtCBR* coding region is controlled by an mRNA leader region that responds to intracellular levels of Mg^{2+} (19, 116). The dual control of *mgtA* and *mgtCBR* expression by both extracytoplasmic and cytoplasmic sensors of Mg^{2+} is in contrast to the control of the *B. subtilis mgtE* gene, which appears to respond exclusively to cytoplasmic Mg^{2+} levels via its Mg^{2+}-responsive riboswitch because it is transcribed from a constitutive promoter (22).

When *Salmonella* experiences an intermediate Mg^{2+} concentration---one that is low enough to promote transcription initiation from the *mgtA* and *mgtCBR* promoters but high enough to prevent transcription from continuing into their respective coding regions---truncated transcripts are generated that correspond to portions of the *mgtA* and *mgtCBR* leader regions. Because the truncated RNA originating from the *mgtA* leader is fairly stable (at least in *E. coli*) (62), this raises the possibility that it might function as a *trans*-acting small regulatory RNA (123). Such a situation, in which a riboswitch can also operate in *trans* to control gene expression, has been described for an S-adenosyl methionine-sensing riboswitch in *Listeria monocytogenes* (70).

SIGNALS OTHER THAN Mg^{2+} CONTROL SYSTEMS THAT RESPOND TO EXTRACYTOPLASMIC Mg^{2+}

In addition to Mg^{2+}, certain cationic antimicrobial peptides (CAMPs) (3, 4, 80) or a mildly acidic pH (97) have been shown to directly activate the PhoQ protein. For instance, the synthetic CAMP C18G promotes expression of PhoP-activated genes in media containing high (1 mM) levels of Mg^{2+} (4). Activation of PhoQ by CAMPs appears to involve displacement of divalent cations (primarily Mg^{2+}) from the metal bridges formed between PhoQ and the phospholipid head groups in the cytoplasmic membrane (4). These findings suggest that activation of PhoQ by CAMPs results from conformational changes that are similar to those evoked by low Mg^{2+}. It is worth noting that although *phoP* and *phoQ* mutants display hypersusceptibility toward particular CAMPs, there is no correlation between the ability of a given CAMP to activate the PhoP/PhoQ system and its capacity to preferentially kill *phoP* and *phoQ* mutants (40).

The Mg^{2+} sensor CsrS is activated by the human antimicrobial peptide LL-37 (44) in a fashion that also requires a patch of negatively charged residues (120). Given that CsrS activation is specific to LL-37, this peptide may constitute a bona fide signal for this regulatory system in the context of a human infection.

Sensing of acidic pH by PhoQ appears to occur through a mechanism distinct from that responsible for sensing CAMPs. Mildly acidic pH (pH 5.5) leads to protonation of specific amino acid residues in the periplasmic domain of PhoQ (97). This results in conformational changes in the periplasmic domain, which are proposed to alter the enzymatic activities carried out by the cytoplasmic domain of PhoQ (97). The PhoP/PhoQ system controls the expression of genes mediating resistance to acid stress in both *Salmonella* (31) and *E. coli* (125). Hence, sensing of acidic pH constitutes a physiologically relevant signal for PhoQ in these two bacterial species (97, 125).

An array of signals modulates the activity and levels of the PhoP/PhoQ system in an indirect fashion. In diverse bacterial species, the PhoP-activated membrane protein MgrB interacts with PhoQ, downregulating its activity (68, 69). This downregulation seems to require the formation of disulfide bonds by the DbsA/DbsB enzymatic system at two highly conserved cysteine residues in MgrB (68, 69). Because the function of MgrB requires oxidation of these two cysteines, it has been proposed that this small protein integrates information about the oxidative potential of the environment into the PhoP/PhoQ system (69). It is also

possible that MgrB modulates PhoQ's activity in response to a physiological signal generated by PhoP. Specifically, PhoP reduces cellular respiration (and, presumably, the oxidative state of the cytoplasmic membrane) when *E. coli* experiences low Mg^{2+} (1). Therefore, PhoP/PhoQ may modulate MgrB signaling by limiting the availability of electron acceptors needed for the reoxidation of the DbsA/DbsB system. In such a scenario, MgrB would dampen PhoQ activity on the basis of the oxidative status of the periplasm.

