Magnesium transport in *Salmonella typhimurium*: biphasic magnesium and time dependence of the transcription of the *mgtA* and *mgtCB* loci

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*Salmonella typhimurium* has three distinct Mg²⁺ transport systems, the constitutive high-capacity CorA transporter and two P-type ATPases, MgtA and MgtB, whose transcription is repressed by normal concentrations of Mg²⁺ in the growth medium. The latter Mg²⁺-transporting ATPase is part of a two-gene operon, *mgtCB*, with *mgtC* encoding a 23 kDa protein of unknown function. Transcriptional regulation using fusions of the promoter regions of *mgtA* and *mgtCB* to *luxAB* showed a biphasic time and Mg²⁺ concentration dependence. Between 1 and 6 h after transfer to nitrogen minimal medium containing defined concentrations of Mg²⁺, transcription increased about 200-fold for *mgtCB* and up to 400-fold for *mgtA*, each with a half-maximal dependence on Mg²⁺ of 0.5 mM. Continued incubation revealed a second phase of increased transcription, up to 2000-fold for *mgtCB* and up to 10 000-fold for *mgtA*. This secondary increase occurred between 6 and 9 h after transfer to defined medium for *mgtCB* but between 12 and 24 h for *mgtA* and had a distinct half-maximal dependence for Mg²⁺ of 0.01 mM. A concomitant increase of at least 1000-fold in uptake of cation was seen between 8 and 24 h incubation with either system, showing that the transcriptional increase was followed by functional incorporation of large amounts of the newly synthesized transporter into the membrane. Regulation of transcription by Mg²⁺ was not dependent on a functional stationary-phase sigma factor encoded by *rpoS*, but it was dependent on the presence of a functional *phoPQ* two-component regulatory system. Whereas *mgtCB* was completely dependent on regulation via *phoPQ*, the secondary late Mg²⁺-dependent phase of *mgtA* transcription was still evident in strains carrying a mutation in either *phoP* or *phoQ*, albeit substantially diminished. Several divalent cations blocked the early phase of the increase in transcription elicited by the decrease in Mg²⁺ concentration, including cations that inhibit Mg²⁺ uptake (Co²⁺, Ni²⁺ and Mn²⁺) and those which do not (Ca²⁺ and Zn²⁺). In contrast, the second later phase of the transcriptional increase was not well blocked by any cation except those which inhibit uptake. Overall, the data suggest that at least two distinct mechanisms for transcriptional regulation of the *mgtA* and *mgtCB* loci exist.

**Keywords**: magnesium transport, magnesium regulation, *phoPQ* two-component system, *Salmonella typhimurium*

**INTRODUCTION**

Magnesium is a vital divalent cation in living organisms, functioning as both a cofactor and a regulator of...
numerous proteins and as a stabilizing factor for membranes, ribosomes and other cellular structures (Altura, 1992; Grubbs & Maguire, 1987; Maguire, 1990; Romani et al., 1993). Studies in mammalian and other systems have shown that Mg\textsuperscript{2+} transport is far more active and the intracellular content of Mg\textsuperscript{2+}, both free and total, is far greater than previously appreciated (Clausen et al., 1991; Grubbs et al., 1985; Maguire, 1990; Romani et al., 1993). In Salmonella typhimurium, three Mg\textsuperscript{2+} transport systems have been identified and designated as CorA, MgtA and MgtB, each encoded by a gene operon, where mgtC confers high capacity. This locus encodes a single protein of the PhoQ protein being a membrane sensor-kinase for Mg\textsuperscript{2+} transport is of interest. Here we report that the derepression of the promoters for both the mgtA and mgtCB loci has a biphasic time and Mg\textsuperscript{2+} concentration dependence, that such derepression results in extremely large increases in both gene transcription and translation of functional transport protein even in the absence of cell growth, and that this response appears to involve a second regulatory pathway, in addition to the phoPQ system.

**METHODS**

**Plasmids and strains.** These are shown in Table 1.

**Buffers.** Luria-Bertani (LB) broth was used for routine cultures with antibiotics supplemented as required (Hmiel et al., 1989; Snively et al., 1989). For luciferase assays as well as \textsuperscript{45}Mg\textsuperscript{2+} transport assays, nitrogen minimal medium supplemented with 1 mM leucine, 0·1% Casamino acids, 0·4% glucose, 1 µg FeSO\textsubscript{4} ml\textsuperscript{-1} and the indicated concentration of Mg\textsuperscript{2+} was used as culture broth (Hmiel et al., 1989). Luciferase assay buffer is 50 mM sodium phosphate buffer, pH 7·5, containing 0·01% (v/v) dodecyl aldehyde.

