How Pathogens Feel and Overcome Magnesium Limitation When in Host Tissues

Anne-Béatrice Blanc-Potard, Eduardo Groisman

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Opinion

How Pathogens Feel and Overcome Magnesium Limitation When in Host Tissues

Anne-Béatrice Blanc-Potard[1,2,*] and Eduardo A. Groisman[3,4,*]

Host organisms utilize nutritional immunity to limit the availability of nutrients essential to an invading pathogen. Nutrients may include amino acids, nucleotide bases, and transition metals, the essentiality of which varies among pathogens. The mammalian macrophage protein Slc11a1 (previously Nramp1) mediates resistance to several intracellular pathogens. Slc11a1 is proposed to restrict growth of *Salmonella enterica* serovar Typhimurium in host tissues by causing magnesium deprivation. This is intriguing because magnesium is the most abundant divalent cation in all living cells. A pathogen's response to factors such as Slc11a1 that promote nutritional immunity may therefore reflect what the pathogen 'feels' in its cytoplasm, rather than the nutrient concentration in host cell compartments.

Highlights

Nutritional immunity is a host defense mechanism that limits nutrient availability to an invading pathogen.

Slc11A1 is a macrophage protein that confers resistance to different pathogens that remain within a phagosome. Slc11A1 was proposed to starve microbes of Fe^{2+} and Mn^{2+} by transporting these metals from the phagosome to the cytoplasm. *Salmonella* experiences Mg^{2+} starvation in host tissues with a functional Slc11A1 protein.

During infection of their eukaryotic hosts, bacterial pathogens respond to changes both in their surroundings and in the cytoplasmic concentration of specific chemicals, which may be triggered by the action of host proteins.

Introduction

Microbes establish various interactions with animal and plant hosts. These interactions may result in the absence of infection, an asymptomatic infection, a symptomatic infection potentially leading to death of the host, or a symbiotic relationship. The outcome of a microbe–host interaction can range from detrimental to highly beneficial, depending on numerous factors, including the number of infectious organisms, the state of host defenses against invading microbes, and previous interactions with the same or different microbes (Box 1).

Eukaryotic hosts utilize diverse mechanisms to fend off infectious microbes. These mechanisms differ in the nature of the antimicrobial response (physical or chemical), the time of occurrence (immediate or delayed), and the specificity (narrow or broad). Hosts compromise microbial survival by producing proteins and peptides with antimicrobial properties [1], generating reactive oxygen and nitrogen species [2], lowering the pH of the compartment harboring an invading microbe [3], and increasing their body temperature [4]. In addition to mounting nonspecific responses shortly after infection, hosts mount delayed, highly directed responses in the form of lymphocytes and their secreted factors.

One host defense strategy with broad specificity is **nutritional immunity** [5]. This strategy limits microbial access to essential nutrients, thereby hindering the ability to grow and divide. Therefore, susceptibility to infection can result from changes in host metabolism or proteins that decrease the availability of nutrients required by an invading microbe. For example, dietary iron or genetic conditions resulting in excess iron in blood can favor bacterial growth in host tissues (Box 2) [6]. Pathogenic microbes, in turn, have evolved mechanisms to acquire nutrients in low abundance and overcome nutritional immunity [7]. Transition metals such as iron, zinc, and manganese play a key role in nutritional immunity by acting as cofactors in various enzymatic reactions, and their depletion can compromise bacterial growth [5]. Concentrations of these transition metals must be tightly regulated because their overabundance hinders cell viability [8,9].

[1]Laboratory of Pathogen Host Interactions, Université Montpellier, case 107, Place Eugène Bataillon, 34095, Montpellier cedex 5, France
[2]CNRS, UMR5235, 34095, Montpellier Cedex 05, France
[3]Department of Microbial Pathogenesis, Yale School of Medicine, 295 Congress Avenue, New Haven, CT 06536, USA
[4]Yale Microbial Sciences Institute, P.O. Box 27389, West Haven, CT 06516, USA

*Correspondence:
anne.blanc-potard@umontpellier.fr (A.-B. Blanc-Potard) and
eduardo.groisman@yale.edu (E.A. Groisman).

Box 1. What Determines Susceptibility to Microbial Infection?

Several factors impact the susceptibility of a given individual to infection by a given pathogen. Age is a critical factor because the immune system is not fully developed in the very young and its activity decreases in the very old. The susceptibility to infection typically increases in individuals with cancer or receiving immunosuppressive drugs. Diet impacts susceptibility to infection in at least two ways: it can serve as nutrients for particular pathogens, and it can foster the proliferation of individual members of the gut microbiome that often protect a host from infection. In addition, previous exposure to a given microbe can alter the immune status of the host by impacting the ability of a different microbe to establish a productive infection.

Here, we explore how nutritional immunity limits pathogen proliferation. We examine how microbes 'know' nutrients are present and what they 'feel' in a host environment. We focus on: (i) **Slc11a1** (for solute carrier 11a1, previously known as Nramp1 for natural resistance-associated macrophage protein 1; see Glossary), a mammalian protein that confers resistance to infection by intracellular pathogens that remain within a membrane-bound acidic vacuole [10]; (ii) the intracellular pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) (Box 3) [11]; and (iii) the notion that Slc11a1 confers resistance to *S.* Typhimurium by causing Mg^{2+} limitation in tissues occupied by this pathogen [12].

The Macrophage Protein Slc11a1 Mediates Resistance to Several Intracellular Pathogens

Slc11a1 is a protein of murine macrophages that localizes to membranes of late endosomes/lysosomes but not to early endosomes [10]. It confers resistance to several intracellular pathogens, including *S.* Typhimurium, *Mycobacterium bovis, Mycobacterium leprae*, and *Leishmania donovani* [13]. These pathogens share the ability to survive and replicate within a mildly acidic macrophage vacuole [14].