PhoQ can also be activated by the small membrane peptide SafA in *E. coli*. Expression of *safA* is controlled by the EvgA/EvgS two component system and, hence, it enables environmental information sensed by EvgA/EvgS to be relayed to PhoP/PhoQ (59). SafA activates PhoQ by interacting with its periplasmic domain (24) and mediating conformational changes distinct from those elicited by low Mg^{2+}, CAMPs, and acidic pH (23). Notably, oxidized ubiquinone has been shown to strongly inhibit the kinase activity of *E. coli* EvgS in vitro (8). PhoP-mediated reduction of respiration (1) is expected to decrease the pools of oxidized ubiquinone, thereby increasing EvgS activity and the expression of SafA. Thus, as with MgrB, the response of EvgA/EvgS to a PhoP-induced physiological signal may be part of a regulatory loop.

Ultimately, the amount of PhoP-P in the cell depends not only on conditions that activate PhoQ but also on those that alter the levels of PhoP. In addition to the autoregulation exerted by PhoP on its own promoter (61, 92, 115), two small RNAs (sRNAs) termed MicA (17) and GcvB (16) regulate *phoPQ* posttranscriptionally in *E. coli*. Both of these sRNAs bind to the 5′ end of the *phoPQ* mRNA and occlude the ribosome binding site of *phoP*, thereby inhibiting translation and targeting the message for degradation (16). MicA is regulated by the extracytoplasmic stress sigma factor RpoE. Hence, repression of PhoP/PhoQ by MicA suggests that PhoP-promoted functions, such as the modification of envelope components, are detrimental when the organism experiences RpoE-inducing conditions (17). GcvB is regulated by the GcvA/GcvR system, which controls genes that encode enzymes involved in glycine cleavage. Because GcvB targets a wide number of mRNAs (16, 17), its role in cellular physiology and the potential connection with the PhoP/PhoQ system is presently not understood.

It is interesting to note that CAMPs, acidic pH, and other signals that activate PhoQ fail to evoke the same set of physiological adaptations that are elicited when bacteria experience Mg^{2+} limitation (40). This suggests the existence of additional factors participating in the differential regulation of certain genes in response to Mg^{2+} starvation versus other PhoQ-activating signals.

SIGNALS OTHER THAN Mg^{2+} LEVELS CONTROL THE EXPRESSION OF Mg^{2+} TRANSPORTERS

In *Salmonella*, the *mgtA* gene and the *mgtCBR* operon are also regulated by signals other than Mg^{2+}. This regulation is mediated primarily by the *mgtA* and *mgtCBR* mRNA leader regions. These mRNA leaders function as complex sensing devices that can detect cytoplasmic signals in addition to low Mg^{2+} and thereby favor or hinder transcription elongation into their respective coding regions. This enables a bacterium to interpret

chemical or physical changes taking place in its surroundings by the effects they have on cytoplasmic components.

First, hyperosmotic stress promotes expression of the *mgtA* and *mgtCBR* coding regions (67, 90) (Figure 4). This induction is mediated by short open reading frames (ORFs) rich in proline codons (*mgtL* and *mgtP*), which are located in the *mgtA* and *mgtCBR* leader regions, respectively (67, 90). These ORFs are located within sections of the mRNA leaders that have the capacity to adopt alternative stem-loop structures. In each case, translation of the short ORF is predicted to favor formation of one of the two stem-loop structures. It has been proposed that *Salmonella* detects hyperosmolarity via a decrease in the levels of proline-charged $tRNA^{Pro}$. This is because proline functions as an osmoprotectant when *Salmonella* experiences hyperosmotic stress, and the total proline content does not change under these conditions (20). Therefore, under hyperosmotic stress, the decreased availability of proline-charged $tRNA^{Pro}$ would cause ribosome stalling at the Pro codons in *mgtL* and *mgtP*, and this would result in the formation of stem-loop structures that favor transcription elongation into the *mgtA* and *mgtCBR* coding regions (67, 90).