**Luciferase assay.** Previous work (Maguire et al., 1992; Smith et al., 1993b; Snively et al., 1995; Tao et al., 1995) has shown that a decrease in the Mg\textsuperscript{2+} concentration in minimal medium greatly increases transcriptional activity at both the mgtA and mgtCB loci. Previous studies were performed using mgtB:: lacZ and mgtA:: lacZ fusions, measuring \(\beta\)-galactosidase activity at a single 6 h time point. The use of the luciferase reporter constructs allows easier continuous monitoring of much smaller volumes of individual cultures over any incubation period. Further, since at 37°C the luciferase protein is unstable, a time course of luciferase activity can give some measure of the temporality of transcriptional activity, unlike \(\beta\)-galactosidase measurements which give only a sum of activity over time without necessarily indicating when the activity occurred. The constructs were tested over a range of supplemental Mg\textsuperscript{2+} concentrations from 0·3 µM to 100 mM Mg\textsuperscript{2+}. We have previously determined that the contaminant Mg\textsuperscript{2+} concentration inherent in nitrogen minimal medium as measured by atomic absorption spectrometry is approximately 15 µM (Hmiel et al., 1986, 1989). However, this amount appears to be bound completely since we can detect effects of added Mg\textsuperscript{2+} concentrations as low as 1–2 µM (Snively et al., 1991b).

The luciferase assay has been previously described (Tao et al., 1995). Briefly, an aliquot of cells (5–50 µl) is mixed with 500 µl luciferase assay buffer at room temperature in a 0·7 ml clear plastic microfuge tube. The capped tube is mixed for 10 s, placed in a carrier scintillation vial, and luciferase activity determined immediately by light counting in a Beckman LS7000 liquid scintillation counter for 0·5 min using a full channel setting. To correct for coincidence detection in the scintillation counter, net c.p.m. activity is calculated as the square root of the c.p.m. detected in the counter. All data are normalized for cell number as represented by simultaneous measurement of turbidity as OD\textsubscript{600}. Luciferase activity was linear over cell densities in the scintillation counter (as OD\textsubscript{600} from 0·001 to at least 1·0) as long as the total c.p.m. was less than 1 × 10\textsuperscript{8}. Activities greater than this amount were measured by dilution so that the total c.p.m. per cell aliquot used was less than 1 × 10\textsuperscript{8}.

It is important to note that the luciferase enzyme is temperature sensitive, being most stable at 28–30°C. Transcriptional data in this report were obtained with cells grown both at 30°C and at 37°C. Control experiments indicated that qualitatively similar results were obtained with cells grown at either temperature although the maximal response obtained at 37°C was somewhat less and slightly more variable, presumably because of ongoing denaturation and proteolysis of the luciferase. The growth temperature also has some effect on the temporal dependence of the transcriptional response, with lower temperatures resulting in a lengthening in the lag period before increases in transcription were observed. Finally, estimation of the fold increase in transcription (and transport itself) is inherently variable because of the extremely low, possibly zero, levels of activity seen in the presence of high extracellular Mg\textsuperscript{2+} concentrations, thus making the denominator in the calculation a small, variable number. However, both the baseline and maximal responses varied slightly with inoculum density and from experiment to experiment. Overall, the most consistent presentation of the data is by fold increase. Representative absolute values for responses are given in the figure legends; \(K_{95}\) values are
Mg\textsuperscript{2+} regulation of mgtA and mgtCB transcription

