MgtA and MgtB: Prokaryotic P-Type ATPases That Mediate Mg\(^{2+}\) Influx

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The gram-negative bacterium *Salmonella typhimurium* possesses three distinct Mg\(^{2+}\) transport systems, encoded by the corA, mgtA, and mgtB loci. The CorA transport system is the constitutive Mg\(^{2+}\) influx system. It can also mediate Mg\(^{2+}\) efflux at very high extracellular Mg\(^{2+}\) concentrations. In contrast, the MgtA and MgtB Mg\(^{2+}\) transport systems are normally expressed only at low extracellular Mg\(^{2+}\) concentrations. A strain of *S. typhimurium* was constructed by mutagenesis which lacks Mg\(^{2+}\) transport and requires 100mM Mg\(^{2+}\) for growth. Using this strain, both the MgtA and MgtB transport systems were cloned by complementation of the strain's inability to grow without Mg\(^{2+}\) supplementation. After sequencing and further genetic analysis, the MgtB system appears to be an operon composed of the mgtC and mgtB genes (5' to 3'). The downstream mgtB gene encodes the 102kDa MgtB protein which by sequence analysis is clearly a P-type ATPase. Interestingly, while MgtB has relatively poor homology to other known prokaryotic P-type ATPases, it is highly homologous to mammalian reticular Ca\(^{2+}\)-ATPases. MgtC is a 22.5kDa hydrophobic membrane protein that lacks homology to any known protein. Transposon insertions in this gene abolish uptake by the MgtB transport system. We hypothesize that MgtC is a subunit of the MgtB ATPase involved either in proper insertion of MgtB into the membrane or possibly in binding of extracellular Mg\(^{2+}\) for delivery to the ATPase subunit. The sequence of the MgtA gene has recently been completed, and it too is a P-type ATPase more similar to eukaryotic than prokaryotic P-type ATPases. Expression of both MgtA and MgtB are highly regulated by the concentration of extracellular Mg\(^{2+}\). Transcription of mgtB can be increased about 1000 fold by lowering Mg\(^{2+}\) from 1 mM to 1 \(\mu\)M. Likewise, when mgtB is expressed from a multicopy plasmid, a similar decrease in extracellular Mg\(^{2+}\) greatly increases transport. Under growth conditions of limiting Mg\(^{2+}\), MgtB becomes the dominant Mg\(^{2+}\) influx system in *S. typhimurium*. Even so, since MgtB (and MgtA) mediate only influx of Mg\(^{2+}\), it is unclear why the cell requires energy from ATP to mediate Mg\(^{2+}\) entry into the cell down a large electrochemical gradient. Further studies of the structure-function and energetics of these novel Mg\(^{2+}\) influx P-type ATPases should yield insights into the function of P-type ATPases in general as well as information about the regulation of cellular Mg\(^{2+}\) fluxes.

KEY WORDS: Magnesium; transport; ATPase, P-type ATPase; *Salmonella typhimurium*; genetics; influx; sequence regulation; homology; prokaryote.

INTRODUCTION

Mg\(^{2+}\) is the second most abundant cation within cells and by far the most abundant divalent cation. It plays important roles in the structural properties of cells, interacts with ATP as the required or preferred substrate for over 300 enzymes, and binds to specific metal sites on a multitude of proteins to alter their properties.\(^{(1-6)}\) Despite its importance, little is known...
about how intracellular Mg\(^{2+}\) is controlled, including how Mg\(^{2+}\) flux across membranes is mediated. A major reason for this limited knowledge is that the techniques available for the study of Mg\(^{2+}\) lag far behind those for other ions. Microelectrodes and dyes sufficiently sensitive and selective for Mg\(^{2+}\) have not yet been optimized. \(^{28}\)Mg\(^{2+}\), the only isotope useful for transport studies, is rarely available, has a 21-hour half-life, and is extremely expensive.

The determination of the structure, mechanism, and control of Mg\(^{2+}\) transport systems would be of great value, not only because of the importance of Mg\(^{2+}\) itself, but also for comparison to other transport systems. Because of the paucity of sensitive techniques with which Mg\(^{2+}\) transport can be measured, classical biochemical means cannot be used to purify a Mg\(^{2+}\) transport protein. Moreover, despite the great advances in mammalian molecular biology, techniques are not yet available for isolation of a Mg\(^{2+}\) transporter from a mammalian cell. Consequently, we have used the powerful genetic techniques available in prokaryotes to identify mutations and subsequently chromosomal loci involved in Mg\(^{2+}\) transport. The model organism chosen has been the gram-negative bacterium Salmonella typhimurium. Our data indicate that S. typhimurium, and likely most gram-negative bacteria, possesses three distinct Mg\(^{2+}\) transport systems, designated the CorA, MgtA, and MgtB, transporters.\(^{7-12}\)