Second, *Salmonella* responds to mildly acidic pH, which it experiences inside acidified macrophage phagosomes, by promoting transcription of the coding region of the *mgtCBR* operon (Figure 4) (66). An acidic pH environment increases the proton concentration gradient across the bacterial cytoplasmic membrane and drives synthesis of ATP through the F_1F_o ATP synthase (50, 104). ATP is sensed by a portion of the *mgtCBR* leader mRNA that is distinct from the portion sensing proline. The ATP-sensing portion harbors an ORF (*mgtM*) that is not conserved at the level of the amino acid sequence but that includes a stretch of conserved adenosine nucleotides (66). An increase in cytoplasmic ATP levels favors transcription elongation into the downstream *mgtCBR* coding region, presumably by furthering one of the alternative stem-loop structures that can be formed by this portion of the leader RNA (Figure 4) (66).

The proposed mechanisms by which the *mgtA* and *mgtCBR* leaders can respond to hyperosmotic stress and mildly acidic pH are reminiscent of transcription attenuation, a regulatory mechanism that entails formation of alternative stem-loop structures in an mRNA leader that control expression of a downstream protein-coding region (41, 55, 65, 76, 84). In classical transcription attenuation, which was discovered in nutrient biosynthetic genes, there is a clear connection between the signal detected and the genes being controlled. For example, a short ORF with two consecutive tryptophan codons in the leader region of the transcript that specifies tryptophan biosynthetic enzymes enables a response to the levels of tryptophan-charged $tRNA^{Trp}$ (124). Similarly, uridine controls transcription of the uridine biosynthetic *pyrB1* gene in a manner dependent on uridine nucleotides in the leader portion of the transcript that includes a short ORF (121). Regulation of *mgtA* and *mgtCBR* appears to operate in an analagous fashion because the conserved proline codons in *mgtL* and *mgtP* are necessary for the response to hyperosmotic stress (67, 90), and conserved adenine nucleotides in the *mgtM* region are required for the response to high ATP (66). Further support for a transcription attenuation-like mechanism is provided by the enhanced expression of the *mgtA* coding region that results from stop codon mutations early in *mgtL* (86, 90). However, in the case of the Mg^{2+} transporter genes, the connection between the

proline and ATP signals sensed by the leader RNA and the function of the gene being regulated is not as obvious as in the control of the *trp* and *pyrB1* genes.

The low Mg^{2+} and low proline signals act synergistically on the *mgtA* leader to promote transcription of the associated coding region (90). By contrast, the effects of the high ATP and low proline signals on the *mgtCBR* leader are additive (67). This might be because the low Mg^{2+} and low proline signals act on the same region of the *mgtA* leader, whereas the high ATP and low proline signals exert their effects on different portions of the *mgtCBR* leader.

Finally, it has been reported that overexpression of the regulatory protein Rob promotes transcription of the *mgtA* gene in *Salmonella* independently of the PhoP/PhoQ system (6) (Figure 4). Rob activates transcription initiation at a different start site than does PhoP. The physiological signal that activates the Rob protein is presently unknown. and inactivation of the *rob* gene does not affect *mgtA* transcription under the conditions tested. Although Rob-activated transcription generates a shorter transcript than that stimulated by PhoP, the sequence information necessary for the *mgtA* leader to exert its regulatory effect on transcription elongation into the coding region is still present.

WHY CERTAIN BACTERIA HAVE MULTIPLE Mg^{2+} TRANSPORTERS

Virtually every combination of Mg^{2+} transporter classes has been observed, although no example is known in which all of the Mg^{2+} transporters in a single species belong to the MgtA class (75). The repertoire of Mg^{2+} transporters varies widely even within groups of related bacterial species. For example, among the enteric bacteria, *Salmonella* harbors CorA, MgtA, and MgtB, *E. coli* has both CorA and MgtA but lacks MgtB, *S. marcescens* has two homologs of MgtE, and *Y. pestis* carries CorA, MgtE, and MgtB.

The import of a particular nutrient or ion via more than one transporter is not unique to Mg^{2+} as *Salmonella* and *E. coli* each encode two distinct proline transporters and three different K^+ transport systems (25, 123a). It is often the case that bacterial genomes encode a constitutively expressed transporter with a moderate affinity for the substrate and another, inducible system with a high affinity for the substrate, which acts as a scavenger when the substrate is scarce. For example, the *E. coli* K^+ transporter Trk is a constitutively expressed, low-affinity, high-capacity channel, whereas Kdp is an inducible, high-affinity, low-capacity ATP-dependent transporter (75, 119). However, the *Salmonella* Mg^{2+} transporters do not fit this model because the reportedly constitutive (i.e., CorA) and inducible (i.e., MgtA and MgtB) systems have essentially the same affinity for Mg^{2+} (Table 1) (118). This raises the question: Why does *Salmonella* harbor multiple Mg^{2+} transporters?