Table 1. *S. typhimurium* strains and plasmids used in this work

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype/plasmid</th>
<th>Reference/source</th>
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<tbody>
<tr>
<td>MM281</td>
<td>DEL485(1eubC) mgtB10::MudJ corA45::MudJ mgtA21::MudJ zhi618::Tn10(Cam\textsuperscript{r})</td>
<td>Hmiel et al. (1989)</td>
</tr>
<tr>
<td>MM387</td>
<td>DEL485(1eubC) corA185::Tn10A16A17(Tet\textsuperscript{r})</td>
<td>Smith et al. (1993a)</td>
</tr>
<tr>
<td>MM101</td>
<td>MM387/pTT5SE</td>
<td>This study</td>
</tr>
<tr>
<td>MM102</td>
<td>MM387/pTT6SE</td>
<td>This study</td>
</tr>
<tr>
<td>MM103</td>
<td>MM387/pTT-CALux</td>
<td>This study</td>
</tr>
<tr>
<td>MM104</td>
<td>MM387/pTT-ALux</td>
<td>This study</td>
</tr>
<tr>
<td>MM106</td>
<td>MM387/pTT-CLux</td>
<td>This study</td>
</tr>
<tr>
<td>MM1267</td>
<td>pboP5170::MudJ (14028s)</td>
<td>E. Groisman*</td>
</tr>
<tr>
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<td>pboQ5172::MudJ (14028s)</td>
<td>E. Groisman</td>
</tr>
<tr>
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<td><em>S. typhimurium</em> 14028s (wild-type)</td>
<td>ATCC</td>
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<td>MM1269/pTT-ALux</td>
<td>This study</td>
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<td>MM1552</td>
<td>pboQ5172::MudJ/pTT-CLux</td>
<td>This study</td>
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Fig. 1 shows control data for the promoter constructs pTT5SE and pTT6SE and for the promoter plasmid containing the corA promoter sequence, pTT-CALux. These plasmids were derived from pTrc99A (Pharmacia) and carry the trc promoter under lac\textsuperscript{p} control. There is no significant alteration in corA transcription by the concentration of extracellular Mg\textsuperscript{2+}. Likewise, transcription from the trc promoter of the parental construct was not affected by Mg\textsuperscript{2+} whether expressed relative to the negative control plasmid or to its activity at 100 mM extracellular Mg\textsuperscript{2+}.

Control experiments for cation toxicity. Cell exposure to high concentrations of many divalent cations is toxic; therefore, in experiments to determine cation specificity of transcriptional response, we used three parameters to gauge toxicity. First, cells carrying the various plasmids were tested by a disk inhibition assay in which a filter paper disk was placed in the centre of a lawn of cells. Cells that are not inhibited by the test ion grow up to the disk while those that are sensitive form a ring some distance from the disk with no visible growth within the ring (Hmiel et al., 1986, 1989). Second, in liquid cultures, we have used the cell density as a guide. In nitrogen minimal medium containing no added Mg\textsuperscript{2+}, cell density (OD\textsubscript{657}) usually increases by a factor of about 3–5 with a starting inoculum of OD\textsubscript{657} 0.01 over the first several hours of incubation and remains constant for the remainder of the 24 h incubation period. (Toxicity results were similar at initial densities of OD\textsubscript{657} 0.02–0.2.) Cations were considered as non-toxic if, at the tested concentration, they did not significantly affect final cell density in liquid culture or cause formation of a centre ring devoid of growth on plates. Based on these considerations (data not shown), Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Mn\textsuperscript{2+} and Zn\textsuperscript{2+} were toxic at approximately the same or slightly higher concentrations than those at which they diminished transcription of the lux\textsubscript{AB} fusions. Ba\textsuperscript{2+} and Sr\textsuperscript{2+} showed no significant toxicity. The effect of Ca\textsuperscript{2+} depends on the system being tested. In cells dependent on the MgtB Mg\textsuperscript{2+} transport system for Mg\textsuperscript{2+} uptake (e.g. mgtA corA double mutants), Ca\textsuperscript{2+} is highly toxic (Hmiel et al., 1986, 1989). In contrast, in cells dependent on the MgtA system (mgtCB corA double mutants), even very

![Graph of Mg\textsuperscript{2+} regulation](image)

estimates. The small number of points on the steep part of the dose response curve made curve-fitting error prone. The estimated K\textsubscript{d} values varied no more than threefold between experiments.
high concentrations of extracellular Ca\(^{2+}\) have no effect on growth.

As a third test of possible cation toxicity, we measured the ability of the various cations to alter transcription of the \textit{treR} promoter carried on pTT-DLux. The \textit{treR} gene is adjacent to and transcribed in the opposite direction from \textit{mgtA} (Tao et al., 1995), with their promoters carried on the same segment of DNA between the two genes. This is probably the most informative assay because it measures the same parameter (transcription) and utilizes the same segment of DNA as the Mg\(^{2+}\)-sensitive promoter carried on pTT-ALux (see above). Using transcription of \textit{treR} as a guide, we concluded (data not shown) that regardless of any effect of divalent cations to inhibit growth, no cation is severely toxic based on inhibition of \textit{treR} transcription. Only Ni\(^{2+}\) represses transcription from pTT-DLux more than 50\% after 24 h incubation.