The CorA system is composed of four distinct chromosomal loci, corA, corB, corC, and corD. The corA locus is composed of a single gene expressing the 42-kDa CorA protein\(^{13}\) which by itself can mediate Mg\(^{2+}\) influx. Concomitant expression from the corB, corC, and corD loci will allow Mg\(^{2+}\) efflux via CorA\(^{12}\) in addition to influx. The CorA transport complex is the major, constitutive Mg\(^{2+}\) transporter in S. typhimurium (and likely all gram-negative bacteria) and is apparently the cell’s only Mg\(^{2+}\) efflux system. Under normal growth conditions in the laboratory, the major role of the CorA system is to mediate Mg\(^{2+}\) influx, at least at extracellular Mg\(^{2+}\) concentrations below 0.3–0.5 mM. At extracellular concentrations of 1 mM or higher, the CorA system function as a gated Mg\(^{2+}\)–Mg\(^{2+}\) exchanger.\(^{8,10}\) The CorA protein, capable of mediating influx by itself, represents a novel transport system, with no sequence homology to any known protein and with no apparent structural similarity to any known transport system.

In contrast, the MgtA and MgtB systems are clearly members of a well-studied family of transport proteins, but their function within the organism is not immediately clear. By sequence analysis, both MgtA and MgtB are P-type ATPases (Ref. 11 and unpublished observations). However, they appear to mediate the influx of Mg\(^{2+}\) down its electrochemical gradient even in aerobically grown cells whose Δψ plus Δμ\(^{+}\) may total ~200 mV negative inside. Moreover, the \(K_a\) for Mg\(^{2+}\) influx via these two systems is not significantly different from that of the CorA system, thus rendering unlikely the possibility that they function (solely) as scavenger systems. This article is a brief review of what we have currently discovered about the function and expression of those two novel ATPases.

**TRANSPORT PROPERTIES OF THE MgtA AND MgtB SYSTEMS**

**Influx**

The MgtA and MgtB transport systems each mediate the uptake of both Mg\(^{2+}\) and Ni\(^{2+}\) (Table I). Because of the extreme cost and relative unavailability of \(^{28}\)Mg\(^{2+}\), \(^{63}\)Ni\(^{2+}\) has been used extensively to characterize uptake by each system.\(^{10}\) The rank order of potency of inhibition by other divalent cations is identical for both Ni\(^{2+}\) and Mg\(^{2+}\) uptake, Mg\(^{2+}\) and Ni\(^{2+}\) are competitive inhibitors of the other’s uptake, and deletion of the Mg\(^{2+}\) transport genes abolishes Mg\(^{2+}\) and Ni\(^{2+}\) uptake by each system. Mn\(^{2+}\) and Co\(^{2+}\) are also competitive inhibitors of uptake for both systems but cannot be transported by either. Ca\(^{2+}\) is a competitive inhibitor of uptake via MgtA with a \(K_i\) approximately 10-fold greater than the \(K_m\) for Mg\(^{2+}\); in contrast, Ca\(^{2+}\) fails to inhibit uptake via MgtB even when added at concentrations more than 1000-fold greater than the \(K_m\)'s for Mg\(^{2+}\) or Ni\(^{2+}\) uptake. In absolute terms, both MgtA and MgtB have a significantly higher \(V_{\text{max}}\) for Mg\(^{2+}\) uptake than for Ni\(^{2+}\). In contrast, the \(K_m\) values for uptake of Mg\(^{2+}\) and Ni\(^{2+}\) are approximately equal for MgtB. With MgtA, the \(K_m\) for Ni\(^{2+}\) uptake is about 5-fold lower than the \(K_m\) for Mg\(^{2+}\) uptake.\(^{8,10}\)

It might be argued from the transport data that the MgtA and/or MgtB systems physiologically mediate the uptake of Ni\(^{2+}\) rather than Mg\(^{2+}\). However, at the Ni\(^{2+}\) concentrations required for uptake, Ni\(^{2+}\) is quite toxic to S. typhimurium and most other gram-negative organisms, rapidly causing cell death. Ni\(^{2+}\) uptake via Mg\(^{2+}\) transport systems is quite common in chemoorganotrophic bacteria.\(^{14,15}\) The physiological requirement for Ni\(^{2+}\) in these bacteria is