Slc11a1 exhibits 65% shared amino acid identity with Scl11a2 (Nramp2), a protein that localizes to early endosomes and does not appear to be involved in resistance to infection [10]. Scl11a2 exhibits a much broader tissue distribution than Scl11a1, which is found solely in the myeloid lineage and neurons [15].

Polymorphism in the *Slc11a1* gene (*SLC11A1* in humans) is associated with susceptibility to infection by microbes in different hosts. These include *Mycobacterium tuberculosis* infection of humans, mice, pigs, and cattle [16]; infection of chickens and mice by *S. enterica* serovars Pullorum and Enteritidis [17]; and *Brucella abortus* infection of cattle [18]. The G169D substitution in the Slc11a1 protein, found in mouse strains frequently used in research (e.g., C57BL6/J and

Box 2. The Virulence Roles of Bacterial Genes Are Host-Specific

In 2009, Malcolm J. Casadaban, the great bacterial geneticist who developed the *lac* gene fusion technology widely used over the last >40 years, succumbed to an infection by the plague agent *Yersinia pestis* [67]. This was surprising given that Dr Casadaban was infected by a mutant strain of *Y. pestis* believed to be attenuated for virulence because it lacked a set of genes necessary for acquisition of iron, an essential nutrient for most organisms. However, this mutant was still virulent for Dr Casadaban because he suffered from hemochromatosis, a genetic disorder that results in high amounts of iron in the blood [6]. Thus, the increased iron concentration in Dr Casadaban's blood restored virulence to an attenuated *Y. pestis* mutant lacking a specific iron importer.

The tragic outcome resulting from the interaction between an 'attenuated' strain and a host with a genetic disorder indicates that virulence is a microbial property defined in the context of the host. Moreover, it argues that a critical property of pathogenic organisms is their ability to obtain essential nutrients from the organs, tissues, and cells explored during infection. Furthermore, it supports the notion of 'nutritional immunity' by which limiting access to essential nutrients allows hosts to fend off microbial infections. In other words, differential susceptibility to infection can result from changes in host metabolism that impact the availability of nutrients that an invading microbe requires to produce energy and cell mass. Pathogenic bacteria, which are often auxotrophic for particular amino acids or vitamins, have evolved efficient mechanisms to acquire nutrients, and thus, overcome nutritional immunity.

Glossary

mRNA leader: the 5′ region of an mRNA up to the start codon of the associated coding region. Certain mRNA leaders can adopt alternative conformations in response to different cytoplasmic signals. These conformations determine whether the associated coding region is transcribed or translated or whether the mRNA is cleaved.

Nutritional immunity: a host defense strategy that limits microbial access to essential nutrients.

Phagosome: a membrane-bound vacuole containing phagocytized microorganisms. The chemical composition of a phagosome is different from that of the cytoplasm.

Slc11A1: (formerly Nramp1) a mammalian protein that mediates resistance to several intracellular pathogens that remain within a phagosome. It is present in the phagosomal membrane; Slc11A1 is believed to be a cation transporter, the direction and specificity of which is still a matter of debate.

Two-component regulatory system: a signaling transduction system prevalent in bacteria and present in all domains of life. It is typically composed of a sensor protein that responds to chemical or physical signals by modifying the phosphorylated state of a cognate regulatory protein that mediates a cellular response to the environment denoted by the signal. The PhoP/PhoQ two-component system governs virulence and Mg^{2+} homeostasis in *S.* Typhimurium.

Box 3. Different Disease Outcomes Can Result from Differences in the Expression of Virulence Genes

A bacterium may be pathogenic in other animals but not in humans or plants, and vice versa. A pathogen may also cause different diseases in different animal hosts. For example, *S.* Typhimurium causes a self-limiting disease of the gastrointestinal tract in healthy human adults. By contrast, it causes a systemic disease in mice, resulting in bacterial growth in the liver and spleen. The infection that *S.* Typhimurium provokes in mice is often used to model typhoid fever, the systemic disease resulting from infection by the human-adapted *S.* Typhi.

Changes in the expression of virulence genes can result in different pathogenicity in a given host. For instance, the *pgtE* gene specifies an outer-membrane protease required for the production of murine typhoid by *S.* Typhimurium. African isolates of *S.* Typhimurium with nucleotide substitutions in the *pgtE* promoter resulting in much higher expression of the PgtE protein caused systemic disease in humans [68]. Therefore, changes in the expression of a virulence gene altered the pathogenicity of *S.* Typhimurium, allowing it to cause, in humans, a disease resembling typhoid fever, as opposed to gastroenteritis.

BALB/c), is not functional and renders mice hypersusceptible to infection by multiple pathogens [10].

How Does Slc11a1 Mediate Resistance to Intracellular Pathogens?