\textit{65Ni\(^{2+}\) transport.} MM281 was transformed by electroporation with plasmids carrying inserts to be tested. The resulting strains were grown overnight in LB broth supplemented with appropriate antibiotics. Subcultures (20 ml) were started by 1:50 inoculation of nitrogen minimal medium containing 1 mM Mg\(^{2+}\). After 8 h growth, the cells from the subculture were collected by centrifugation at 1000 \(g\) for 5 min and washed twice in the same volume of nitrogen minimal medium containing no added Mg\(^{2+}\). New subcultures were then started from the washed cells by resuspension in nitrogen minimal medium from the same volume of nitrogen minimal medium containing no added Mg\(^{2+}\). New subcultures were then started from the washed cells by resuspension in nitrogen minimal medium containing the indicated Mg\(^{2+}\) concentration to a final OD\(_{600}\) of 0.1. After incubation for the indicated time, cells were collected and washed three times in nitrogen minimal medium without added Mg\(^{2+}\) before suspension in the same medium at a final OD\(_{600}\) of 1.0 for use in the transport assay (Snively et al., 1989; Grubbs et al. 1989).

### RESULTS

#### Regulation of \textit{mgtCB}

Regulation of the \textit{mgtCB} promoter (pTT-CLux) over the first several hours of incubation at 37 °C is shown in Fig. 2. An increase in transcription can be seen as early as 1 h after resuspending cells in low Mg\(^{2+}\) concentrations. A marked increase occurs beginning after about 2 h incubation, reaching about 200-fold for \textit{mgtCB} by 6 h. The apparent half-maximal (extracellular) Mg\(^{2+}\) concentration \((K_{50})\) at which this increase in transcription occurs is 0.5–1 mM. Continued incubation for a total of 24 h provided an additional increase in transcription of \textit{mgtCB} (Fig. 3). Beginning at approximately 7 h incubation, a second phase of transcriptional activity ensues, reaching a maximum of 1500–2000-fold in most experiments. The \(K_{50}\) for Mg\(^{2+}\) of this second phase is about 10 \(\mu\)M, significantly lower than that of the initial phase. The time course of the increase in activity is clearly at least biphasic, as shown in the inset to Fig. 3.

#### Regulation of \textit{mgtA}

Identical experiments with the \textit{mgtA} promoter construct (pTT-ALux) in cells grown at 37 °C gave results qualitatively comparable to those with the \textit{mgtCB} promoter.
3–4 h incubation is delayed with mgtA until 5–6 h incubation. While the overall activity of mgtA during this initial phase is significantly higher than that of mgtCB, the $K_{m}$ for Mg$^{2+}$ is similar, about 0.5–1 mM. As with the initial response, the second phase of the transcriptional response was delayed for mgtA compared to mgtCB, occurring after at least 10 h incubation, and was greater for mgtA (Fig. 5) than for mgtCB (Fig. 3). Increases of up to 10000-fold have been routinely measured. As with mgtCB, the $K_{m}$ of Mg$^{2+}$ for this second phase is significantly lower than for the first phase. The variability in the assay and their lengthy time course make determination of the second $K_{m}$ for mgtA more difficult, but the $K_{m}$ values for both mgtA and mgtCB appear similar at approximately 10 mM Mg$^{2+}$.

**Regulation of Mg$^{2+}$ transport**

The question of whether these large increases in transcription result in a similarly large translation into functional protein was addressed using $^{63}$Ni$^{2+}$ uptake as surrogate for the unavailable $^{28}$Mg$^{2+}$ (Snavel et al., 1989, 1991b). We have previously shown that activation of the (single-copy) chromosomally encoded mgtCB locus results in significant increases in $^{48}$Mg$^{2+}$ and $^{63}$Ni$^{2+}$ uptake when the Mg$^{2+}$ concentration of the medium is decreased (Snavel et al., 1991b). Since the luciferase reporter plasmids assayed above are pBR322-based and therefore have a relatively high copy number, $^{63}$Ni$^{2+}$ uptake was assayed under conditions as similar as possible to the promoter plasmids using pDS107 (Smith et al., 1993b), a pBR322-based plasmid expressing the intact mgtCB operon, carried in MM281. Since the chromosomal Mg$^{2+}$ transport genes have been inactivated in MM281 (Hmiel et al., 1989, Snavely et al., 1989), the strain is dependent on MgtB protein encoded by pDS107 for Mg$^{2+}$ uptake and Mg$^{2+}$-independent growth. When extracellular Mg$^{2+}$ is lowered, Mg$^{2+}$ uptake measured as $^{63}$Ni$^{2+}$ accumulation markedly increases, with a time course similar to that of the transcriptional increase (Fig. 6). Similar increases in transport via the MgtA system are also seen under these incubation conditions (data not shown). The degree of increase is a function of the extracellular Mg$^{2+}$ concentration (Maguire et al., 1992; data not shown). Estimation of the exact fold increase is difficult however because of the low initial level of uptake.