The presence of multiple transporters in a given organism could be interpreted as an example of functional redundancy, i.e., the presence of multiple proteins that carry out the same task. This idea is supported by the phenotypes of *Salmonella* mutants deficient in any one or two of its three Mg^{2+} transporters, which can grow in standard laboratory media without Mg^{2+} supplementation (56). This is in contrast to the 50 mM Mg^{2+} required for growth of a mutant that lacks all three Mg^{2+} transporters (56). An alternative view is that an organism harbors multiple Mg^{2+} transporters because each transporter is required at one

time or another to satisfy the bacterium's needs in the various environments it explores, such as inside and outside animal hosts. In agreement with this notion, the *Salmonella* Mg^{2+} transporters differ in substrate preferences, susceptibility to inhibition by other cations, energy sources, the temperatures at which they are active, and their abilities to export Mg^{2+} (Table 1). These properties likely make a given transporter more useful than the others under certain conditions.

Co^{2+}, Ni^{2+}, Zn^{2+}, and Mn^{2+} can potently inhibit one or more Mg^{2+} transporters, either by competing as substrates or by inhibiting Mg^{2+} uptake without being transported (Table 1) (110, 111). Each inhibitory cation affects the individual Mg^{2+} transporters differently. For example, Co^{2+} inhibits all bacterial Mg^{2+} transporters but enters the cell only via CorA and/or MgtE (75), whereas Zn^{2+} potently inhibits MgtA but not MgtB (111). The ability of Mg^{2+} transporters to move non-Mg^{2+} cations might be a by-product of the Mg^{2+} ion's chemical and geometric properties (75). This notion is supported by the existence of dedicated uptake systems for other divalent cations and by the observation that the Mg^{2+} transporters can take up non-Mg^{2+} cations only when they reach concentrations that are toxic to the cell (57).

A bacterium may require multiple Mg^{2+} transporters in order to survive under conditions in which a given inhibitory cation becomes abundant and/or the extracellular Mg^{2+} concentration becomes low enough for certain toxic cations to compete effectively with Mg^{2+}. For example, it has been proposed that when *Salmonella* experiences low Mg^{2+} environments, it uses the PhoP-activated Mg^{2+} transporters MgtA and MgtB, rather than CorA, to take up Mg^{2+} because CorA appears to be less able than MgtA and MgtB to discriminate against Fe^{2+} (11). In support of this idea, a *phoP* null mutant is hypersusceptible to killing by Fe^{2+}, and wild-type levels of resistance can be restored upon mutation of the *corA* gene (11). Indeed, the *E. coli* CorA protein has been implicated in Fe^{2+} uptake (49), although it is presently unclear whether CorA can itself transport Fe^{2+} (88).

In sum, although CorA, MgtA, MgtB, and MgtE can and do move Mg^{2+} from the periplasm into the cytoplasm, factors other than the concentration of Mg^{2+} per se can impact a particular transporter's effect on the health of a bacterium and/or the ability of a transporter to carry out Mg^{2+} uptake. Thus, a given bacterium may rely on multiple Mg^{2+} transporters to prosper in the various environments it encounters.

Differential Expression of Mg^{2+} Transporters

The distinct properties of individual bacterial Mg^{2+} transporters suggest that they are useful in different situations. In support of this notion, they are expressed under different conditions (Figure 5). For instance, during growth in high Mg^{2+}, there is no expression of the *mgtA* and *mgtB* genes and Mg^{2+} enters the cell via CorA (Figure 5*a*). By contrast, when *Salmonella* experiences low Mg^{2+}, transcription of *mgtA* and *mgtB* is induced (Figure 5*b*). This suggests that, under low Mg^{2+} conditions, the P-type ATPases encoded by these two genes are required by or beneficial to the cell, and/or the constitutively expressed CorA protein is not active or its activity might be harmful. *Salmonella* uses transporters that derive energy from ATP hydrolysis to import Mg^{2+} under low Mg^{2+} conditions because these

conditions also result in a reversal of membrane potential (1), which would hinder Mg^{2+} uptake by CorA (Figure 5*b*).