The Slc11a1 protein has 12 predicted transmembrane domains and appears to function as a divalent cation transporter [10]. Despite general agreement that Slc11a1 localizes to the phagosomal membrane, there is ongoing debate about the substrates that Slc11a1 transports and the direction of transport (Figure 1). For example, expression of the *Slc11a1* gene in *Xenopus* oocytes suggests that Slc11a1 operates as an H^+/bivalent antiporter that can flux bivalent cations in either direction, depending on the pH on either side of the membrane [19]. In an acidic **phagosome**, Slc11a1 would transport Fe^{2+} and other metal ions from the cytoplasm into the phagosome because cytoplasmic pH is higher than phagosomal pH [19]. Iron accumulation in the phagosome would favor formation of hydroxyl ions from hydrogen peroxide via the Fenton reaction, resulting in microbial death [20]. By contrast, others have argued that Slc11a1 transports bivalent cations in the opposite direction [5], thereby hindering microbial growth by lowering the phagosomal concentration of bivalent cations below a threshold critical for microbial replication. Some transport properties of Slc11a1 have been extrapolated from those established with Slc11a2, also known as DMT1 or DCT1, a Fe^{2+} transporter [21]. Slc11a2, which exists in different isoforms and subcellular locations, is the major iron transporter in most cells [22]. However, the

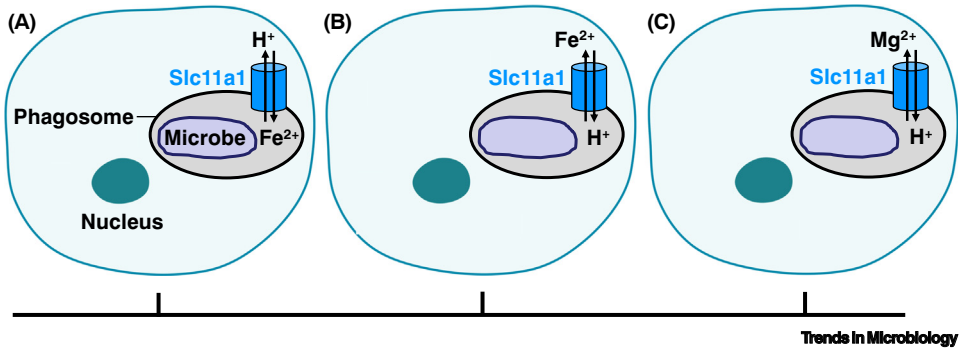

Figure 1. The Function of Phagosomal Membrane Protein Slc11a1 in Macrophages. Slc11a1-mediated protection from intracellular microbes has been ascribed to its ability: (A) to transport Fe^{2+} into the phagosome in exchange for H^+, thereby promoting iron-mediated toxicity; (B) to transport Fe^{2+} from the phagosome to the cytoplasm in exchange for H^+, thereby starving microbes of iron; and (C) to starve microbes for magnesium inside the phagosome, possibly by transporting Mg^{2+} from the phagosome to the cytoplasm in exchange for H^+. Because the composition of the phagosome is not constant, a given Slc11a1 protein may perform one or more of the proposed functions at different times after microbe internalization.

purified Slc11a1 protein is not yet confirmed as a transporter. Therefore, proposed mechanisms by which Slc11a1 hinders microbial growth are often based on the behavior of bacterial mutants and bacterial genes in hosts harboring functional or defective *Slc11a1* genes.

Slc11a1 was suggested to transport Fe^{2+} and Mn^{2+} from the phagosome containing *S.* Typhimurium into the cytoplasm of macrophages [23]. This proposal was based on the behavior of *S.* Typhimurium *sitA* and *mntH* single mutants, deficient for transport of Fe^{2+} and Mn^{2+}, respectively, and attenuated for virulence in *Slc11a1*$^{+}$/$^{+}$ but not in *Slc11a1*$^{-}$/$^{-}$ mice [23]. As expected, a *sitA mntH* double mutant was also attenuated for virulence [23]. Supporting the notion that Slc11a1 provokes limitation for Fe^{2+} and Mn^{2+}, expression of the *sitA* and *mntH* genes was more upregulated in macrophages harboring the wild-type *Slc11a1* gene than those with nonfunctional alleles of *Slc11a1* [23].

Others reported that the fitness of a *S.* Typhimurium *sitABCD mntH feoABC entC* quadruple mutant was strongly compromised in *Slc11a1*$^{+}$/$^{+}$ compared to *Slc11a1*$^{-}$/$^{-}$ mice [12]. The quadruple mutant lacks genes specifying two additional iron-uptake systems relative to the *mntH sitA* double mutant discussed above. By contrast, a *S.* Typhimurium *sitABCD mntH zupT* triple mutant behaved similarly in *Slc11a1*$^{+}$/$^{+}$ and *Slc11a1*$^{-}$/$^{-}$ mice [12]. Because *zupT* specifies a Mn^{2+} transporter (but can also transport Zn^{2+} and Co^{2+}) [24,25], these results suggest that Slc11a1 hinders *S.* Typhimurium replication inside macrophages by limiting availability of Fe^{2+} rather than Mn^{2+}.

In addition, Slc11a1 is proposed to disrupt bacterial Mg^{2+} homeostasis (Figure 1). A *S.* Typhimurium mutant defective in the Mg^{2+} transporter-specifying gene *mgtB* [26] was attenuated for virulence in *Slc11a1*$^{+}$/$^{+}$ but not *Slc11a1*$^{-}$/$^{-}$ mice [27]. Similar behavior was exhibited by a *S.* Typhimurium mutant lacking the *mgtC gene* [28], which specifies a protein required for normal growth in low-Mg^{2+} laboratory media [29,30]. Furthermore, comparison of a set of isogenic *S.* Typhimurium strains with mutations in Mg^{2+} transporter genes in congenic *Slc11a1* mice revealed an intriguing correlation between bacterial and host genotypes. That is, the replication rate of wild-type *S.* Typhimurium in *Slc11a1*$^{+}$/$^{+}$ mice was similar to that of a *S.* Typhimurium double mutant with a defective *mgtB* gene and impaired *mgtA* expression in *Slc11a1*$^{-}$/$^{-}$ mice [12]. (The *mgtA* gene specifies a Mg^{2+} transporter that shares 50% amino acid identity with MgtB [26].) In other words, wild-type *S.* Typhimurium experiences a replication restriction in mice with functional *Slc11a1* genes equivalent to that felt by a *S.* Typhimurium mutant defective in the Mg^{2+} transporters MgtA and MgtB in mice with nonfunctional *Slc11a1* genes [12].