**Effect of phoPQ on transcription**

The finding by Grosman and colleagues (Garcia-Vescovi et al., 1996; Soncini et al., 1996) that the phoPQ two-component regulatory system, important for virulence in Salmonella and other enteric bacteria (Miller et
transport system. Strain MM281 carrying pDS107 (Smith et al., 1993b) was grown overnight in nitrogen minimal medium containing 1 mM Mg\(^{2+}\). The cells were washed twice in the same medium without added Mg\(^{2+}\), resuspended in the same medium at OD\(_{600}\) 0.1, transferred into flasks containing medium at 37 °C either without added Mg\(^{2+}\) or containing 1 mM Mg\(^{2+}\), and incubated at 37 °C. At the indicated times, triplicate 1 ml aliquots were withdrawn from each flask, centrifuged for 10 s in a microfuge, resuspended in nitrogen minimal medium containing 100 \(\mu\)M \(^{65}\text{Ni}^{2+}\) and no added Mg\(^{2+}\). Uptake was measured as previously described for 20 min at 37 °C (Snively et al., 1989; Grubbs et al., 1989). The basal level of uptake in cells grown in 1 mM Mg\(^{2+}\) was 100–200 net c.p.m. \(^{65}\text{Ni}^{2+}\) per 10\(^8\) cells over the 20 min incubation period over a scintillation counter background of 100–200 c.p.m. The fold increase at each time point was calculated by dividing the uptake in cells grown without added Mg\(^{2+}\) by uptake in the cells grown with added Mg\(^{2+}\) after normalizing for cell number. The uptake in cells grown in 1 mM Mg\(^{2+}\) varied less than twofold over the time course of the experiment. The variation in uptake at each time point was \(<5\%\) for cells grown without added Mg\(^{2+}\) and \(<20\%\) for cells grown in 1 mM Mg\(^{2+}\). The inset shows the same data at the early time points on an expanded scale.

**Fig. 6.** Time course of induction of Mg\(^{2+}\) uptake by the Mgt8 transport system. Strain MM281 carrying pDS107 (Smith et al., 1993b) was grown overnight in nitrogen minimal medium containing 1 mM Mg\(^{2+}\). The cells were washed twice in the same medium without added Mg\(^{2+}\), resuspended in the same medium at OD\(_{600}\) 0.1, transferred into flasks containing medium at 37 °C either without added Mg\(^{2+}\) or containing 1 mM Mg\(^{2+}\), and incubated at 37 °C. At the indicated times, triplicate 1 ml aliquots were withdrawn from each flask, centrifuged for 10 s in a microfuge, resuspended in nitrogen minimal medium containing 100 \(\mu\)M \(^{65}\text{Ni}^{2+}\) and no added Mg\(^{2+}\). Uptake was measured as previously described for 20 min at 37 °C (Snively et al., 1989; Grubbs et al., 1989). The basal level of uptake in cells grown in 1 mM Mg\(^{2+}\) was 100–200 net c.p.m. \(^{65}\text{Ni}^{2+}\) per 10\(^8\) cells over the 20 min incubation period over a scintillation counter background of 100–200 c.p.m. The fold increase at each time point was calculated by dividing the uptake in cells grown without added Mg\(^{2+}\) by uptake in the cells grown with added Mg\(^{2+}\) after normalizing for cell number. The uptake in cells grown in 1 mM Mg\(^{2+}\) varied less than twofold over the time course of the experiment. The variation in uptake at each time point was \(<5\%\) for cells grown without added Mg\(^{2+}\) and \(<20\%\) for cells grown in 1 mM Mg\(^{2+}\). The inset shows the same data at the early time points on an expanded scale.

**Fig. 7.** Effect of Mg\(^{2+}\) and phoPlphoQ on transcription from the mgtA and mgtCB promoters measured after 6 h incubation. The parental strain for these studies was S. typhimurium 14028s which carries a wild-type rpoS allele. Transcription was measured as described in Methods and the legend to Fig. 2 after incubation with the indicated Mg\(^{2+}\) concentrations. At each time the data were normalized to cell density (OD\(_{600}\) and to the apparent transcription at 100 mM Mg\(^{2+}\). Absolute values of activity were similar in this experiment to those noted in the legends to Figs 2 and 3. ○, pTT-CLux; ■, pTT-CLux/phiP; ▲, pTT-CLux/phiQ; ●, pTT-Alux; ★, pTT-Alux/phiP; □, pTT-Alux/phiQ.