In addition to low Mg^{2+}, expression of the *mgtA* and *mgtB* genes is promoted when cells experience hyperosmotic stress (67, 90). This condition decreases membrane potential (21) and thus Mg^{2+} uptake by CorA (1)), but does not affect ATP-driven transport. Therefore, MgtA and MgtB can support uptake of Mg^{2+} under hyperosmotic stress. In spite of their similarities, MgtA and MgtB do not appear to operate at the same time because the *mgtCBR* operon specifies MgtR, a peptide that binds to the MgtA protein and promotes its degradation (14). In addition, MgtB protein is active at 37°C but not at 20°C, whereas MgtA can operate at both temperatures (111). Furthermore, the *mgtB* coding region is actively transcribed at mildly acidic pH, a condition that *Salmonella* experiences inside a macrophage phagosome, whereas the *mgtA* coding region is not (66). Thus, MgtB might be the primary Mg^{2+} uptake system when *Salmonella* is inside a mammalian cell (Figure 5*c*).

Mg^{2+} AND VIRULENCE

The PhoP/PhoQ system was first identified in *Salmonella* as a regulator of virulence and necessary for survival inside macrophages (29, 33, 38, 79). The discovery that this system controls expression of Mg^{2+} transporters suggested a connection between Mg^{2+} homeostasis and virulence (34). PhoP-activated promoters are highly induced when *Salmonella* is inside a macrophage phagosome (53), indicating that *Salmonella* experiences activating conditions for PhoQ in this compartment. It has been proposed that low Mg^{2+} is the signal that activates PhoQ inside a phagosome (36). However, others have argued that antimicrobial peptides are the relevant signal because a *Salmonella* strain harboring a PhoQ derivative that retained the ability to respond to low Mg^{2+} but not to a specific antimicrobial peptide was mildly attenuated for virulence (96).

In addition to *Salmonella*, the PhoP/PhoQ system is required for virulence in a variety of bacterial pathogens. These include the bubonic plague agent *Y. pestis* (87), the etiologic agent of bacillary dysentery *Shigella flexneri* (83), the plant pathogen *Erwinia carotovora* (30), the fish pathogen *Edwardsiella tarda* (72), and the human opportunistic pathogen *Pseudomonas aeruginosa* (35). PhoQ is activated by low Mg^{2+} in all these species, and PhoP controls expression of Mg^{2+} transporters in many of them. Furthermore, as discussed above, the Mg^{2+}-responding CsrR/CsrS regulatory system is a critical regulator of virulence in GAS (15).

We hypothesize that the PhoP-activated Mg^{2+} transporter MgtB likely contributes to *Salmonella* pathogenicity because the *mgtCBR* operon is located in the SPI-3 pathogenicity island, a *Salmonella*-specific gene cluster that harbors genes required for virulence (7). Moreover, the MgtB protein functions at 37°C but not at 20°C (111) and thus probably acts during infection of warm-blooded hosts but not in non-host environments. In contrast to *mgtB*, the *mgtA* gene shows a wider phylogenetic distribution within enteric species, including organisms not normally associated with animals (92).

MgtB is coexpressed with MgtC (113), a protein required for survival inside macrophages and for *Salmonella* to cause a lethal infection in mice (7). The *mgtC* gene is one of the most

highly induced genes when *Salmonella* is within a macrophage phagosome (26). Induction of *mgtC* gene expression inside macrophages can be ascribed to the mildly acidic phagosomal pH, which creates a proton concentration gradient across *Salmonella*'s cytoplasmic membrane. This generates an increase in the levels of cytoplasmic ATP, which is detected by the *mgtCBR* leader (66). In addition, the *mgtCBR* leader responds to proline-charged $tRNA^{Pro}$ (67), which could mean that *Salmonella* experiences low proline levels and/or hyperosmotic stress during infection.

Virulence phenotypes have also been associated with Mg^{2+} transporters whose expression is not dependent on PhoP. CorA was recently shown to be required for *Salmonella* virulence in mice (89). CorA is also the only *Salmonella* Mg^{2+} transporter required for invasion of epithelial cells, possibly because PhoP is not activated until after invasion (89, 108). However, it is presently unclear how CorA contributes to *Salmonella* pathogenicity. In addition, the Mg^{2+} transporter MgtE plays a role in virulence-associated phenotypes in some bacteria. For example, MgtE is required for adherence to surfaces and biofilm formation in the fish pathogen *Aeromonas hydrophila* (77), and expression of a type III secretion system in *P. aeruginosa* (2).