As stated above, Slc11a1 localizes to the phagosomal membrane [10], and its expression in *Xenopus* oocytes results in divalent cation movement across a membrane [19]. The behavior of isogenic *S.* Typhimurium strains defective in the Mg^{2+} transporters MgtA and MgtB in congenic *Slc11a1* mice [12] raises the possibility that the Slc11a1 protein is a Mg^{2+} transporter. In this scenario, Slc11a1 would lower Mg^{2+} concentration in the phagosome by transporting Mg^{2+} from the phagosome into the cytoplasm. A bacterial protein distantly related to Slc11a1 appears to transport Mg^{2+} because it restored growth in low Mg^{2+} to a mutant *Bacillus subtilis* strain lacking all known Mg^{2+} transporter genes [31]. Magnesium transport assays are required to determine whether Slc11a1 does transport Mg^{2+}. Alternatively, Slc11a1 may alter vacuolar trafficking inside macrophages [32] by limiting Mg^{2+} availability to the phagosome harboring *S.* Typhimurium [12]. That Slc11A1 might provoke Mg^{2+} limitation in *S.* Typhimurium is intriguing because, in contrast with transition metals maintained at relatively low concentrations, Mg^{2+} is the most abundant divalent metal in living cells, present at concentrations of 0.5–2.0 mM across organs, cell types, and subcellular compartments [33,34].

The Connection among Mg^{2+} Homeostasis, *S.* Typhimurium Virulence, and Slc11a1

S. Typhimurium survival within macrophages is directly correlated with its ability to cause a lethal infection in mice [35]. This ability requires the virulence control system PhoP/PhoQ, a **two-component regulatory system** that consists of the sensor PhoQ and its cognate regulator PhoP [36] (Figure 2). That PhoP-activated genes are highly induced inside macrophages [37] and in laboratory media of low Mg^{2+} [38] had led to the proposal that Mg^{2+} concentration in the *Salmonella*-containing vacuole is low enough to activate the sensor PhoQ, and thus, promote *S.* Typhimurium virulence [39]. Although PhoQ does sense periplasmic Mg^{2+} [38,40], as discussed next, decreased Mg^{2+} concentration in the *Salmonella*-containing vacuole is not the signal activating PhoP/PhoQ inside macrophages.

Mg^{2+} concentration in the *Salmonella*-containing vacuole is ~1 mM at 1 h post bacterial internalization by macrophages [41], a concentration too high to fully activate the PhoP/PhoQ system in laboratory media [38]. (Unfortunately, Mg^{2+} concentration at 5 h post bacterial internalization, when the PhoP/PhoQ system is fully activated [35,42], is unknown).

Mildly acidic pH, as opposed to low Mg^{2+}, appears to be the signal activating the sensor PhoQ when *S.* Typhimurium is inside macrophages. Inhibiting acidification of the *Salmonella*-containing vacuole hinders both transcription of PhoP-activated genes [37] and *S.* Typhimurium survival inside macrophages [43]. Moreover, strains defective in PhoQ activation by mildly acidic pH, but retaining a wild-type response to changes in Mg^{2+} concentration, are attenuated for virulence in mice [44,45]. So, while PhoQ can be activated by multiple signals, including low Mg^{2+} [38] and antimicrobial peptides [46], PhoQ's ability to respond to mildly acidic pH is essential