Transcriptional activity (luciferase activity per OD\(_{600}\) unit).

transcription, induced by Mg\(^{2+}\) concentrations in the low micromolar range, that remains in the presence of a phoP or phiQ mutation. This dose dependence is also consistent with the concentration dependence of the interaction of Mg\(^{2+}\) with the PhoQ membrane sensor protein, which is in the range of 0.5–1.0 mM (Garcia-Vescovi et al., 1996; Vescovi et al., 1997). Together, these data suggest that a second level of transcriptional control exists for mgtA but that a similar second level of control may not exist for mgtCB.

**Relationship of growth and transcriptional response**

The transcriptional results presented above are somewhat complicated by the fact that at very low Mg\(^{2+}\) concentration in the growth medium, Mg\(^{2+}\) becomes growth limiting. We have examined this by measuring the medium Mg\(^{2+}\) concentration during growth (Fig. 8). In Mg\(^{2+}\) concentrations above about 0.2 mM in the supplemented nitrogen minimal medium described in Methods, Mg\(^{2+}\) is not growth limiting. With cell growth, an initial Mg\(^{2+}\) concentration in the medium of 1 mM falls rapidly to about 150–200 \(\mu\)M within 2 h and remains constant throughout the remainder of the incubation period even though the cells continue to increase in density for at least 6–8 h (data not shown). Cell viability remains constant between 2 and 24 h. In Mg\(^{2+}\) concentrations of 0.1 mM or below, Mg\(^{2+}\) concentration in the medium falls to about 5–10 \(\mu\)M over at least 6 h. With such low Mg\(^{2+}\) concentrations, OD\(_{600}\) doubles within 2–3 h and cell growth ceases even before
Mg²⁺ has fallen to its lowest level. Again, both Mg²⁺ concentration and cell viability remain constant for at least 24 h (data not shown). The initial cell density had little effect on these time courses, affecting only the final cell density. Interestingly, there was no difference in medium Mg²⁺ level or the time course of its depletion when tested in wild-type versus corA strains. This would imply that the source of Mg²⁺ (CorA versus the Mgt systems) has no direct influence on the cell’s Mg²⁺ requirement.

Regulation by other cations

Both MgtA and MgtB mediate Mg²⁺ and, non-physiologically, Ni²⁺ influx, but each transporter has a distinct cation inhibition profile. Previous work has also shown that the initial phase of transcription of both mgtA and mgtCB induced by decreasing Mg²⁺ concentration could be blocked by the addition of 1 mM extracellular Ca²⁺ (Snavely et al., 1991b), as measured using mgtB::lacZ fusions. Thus it was of interest to determine how other cations affected transcription of these loci. We therefore tested the Group IIa divalent cations Ba²⁺, Sr²⁺ and Ca²⁺ and the transition metal cations Ni²⁺, Co²⁺, Mn²⁺ and Zn²⁺ for their ability to alter transcription at the mgtA and mgtCB loci. First, no cation could, of itself, elicit an increase in transcription when added in the absence of either low or high Mg²⁺ concentrations (data not shown). Second, a cation’s ability to inhibit the increase in transcriptional activity elicited by the absence of extracellular Mg²⁺ depended on the specific cation tested. Of the Group IIa cations, Ca²⁺ but not Sr²⁺ (Fig. 9) blocks the initial increase in mgtA or mgtCB transcription. Ba²⁺ also was unable to block this initial increase (data not shown). About 1 mM Ca²⁺ is required for complete inhibition of transcription at mgtA and mgtCB (data not shown; Snavely et al., 1991b). Since Ca²⁺ is not toxic in strains dependent on Mg²⁺ uptake at concentrations that block the initial increase in transcription, the ability to repress the initial phase of transcription at both the mgtA and mgtCB loci is likely a specific effect of the cation, most probably mediated by binding to PhoQ (see below) which recognizes both Mg²⁺ and Ca²⁺. In contrast to its ability to inhibit the initial phase of increased transcription, Ca²⁺ is largely ineffectual in blocking the second later phase of transcriptional activity completely (Fig. 9).

Among the transition metal cations, Co²⁺ blocks the increase in transcription at either locus (Fig. 9 and data not shown). Significant inhibition can be seen at <10 μM Co²⁺, almost complete inhibition is seen at 100 μM Co²⁺ (data not shown), and both the early and late phases of the transcriptional response are inhibited. Test strain MM387, carrying a corA mutation, is

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**Fig. 8.** Mg²⁺ content of growth medium during growth of cells. MM1269 (wild-type) and MM387 (corA) were inoculated at OD₅₆₀ 0.1 into a 25 ml flask with the supplemented nitrogen minimal medium as described in Methods and grown at 37 °C with either 1 mM or 0.1 mM Mg²⁺ added to the medium. At each time point, a 1 ml aliquot was withdrawn, the cells removed by spinning in a microfuge for 1 min, and 0.5 ml of the supernatant withdrawn for analysis by atomic absorption using a Ca²⁺/Mg²⁺ lamp and standards made in the same medium. Values are corrected for the approximately 15 μM Mg²⁺ inherent to the nitrogen minimal medium (Hmiel et al., 1989). ▶ Wild-type (1 mM Mg²⁺); ●, corA (1 mM Mg²⁺); △, wild-type (100 mM Mg²⁺); ■, corA (100 mM Mg²⁺).