CONCLUSIONS AND PERSPECTIVES

Bacteria have the means to assess Mg^{2+} levels in their surroundings as well as in their cytoplasm. This ability to sense Mg^{2+} enables bacteria to modulate the expression of Mg^{2+} transporters and of proteins that promote chemical changes in the bacterial cell that are thought to enhance Mg^{2+} availability. The presence of multiple Mg^{2+} transporters in a given bacterial species is an indication that the organism has the ability to prosper in multiple environments that differ in chemical and/or physical properties. Mg^{2+} can act as a signaling molecule in spite of its central role in a variety of biochemical reactions and the stability of diverse cellular structures. The ability of bacterial pathogens to detect the levels of free Mg^{2+} in host tissues may provide new insights into how pathogens acquire Mg^{2+} during infection.

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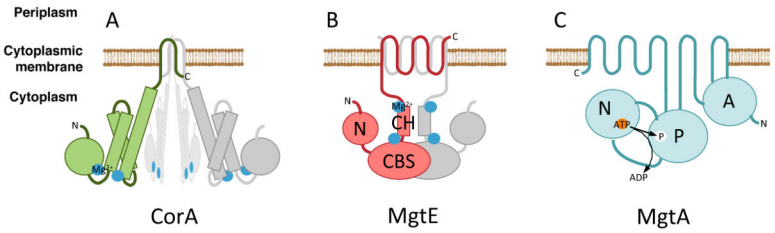

Figure 1.

Schematic representation of the three bacterial Mg^{2+} transporter classes. (*a*) CorA is a homopentamer (one monomer has been omitted for clarity) in which each monomer contains a large N-terminal cytoplasmic domain and two transmembrane helices. Putative regulatory Mg^{2+} binding sites are located at monomer-monomer interfaces (*blue circles*). Loss of Mg^{2+} at these sites is thought to induce a conformational change that leads to the opening of a hydrophobic gate within the transmembrane region and passage of Mg^{2+} through the channel (46, 71, 94). (*b*) MgtE is a homodimer in which the cytoplasmic portion of each monomer consists of an N domain (N), a tandemly repeated cystathionine β-synthase domain (CBS), and a connecting helix (CH). Upon loss of Mg^{2+} at the putative regulatory binding sites (*blue circles*), the cytoplasmic domains become more flexible and undergo a conformational change that disrupts the interaction between the connecting helix and the transmembrane domains to facilitate pore opening (52, 82). (*c*) MgtA is a monomeric P-type ATPase whose topology is thought to be analogous to that of the sarcoplasmic reticulum Ca^{2+} ATPase. The cytoplasmic portion of the protein contains a nucleotide binding domain (N), a phosphorylation domain (P), and an actuator domain (A). During each transport cycle, the γ phosphate (*white circle*) from an ATP (adenosine triphosphate) molecule (*orange circle*) is transferred to the P domain. This causes a conformational change in the A domain that allows Mg^{2+} to transverse the membrane. Finally, the A domain dephosphorylates the P domain to complete the cycle (107a, 119a).

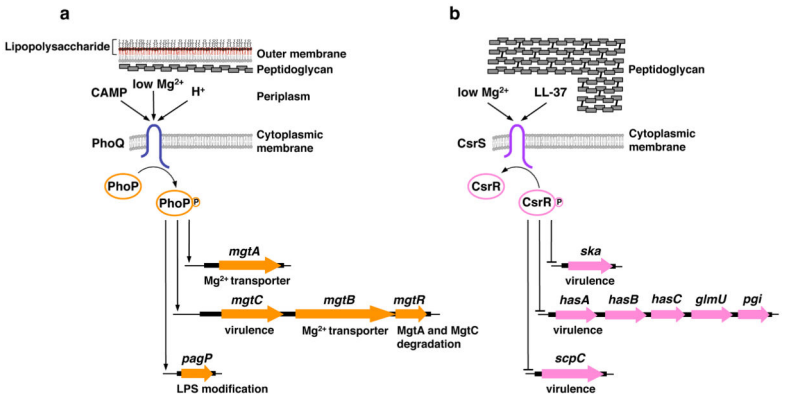

Figure 2.