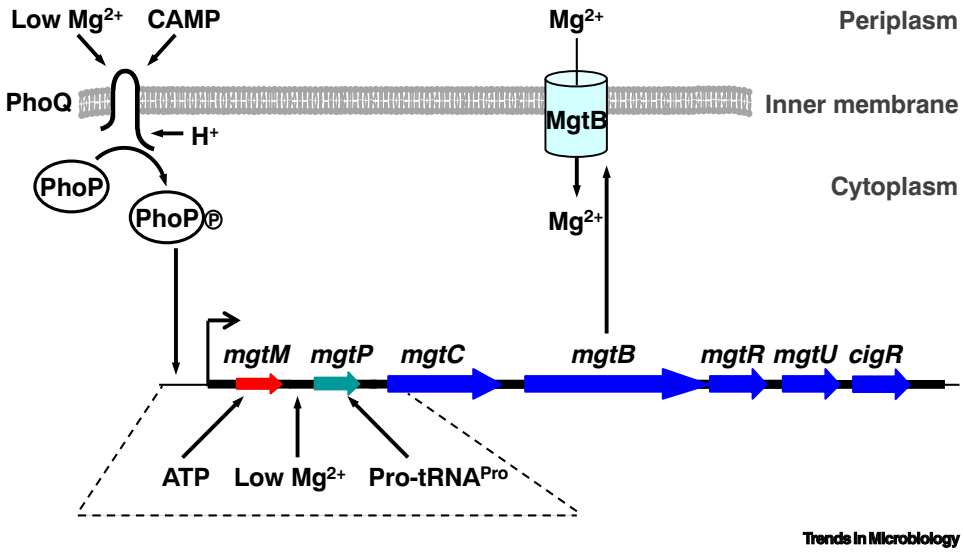

Figure 2. A Model for the Control of Expression of MgtB, a Mg^{2+} Transporter from *S.* Typhimurium Necessary for Bacterial Survival in Host Cells with a Functional Slc11a1 Protein. The sensor PhoQ responds to low Mg^{2+} or cationic antimicrobial peptides (CAMPs) in the periplasm or to mildly acidic pH in the bacterial cytoplasm by promoting phosphorylation of PhoP, a transcriptional activator of the *mgtCBRUcigR* promoter. The *mgtC* coding region (dark blue arrow) is preceded by an unusually long leader sequence harboring two short open reading frames (ORFs). Transcription elongation into the *mgtC* coding region is favored by high cytoplasmic ATP, high osmolarity, or low cytoplasmic Mg^{2+}. The unusually long intergenic region that separates the *mgtC* and *mgtB* coding regions may allow differential expression of the corresponding genes. The *mgtR* gene encodes a short peptide that promotes proteolysis of the MgtB and MgtC proteins by the protease FtsH. The *mgtU* gene specifies a short peptide that hinders MgtB proteolysis by FtsH. Black broken arrow denotes the PhoP-activated transcription start site. Broken lines indicate the DNA region fused to the fluorescent reporter used to explore how MgtB expression is affected by *Slc11a1* genotype [12].

for *S.* Typhimurium to activate the master regulator PhoP and cause disease in a mammalian host [44,45].

The PhoP/PhoQ system is similarly activated in *Slc11a1*$^{+}$/$^{+}$ and *Slc11a1*$^{-}$/$^{-}$ macrophages [23]. This may reflect that the *Salmonella*-containing vacuole acidifies to a similar extent in these macrophages [32]. Mutants in *phoP* or *phoQ* are attenuated for virulence in both *Slc11a1*$^{+}$/$^{+}$ and *Slc11a1*$^{-}$/$^{-}$ mice [44,45,47,48]. Moreover, the vast majority of proteins encoded by PhoP-activated genes are present in similar amounts in the spleens of *Slc11a1*$^{+}$/$^{+}$ and *Slc11a1*$^{-}$/$^{-}$ mice [12]. Therefore, a functional Slc11a1 protein is not necessary to activate the *S.* Typhimurium PhoP/PhoQ system in the murine spleen.

The PhoP-activated *mgtB* gene constitutes a notable exception to the behavior discussed above. *mgtB* expression and *S.* Typhimurium growth rate are inversely correlated in *Slc11a1*$^{+}$/$^{+}$ and *Slc11a1*$^{+}$/$^{-}$ but not *Slc11a1*$^{-}$/$^{-}$ mice [12]. In addition, the abundance of the MgtB protein was higher in spleens of *Slc11a1*$^{+}$/$^{+}$ than in those of *Slc11a1*$^{-}$/$^{-}$ mice [12]. Why, then, does the *Slc11a1* genotype impact expression of *mgtB* but not of other PhoP-activated genes?

mgtB, the second gene in the *mgtCmgtBmgtRmgtUcigR* operon [49–52], harbors an unusually long leader region that includes two short open reading frames (ORFs) (Figure 2). Translation of these ORFs determines whether transcription terminates within the **mRNA** leader or proceeds into the coding region [53]. Transcription elongation into the *mgtCmgtBmgtRmgtUcigR* coding region is favored by an increase in concentration of cytoplasmic adenosine triphosphate (ATP), which *S.* Typhimurium experiences in mildly acidic pH [54]; by a decrease in charged Pro-tRNA^{Pro} resulting from hyperosmotic stress [55]; and when the cytoplasmic Mg^{2+} concentration drops below a certain threshold [56]. In other words, although the PhoP protein directly activates transcription from the promoter of the *mgtCmgtBmgtRmgtUcigR* operon [57], *mgtB* expression also responds to specific cytoplasmic signals acting on the leader region of this transcript [53–56]. The latter regulation is critical for normal *S.* Typhimurium infection because an engineered strain lacking the leader region displayed decreased survival inside macrophages despite producing the MgtC and MgtB proteins in higher amounts than the wild-type strain [58].

When the effect of the *Slc11a1* genotype on *mgtB* expression was examined [12], investigators used a DNA fragment consisting of the PhoP-dependent promoter, leader region, and first 45 nt of the *mgtC* coding region fused to a promoterless *bfp* gene (Figure 2). Although this reporter reflects transcriptional control of the preceding gene *mgtC*, it fails to fully recapitulate *mgtB* expression. This is because the DNA fragment used does not include the 219 nt separating the *mgtC* stop codon from the *mgtB* start codon [49,50]. This unusually long intergenic region within the *mgtCmgtBmgtRmgtUcigR* operon includes the promoter for AmgR, an antisense RNA that downregulates the abundance of both MgtB and MgtC despite lacking complementarity to the *mgtB* portion of the transcript [50].

In addition to the mechanisms that regulate MgtB synthesis, two peptides dictate MgtB protein stability [52]. Encoded in the same operon as *mgtB,* MgtR promotes MgtB degradation by the protease FtsH [52], whereas MgtU protects MgtB from FtsH [52]. Like the *mgtB* null mutant, an *mgtU* null mutant is defective for replication in *Slc11a1*$^{+}$/$^{+}$ macrophages but behaves like wild-type *S.* Typhimurium or the *mgtR* mutant in *Slc11a1*$^{-}$/$^{-}$ macrophages [52]. Both the *mgtB* and *mgtU* mutants fail to reach a wild-type optical density when incubated in laboratory media of low Mg^{2+}, but the *mgtA* mutant displays a wild-type behavior [52]. These data suggest that the MgtB protein helps *S.* Typhimurium to overcome the low Mg^{2+} 'feeling' it experiences in *Slc11a1*$^{+}$/$^{+}$ macrophages.