**Fig. 9.** Effect of various cations on transcription from the mgtCB promoter in cells carrying pTT-CLux. Transcription was measured as described in Methods. Cell aliquots were resuspended in the indicated concentrations of cation, transcription measured after incubation for 6 h (1) or 24 h (2) and the values normalized to transcription at the respective time in the absence of Mg²⁺. Activity of luxAB was measured in triplicate aliquots as described in Methods. The single experiment shown is representative of two such experiments. Triplicate aliquots measured for each individual culture varied ±10% of the absolute value and were similar under all conditions tested. Three independent cell cultures with no added Mg²⁺ were assayed in this experiment and shown as the control with the bars indicating SEM. The percentage transcription relative to the no added Mg²⁺ control is shown by the number above each bar. Absolute values of transcriptional activity were similar to those noted in the legends to Figs 2 and 3.
resistant to Co²⁺ concentrations up to 350 µM in the growth medium, although growth slows somewhat above 150 µM Co²⁺. Thus the inhibition of transcription by Co²⁺ could be a combination of a direct effect on transcription of these specific genes and an indirect effect via general cell toxicity, but any toxic effect of Co²⁺ is probably minimal since Co²⁺ had little effect on treR transcription (see Methods). By the same argument, only a portion of the ability of Ni²⁺, Mn²⁺ and Zn²⁺ to diminish transcription (Fig. 9) can be attributed to toxicity. Except possibly for Ca²⁺, transcriptional inhibition by divalent cations other than Mg²⁺ is rather unlikely to be physiologically relevant since the concentrations required are much greater than the organism is likely to encounter routinely in its various environments. Even for Ca²⁺, the levels required for repression are relatively high.

Since some reports have suggested that sulfate rather than or in addition to Mg²⁺ may play a role in gene expression (Gross & Rappuoli, 1989; Kertesz et al., 1993; Scarlato & Rappuoli, 1991), we tested the effect of anions on mgtA and mgtCB transcription as a control. The ability of Mg²⁺ to repress transcription was independent of chloride or sulfate as the counter-ion; further, sulfate alone had no effect as seen by the inability of 10 mM Na₂SO₄ to alter transcription (data not shown).

**DISCUSSION**

The data presented in this report confirm and extend our previous work on the ability of Mg²⁺ to regulate transcription of its transport genes. The regulation is obviously complex. Distinct transcriptional effects can be seen at different times and different Mg²⁺ concentrations. Divalent cations other than Mg²⁺, but not anions, also alter transcription. Despite some degree of cell toxicity from other cations, a major proportion of this transcriptional inhibition is probably a direct regulatory effect at the mgtA and mgtCB loci. Nonetheless, transcriptional inhibition by cations other than Mg²⁺ is unlikely to be of major physiological significance since relatively high concentrations of cation are required.

The mechanism of these effects on transcription is only partially known. The phoPQ two-component regulatory system (Garcia-Vescovi et al., 1996; Sercinii et al., 1996) would appear to mediate the initial earlier transcriptional response based on the Mg²⁺ concentration required for half-maximal induction and the effect of phoP and phoQ mutations. These data are consistent with previous data (Miller et al., 1989; Johnston et al., 1996; Groisman et al., 1989, Gunn & Miller, 1996; Guo et al., 1997; Sercinii et al., 1996; Sercinii & Groisman, 1996, Garcia-Vescovi et al., 1996) showing that the phoPQ system is involved in both induction and repression of a relatively large set of genes important for virulence. However, the phoPQ system cannot be the only regulatory pathway involved. The most direct evidence for this is the ability of very low extracellular Mg²⁺ concentrations to induce mgtA transcription in the presence of a phoP or phoQ mutation. While the presence of a phoP/Q mutation markedly diminishes the transcriptional response at mgtA, it is clearly not abolished and still has a dose dependence on Mg²⁺ (Fig. 7). Additional evidence for a second regulatory pathway might be deduced from the fact that the second, later phase of mgtA and mgtCB transcription has a distinct half-maximal Mg²⁺ concentration dependence, well below the demonstrated affinity for Mg²⁺ interaction with phoQ. This interpretation is complicated however by the results in Fig. 7 showing complete dependence of mgtCB response on the presence of a functional PhoPQ system. The cation sensitivity data (Fig. 8) suggest that the phase of regulation with a half-maximal Mg²⁺ dependence of about 10 µM appears more selective for Mg²⁺, while that operative at 1 mM Mg²⁺ may be sensitive to several divalent cations. mgtA and mgtCB are clearly not the only loci regulated by changes in extracellular Mg²⁺ concentrations and it would be of interest to determine if other phoPQ-regulated loci respond with a biphasic pattern. Other examples of apparent Mg²⁺ regulation of gene expression have also been reported (Sercinii & Rappuoli, 1991; Kiyota et al., 1989; Grozzi et al., 1991; Phinney & Hoober, 1992; O'Halloran, 1993).