Sensing of extracytoplasmic Mg^{2+} by bacterial sensor proteins. (*a*) In the gram-negative bacterium *Salmonella*, the PhoQ sensor kinase is activated by low extracytoplasmic Mg^{2+}, cationic antimicrobial peptides (CAMPs), and mildly acidic pH. Active PhoQ phosphorylates the cytoplasmic response regulator PhoP, which promotes transcription of a number of genes involved in virulence and adaptation to low Mg^{2+}. (*b*) In the gram-positive Group A *Streptococcus*, the CsrS sensor kinase is inactivated by low extracytoplasmic Mg^{2+}, leading to the dephosphorylation of the cytoplasmic response regulator CsrR and the derepression of virulence genes. CsrS is also activated by the human CAMP LL-37.

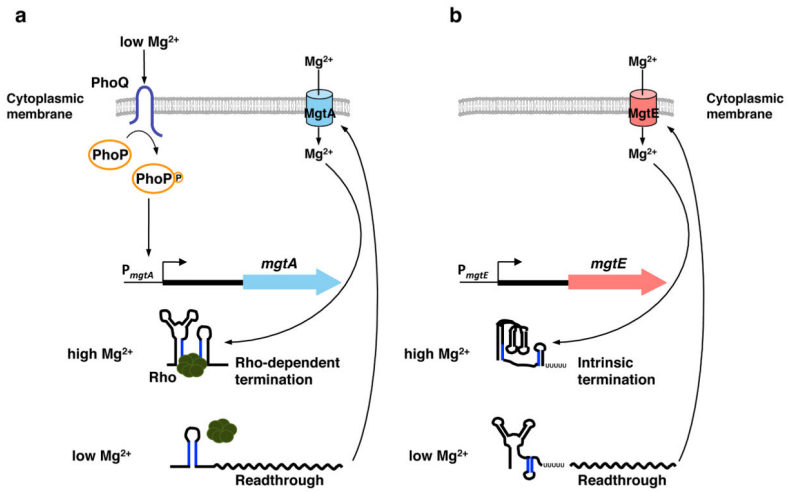

Figure 3.

Sensing of cytoplasmic Mg^{2+} by RNA Mg^{2+} sensors. (*a*) In addition to the control of transcription initiation by extracytoplasmic Mg^{2+} via the PhoP/PhoQ system, the leader region of the *Salmonella* Mg^{2+} transporter gene *mgtA* contains a riboswitch that controls transcription elongation into the *mgtA* coding region in response to cytoplasmic Mg^{2+}. When cytoplasmic Mg^{2+} levels are high, Mg^{2+} binding to the *mgtA* leader RNA favors an RNA conformer that can interact with the transcription termination factor Rho, permitting Rho to terminate transcription within the *mgtA* leader region. When the level of Mg^{2+} in the cytoplasm falls below a particular threshold, the *mgtA* leader RNA adopts an alternative conformation that is unable to interact with Rho, allowing transcription to continue into the *mgtA* coding region. (*b*) Transcription of the *Bacillus subtilis* Mg^{2+} transporter gene *mgtE* is governed by a Mg^{2+}-sensing riboswitch. When levels of Mg^{2+} in the cytoplasm are high, Mg^{2+} binds to the *mgtE* leader region and promotes compaction of the RNA into a structure that includes an intrinsic transcription terminator. This drives transcription termination within the *mgtE* leader region without the need for the Rho protein. When cytoplasmic Mg^{2+} is low, the RNA adopts an alternative conformation that prevents formation of the intrinsic terminator, and thus permits transcription elongation into the *mgtE* coding region. In contrast to *mgtA*, transcription initiation at the *mgtE* promoter does not respond to Mg^{2+}.