In sum, *S.* Typhimurium's ability to grow in host tissues and cause disease in *Slc11a1*$^{+/+}$ mice requires the transport ability of the Mg^{2+} transporter MgtB [12]. The Slc11a1 protein creates an environment in the *S.* Typhimurium cytoplasm that furthers abundance of MgtB but not MgtA (or not to the same extent). These two proteins are differentially expressed even though the *mgtA* promoter is also transcriptionally activated by PhoP [57,59] and the *mgtA* mRNA includes a leader sequence that favors transcription of the *mgtA* coding region when *S.* Typhimurium experiences a decrease in charged Pro-tRNA^{Pro} resulting from hyperosmotic stress [60] or when the cytoplasmic Mg^{2+} concentration drops below a certain threshold [56]. While the *mgtA* gene is widespread within the family Enterobacteriaceae in pathogenic and nonpathogenic species [26], *mgtB* exhibits sporadic distribution and is found within the SPI-3 pathogenicity island of *S.* Typhimurium [29,61]. In addition, MgtR promotes MgtA degradation by FtsH [62], but MgtU neither binds to MgtA nor alters its stability [52]. The virulence behavior of the *S.* Typhimurium strain with a defective MgtB protein [12] suggests that acquisition of the *mgtB* gene was critical in the evolution of *S.* Typhimurium because it enabled survival in mammalian hosts with a wild-type *Slc11a1* gene.

Host Cells May Promote Virulence Gene Expression by Triggering Specific Stresses in the Cytoplasm of a Pathogen

It is increasingly clear that bacteria require sensors for cytoplasmic signals to respond to stresses inside mammalian cells. For example, the leader regions of some mRNAs detect changes in cytoplasmic components by modifying expression of associated coding regions. A leader region may detect a ligand directly in the case of riboswitches, sense the coupling/uncoupling of transcription and translation of a short open reading frame in the leader region, or interact with a small regulatory RNA(s) or RNA-binding protein whose expression responds to specific cytoplasmic signals [63,64].

As discussed above, the leader region of the *mgtCmgtBmgtmgtURcigR* transcript enables *S.* Typhimurium to respond to increases in bacterial ATP concentration resulting from acidification of the *Salmonella*-containing vacuole and to the availability of nutrients whose metabolism increases ATP amounts [54]. This leader also responds to a decrease in proline-charged tRNA^{Pro} triggered by hyperosmotic stress or a decrease in proline availability [55]. That proline is in low abundance inside the *Salmonella*-containing vacuole is supported by the finding that proline auxotrophs are defective for proliferation inside macrophages [55,65]. However, because the mouse *Slc11a1* genotype has a larger impact on the *Salmonella mgtB* mutant than the *mgtC* mutant, there must be specific cytoplasmic signals that differentially affect the abundance or activity of the MgtB and MgtC proteins.

In addition to the role that leader mRNAs play in signal sensing, certain protein sensors control bacterial virulence in response to cytoplasmic signals despite having sensing domains outside the cytoplasm. For example, mildly acidic pH, such as that inside a macrophage phagosome [37], activates the sensor PhoQ via its cytoplasmic domain [44]. *S*. Typhimurium mutants with variant PhoQ proteins defective in response to mildly acidic pH are attenuated for virulence despite retaining the ability to activate PhoQ by low Mg^{2+} and certain antimicrobial peptides via their extracytoplasmic domain [44]. In other words, the PhoQ protein responds to a signal in the bacterium's own cytoplasm generated by acidification in its surroundings.

Some bacterial responses to stress reflect downstream effects of the initial stress. For example, microbes trigger a phosphate-starvation response when protein synthesis decreases [66] because phosphate is not liberated from nucleotide triphosphates during low protein synthesis. Critically, this response takes place in abundant phosphate in the growth media. Therefore, a phosphate-starvation response does not necessarily mean that an organism is experiencing

phosphate starvation.

Concluding Remarks

In the emerging field of nutritional immunity it is critical to decipher how host factors restrict bacterial growth. How the Slc11A1 protein confers resistance to *S.* Typhimurium reflects the complexity of the bacterial response to nutritional limitation imposed by this protein in macrophages. On the one hand, there is a connection between the Slc11a1 host genotype and the behavior of *S.* Typhimurium mutants defective for growth in low Mg^{2+} media. On the other hand, the recent hypothesis of Slc11a1 restricting magnesium inside phagosomes by transporting Mg^{2+} out of the phagosome is challenged by the lack of direct experimental evidence, and the mechanism of Slc11A1-mediated resistance to bacterial infections remains unclear (Figure 1) (see Outstanding Questions). Critically, studies to understand Slc11a1 action bring to light the important idea that bacterial pathogens respond not only to changes in their surroundings but also to bacterial cytoplasmic chemicals, the abundance or availability of which may be modulated by host factors even when the abundance of the chemicals does not change in the bacterial surroundings. A deeper understanding of the molecular mechanisms of nutritional immunity can provide novel applications for human health.

Outstanding Questions

How does the macrophage protein Slc11A1 restrict pathogen growth inside a phagosome?

Does Slc11A1 activity change over time during infection?

Can the Slc11A1 protein transport magnesium across the phagosomal membrane?

What is the magnesium concentration in the *Salmonella*-containing vacuole at various times after bacterial internalization by macrophages?

What promotes the increase in abundance of the *Salmonella* MgtB protein in the spleens of *Slc11a1*$^{+/-}$ mice versus *Slc11a1*$^{-/-}$ mice?