The time courses of the responses of mgtA and mgtCB suggest that the initial phase of Mg²⁺-dependent transcriptional response occurs during or near the end of active growth, but that the second phase, seen after several hours’ incubation and at low extracellular Mg²⁺ concentrations, occurs only after cell growth has ceased (and while extracellular Mg²⁺ concentration remains constant). The question therefore arises whether the second phase of response seen with both mgtA and mgtCB is a stationary-phase response in some part. We do not believe it is. First, cells grown in moderate levels of Mg²⁺ to stationary phase do not show induction of mgtA or mgtCB regardless of how long they are left at high density. Second, more directly, the stationary-phase sigma factor encoded by rpoS has no effect on transcription of mgtA or mgtCB. The experiments shown in Figs 2–5 were performed in a strain background using S. typhimurium LT2 as parent. This nominal wild-type strain carries an rpoS mutation (Wilmes-Riesenberg et al., 1997). (We have confirmed by catalase assay and introduction of a wild-type allele of rpoS that our strain of S. typhimurium LT2 is rpoS.) In contrast, the data of Fig. 7 were obtained in a strain background using S. typhimurium 14028s as parent, which is a virulent strain with a wild-type rpoS allele. From these data, there is little if any difference in the transcriptional response of mgtA or mgtCB in wild-type and rpoS strains. Thus we conclude that neither phase of the Mg²⁺-associated transcription response at mgtA and mgtCB is necessarily tied to growth state.

These data also suggest that MgtA and MgtB are not (solely) scavenger systems for Mg²⁺. Classically, multiple transport systems for nutrients consist of a relatively poor-affinity transporter(s) operative during
growth in media rich in that nutrient while a high-affinity transporter is expressed in media with a very low concentration of that substance. Classic examples are the K⁺ transporters of Escherichia coli. The TRK system has an affinity of 1–2 mM for K⁺ uptake, while in low extracellular K⁺ the Kdp P-type ATPase is induced and has an affinity for K⁺ of 1–3 μM (Altendorf & Epstein, 1994; Epstein et al., 1995). The MagA and MgtB systems do not fit this model. First, all three Mg²⁺ transporters of S. typhimurium and E. coli have affinities for Mg²⁺ of between 5 and 30 μM. Second, as noted above, an increase in magA and mgtCB transcription is seen at extracellular Mg²⁺ concentrations at which the constitutive CorA system is still capable of supplying sufficient Mg²⁺. Third, unlike scavenger systems, both MagA and MgtB transport the physiologically relevant cation down its electrochemical gradient. Even at very low extracellular Mg²⁺, the electrochemical gradient for Mg²⁺ should still be directed inward. The purpose of using ATP is therefore unclear. Fourth, it is unclear why the cell would possess two Mg²⁺ uptake carriers. Although they have similar properties and regulation, the nucleotide sequences of magA and mgtCB, and the amino acid sequences of their products, are not exceptionally similar. This suggests that the alleles must have been maintained separately within S. typhimurium for a considerable time in evolutionary terms or that one of the ATPases has been acquired by Salmonella by horizontal transfer. Presumably the cell would not maintain two such functionally similar alleles unless one or both had some specific, useful property. Thus, while the extraordinarily large transcriptional responses of these loci indicate that they play a role in providing Mg²⁺ at very low extracellular concentrations, this may not be their only role. Indeed, it is possible that Mg²⁺ transport is a secondary function, with their primary role yet to be discovered.

Finally, it must be noted that the function of the mgtC gene is currently unknown. The MgtC protein sequence suggests that the gene is expressed under some specific growth or metabolic condition. Regardless of the mechanism of regulation and the growth conditions under which regulation of these loci occur, these data demonstrate that extracellular Mg²⁺ can have a profound influence on gene expression, indicating that Mg²⁺ may play an important role in cellular metabolic homeostasis and gene expression.

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