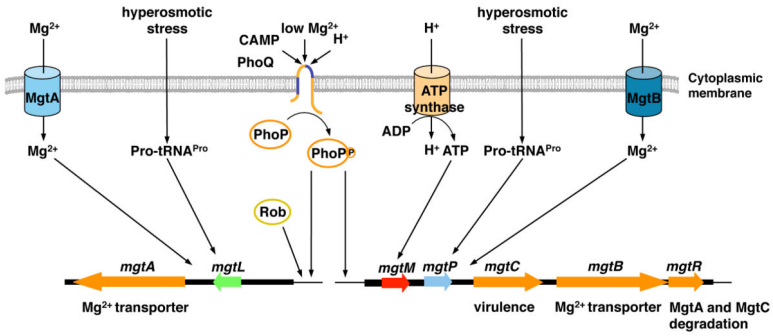

Figure 4.

Signals regulating expression of Mg^{2+} transporters. Transcription initiation at the Mg^{2+} transporter loci *mgtA* and *mgtCBR* is controlled by the PhoP/PhoQ two-component system, which is activated by low extracytoplasmic Mg^{2+}, mildly acidic pH, and specific cationic antimicrobial peptides (CAMPs). Rob promotes *mgtA* transcription in response to an unknown signal(s). In addition, the *mgtA* and *mgtCBR* transcripts include long leader sequences that respond to low cytoplasmic Mg^{2+} by stimulating transcription elongation into their respective coding regions. Transcription elongation into the coding regions is also controlled by the coupling of transcription of the *mgtA* and *mgtCBR* leaders with translation of short open reading frames designated *mgtL* (in the *mgtA* leader) and *mgtM* and *mgtP* (in the *mgtCBR* leader), which affect the formation of alternative stem-loop structures in the RNA. Which secondary structures form is determined by the cytoplasmic levels of proline-charged $tRNA^{Pro}$ (for both *mgtA* and *mgtCBR*) and ATP (for *mgtCBR*), and these conditions result from hyperosmotic stress and phagosome acidification, respectively.

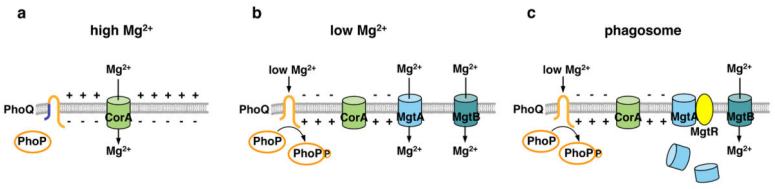

Figure 5.

Expression and activity of *Salmonella* Mg^{2+} transporters under various conditions. (*a*) When *Salmonella* experiences high Mg^{2+}, CorA transports Mg^{2+} using membrane potential as an energy source. The *mgtA* and *mgtB* genes are not expressed. (*b*) When Mg^{2+} levels are low, the PhoP/PhoQ system is activated and reverses the membrane potential (1), thereby hindering the activity of CorA. PhoP activates transcription of the *mgtA* and *mgtB* genes, and if cytoplasmic Mg^{2+} is low enough, transcription of the coding regions ensues. The P-type ATPases MgtA and MgtB transport Mg^{2+} using ATP hydrolysis as an energy source. (*c*) Inside an acidic macrophage phagosome, or under other conditions that result in high expression of the *mgtCBR* operon, only MgtB would transport Mg^{2+} because the MgtR peptide binds to the MgtA protein and leads to its proteolysis (14).

Table 1

Properties of Mg^{2+} transporters

Transporter	Distribution	Km* for Mg^{2+} (3M)	Energy source	Substrate preference	Inhibitory cations	Other	References
MgtA	Bacteria	30	ATP hydrolysis	$Ni^{2+} > Mg^{2+}$	Zn^{2+}, Co^{2+}		(74, 75, 111)
MgtB	Bacteria	6	ATP hydrolysis	$Ni^{2+} \pm Mg^{2+}$	Co^{2+}, Mn^{2+}	Inactive at 20°C	(74, 75, 111)
CorA	Bacteria, Archaea, Eukarya	15	Membrane potential	$Mg^{2+} \pm Co^{2+} > Ni^{2+}$	Mn^{2+}	Capable of Mg^{2+} efflux	(9, 63, 73, 75, 111)
MgtE	Bacteria, Archaea, Eukarya	50	Membrane potential	$Mg^{2+} > Co^{2+}$	Zn^{2+}, Ca^{2+}, Mn^{2+}, Sr^{2+}		(75, 110, 122)

*K_m: The concentration of substrate that leads to half-maximal velocity.