References

1. Mookherjee, N. *et al.* (2020) Antimicrobial host defence peptides: functions and clinical potential. *Nat. Rev. Drug Discov.* 19, 311–332
2. Eisenreich, W. *et al.* (2013) Metabolic host responses to infection by intracellular bacterial pathogens. *Front. Cell. Infect. Microbiol.* 3, 24
3. Kissing, S. *et al.* (2018) Vacuolar ATPase in phago(lyso)some biology. *Int. J. Med. Microbiol.* 308, 58–67
4. Evans, S.S. *et al.* (2015) Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat. Rev. Immunol.* 15, 335–349
5. Lopez, C.A. and Skaar, E.P. (2018) The impact of dietary transition metals on host–bacterial interactions. *Cell Host Microbe* 23, 737–748
6. Ganz, T. (2018) Iron and infection. *Int. J. Hematol.* 107, 7–15
7. Sheldon, J.R. *et al.* (2016) Iron acquisition strategies of bacterial pathogens. *Microbiol. Spectr.* 4, 1–32
8. Palmer, L.D. and Skaar, E.P. (2016) Transition metals and virulence in bacteria. *Annu. Rev. Genet.* 50, 67–91
9. Sheldon, J.R. and Skaar, E.P. (2019) Metals as phagocyte antimicrobial effectors. *Curr. Opin. Immunol.* 60, 1–9
10. Wessling-Resnick, M. (2015) Nramp1 and other transporters involved in metal withholding during infection. *J. Biol. Chem.* 290, 18984–18990
11. Fabrega, A. and Vila, J. (2013) *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clin. Microbiol. Rev.* 26, 308–341
12. Cunrath, O. and Bumann, D. (2019) Host resistance factor SLC11A1 restricts *Salmonella* growth through magnesium deprivation. *Science* 366, 995–999
13. Canonne-Hergaux, F. *et al.* (1999) The Nramp1 protein and its role in resistance to infection and macrophage function. *Proc. Assoc. Am. Physicians* 111, 283–289
14. Amer, A.O. and Swanson, M.S. (2002) A phagosome of one's own: a microbial guide to life in the macrophage. *Curr. Opin. Microbiol.* 5, 56–61
15. Blackwell, J.M. *et al.* (2001) SLC11A1 (formerly NRAMP1) and disease resistance. *Cell. Microbiol.* 3, 773–784
16. Liu, K. *et al.* (2017) Association between SLC11A1 (NRAMP1) polymorphisms and susceptibility to tuberculosis in Chinese Holstein cattle. *Tuberculosis (Edinb)* 103
17. Caron, J. *et al.* (2002) Identification of genetic loci controlling bacterial clearance in experimental *Salmonella enteritidis* infection: an unexpected role of Nramp1 (Slc11a1) in the persistence of infection in mice. *Genes Immun.* 3, 196–204
18. Borriello, G. *et al.* (2006) Genetic resistance to *Brucella abortus* in the water buffalo (*Bubalus bubalis*). *Infect. Immun.* 74, 2115–2120
19. Goswami, T. *et al.* (2001) Natural-resistance-associated macrophage protein 1 is an H^+/bivalent cation antiporter. *Biochem. J.* 354, 511–519
20. Koskenkorva-Frank, T.S. *et al.* (2013) The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. *Free Radic. Biol. Med.* 65, 1174–1194
21. Gunshin, H. *et al.* (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388, 482–488
22. Yanatori, I. and Kishi, F. (2019) DMT1 and iron transport. *Free Radic. Biol. Med.* 133, 55–63
23. Zaharik, M.L. *et al.* (2002) Host–pathogen interactions: Host resistance factor Nramp1 up-regulates the expression of *Salmonella* pathogenicity island-2 virulence genes. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15705–15710
24. Grass, G. *et al.* (2005) The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. *J. Bacteriol.* 187, 1604–1611
25. Grass, G. *et al.* (2002) ZupT is a Zn(II) uptake system in *Escherichia coli*. *J. Bacteriol.* 184, 864–866
26. Groisman, E.A. *et al.* (2013) Bacterial Mg^{2+} homeostasis, transport, and virulence. *Annu. Rev. Genet.* 47, 625–646
27. Choi, E. *et al.* (2017) Elongation factor P restricts *Salmonella*'s growth by controlling translation of a Mg(2+) transporter gene during infection. *Sci. Rep.* 7, 42098
28. Alix, E. *et al.* (2008) Interplay between MgtC and PagC in *Salmonella enterica* serovar Typhimurium. *Microb. Pathog.* 45, 236–240
29. Blanc-Potard, A.B. and Groisman, E.A. (1997) The *Salmonella* selC locus contains a pathogenicity island mediating intramacrophage survival. *EMBO J.* 16, 5376–5385
30. Pontes, M.H. *et al.* (2015) *Salmonella* promotes virulence by repressing cellulose production. *Proc. Natl. Acad. Sci. U. S. A.* 112, 5183–5188
31. Shin, J.H. *et al.* (2014) Transport of magnesium by a bacterial Nramp-related gene. *PLoS Genet.* 10, e1004429
32. Cuellar-Mata, P. *et al.* (2002) Nramp1 modifies the fusion of *Salmonella typhimurium*-containing vacuoles with cellular endomembranes in macrophages. *J. Biol. Chem.* 277, 2258–2265

33. Romani, A.M. (2011) Cellular magnesium homeostasis. *Arch. Biochem. Biophys.* 512, 1–23
34. Romani, A.M. (2013) Magnesium homeostasis in mammalian cells. *Met. Ions Life Sci.* 12, 69–118
35. Fields, P.I. *et al.* (1986) Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. *Proc. Natl. Acad. Sci. U. S. A.* 83, 5189–5193
36. Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* 183, 1835–1842
37. Alpuche-Aranda, C.M. *et al.* (1992) *Salmonella typhimurium* activates virulence gene transcription within acidified macrophage phagosomes. *Proc. Natl. Acad. Sci. U. S. A.* 89, 10079–10083
38. Garcia Vescovi, E. *et al.* (1996) Mg^{2+} as an extracellular signal: environmental regulation of *Salmonella* virulence. *Cell* 84, 165–174
39. Groisman, E.A. (1998) The ins and outs of virulence gene expression: Mg^{2+} as a regulatory signal. *Bioessays* 20, 96–101
40. García Véscovi, E. *et al.* (1997) Characterization of the bacterial sensor protein PhoQ. *J. Biol. Chem.* 272, 1440–1443
41. Martin-Orozco, N. *et al.* (2006) Visualization of vacuolar acidification-induced transcription of genes of pathogens inside macrophages. *Mol. Biol. Cell* 17, 498–510
42. Choi, J. and Groisman, E.A. (2020) *Salmonella* expresses foreign genes during infection by degrading their silencer. *Proc. Natl. Acad. Sci. U. S. A.* 117, 8074–8082
43. Rathman, M. *et al.* (1996) Acidification of phagosomes containing *Salmonella typhimurium* in murine macrophages. *Infect. Immun.* 64, 2765–2773
44. Choi, J. and Groisman, E.A. (2016) Acidic pH sensing in the bacterial cytoplasm is required for *Salmonella* virulence. *Mol. Microbiol.* 101, 1024–1038
45. Choi, J. and Groisman, E.A. (2017) Activation of master virulence regulator PhoP in acidic pH requires the *Salmonella*-specific protein UgtL. *Sci. Signal.* 10, eaan6284
46. Bader, M.W. *et al.* (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* 122, 461–472
47. Groisman, E.A. *et al.* (1992) Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. *Proc. Natl. Acad. Sci. U. S. A.* 89, 11939–11943
48. Miller, S.I. *et al.* (1989) A two-component regulatory system (phoP phoQ) controls *Salmonella typhimurium* virulence. *Proc. Natl. Acad. Sci. U. S. A.* 86, 5054–5058
49. Snavely, M.D. *et al.* (1991) The mgtB Mg2+ transport locus of *Salmonella typhimurium* encodes a P-type ATPase. *J. Biol. Chem.* 266, 815–823
50. Lee, E.J. and Groisman, E.A. (2010) An antisense RNA that governs the expression kinetics of a multifunctional virulence gene. *Mol. Microbiol.* 76, 1020–1033
51. Yeom, J. *et al.* (2018) A protein that controls the onset of a *Salmonella* virulence program. *EMBO J.* 37, e96977
52. Yeom, J. *et al.* (2020) Small proteins regulate Salmonella survival inside macrophages by controlling degradation of a magnesium transporter. *Proc. Natl. Acad. Sci. U. S. A.* Published online August 4, 2020. http://doi.org/10.1073/pnas.2006116117
53. Lee, E.J. and Groisman, E.A. (2012) Tandem attenuators control expression of the *Salmonella* mgtCBR virulence operon. *Mol. Microbiol.* 86, 212–224
54. Lee, E.J. and Groisman, E.A. (2012) Control of a *Salmonella* virulence locus by an ATP-sensing leader messenger RNA. *Nature* 486, 271–275
55. Lee, E.J. *et al.* (2014) Control of a *Salmonella* virulence operon by proline-charged tRNA(Pro). *Proc. Natl. Acad. Sci. U. S. A.* 111, 3140–3145
56. Cromie, M.J. *et al.* (2006) An RNA sensor for intracellular Mg(2+). *Cell* 125, 71–84
57. Zwir, I. *et al.* (2012) The promoter architectural landscape of the *Salmonella* PhoP regulon. *Mol. Microbiol.* 84, 463–485
58. Park, M. *et al.* (2019) The mgtCBR mRNA leader secures growth of *Salmonella* in both host and non-host environments. *Front. Microbiol.* 10, 2831
59. Lejona, S. *et al.* (2003) Molecular characterization of the Mg^{2+}-responsive PhoP-PhoQ regulon in *Salmonella enterica*. *J. Bacteriol.* 185, 6287–6294
60. Park, S.-Y. *et al.* (2010) An mRNA leader that employs different mechanisms to sense disparate intracellular signals. *Cell* 142, 737–748
61. Blanc-Potard, A.-B. *et al.* (1999) The SPI-3 pathogenicity island of *Salmonella enterica*. *J. Bacteriol.* 181, 998–1004
62. Choi, E. *et al.* (2012) The MgtR regulatory peptide negatively controls expression of the MgtA Mg^{2+} transporter in *Salmonella enterica* serovar Typhimurium. *Biochem. Biophys. Res. Commun.* 417, 318–323
63. Henkin, T.M. (2008) Riboswitch RNAs: using RNA to sense cellular metabolism. *Genes Dev.* 22, 3383–3390
64. Henkin, T.M. and Yanofsky, C. (2002) Regulation by transcription attenuation in bacteria: how RNA provides instructions for transcription termination/antitermination decisions. *BioEssays* 24, 700–707
65. Popp, J. *et al.* (2015) Role of host cell-derived amino acids in nutrition of intracellular *Salmonella enterica*. *Infect. Immun.* 83, 4466–4475
66. Pontes, M.H. and Groisman, E.A. (2018) Protein synthesis controls phosphate homeostasis. *Genes Dev.* 32, 79–92
67. Demeure, C.E. *et al.* (2019) *Yersinia pestis* and plague: an updated view on evolution, virulence determinants, immune subversion, vaccination, and diagnostics. *Genes Immun.* 20, 357–370
68. Hammarlof, D.L. *et al.* (2018) Role of a single noncoding nucleotide in the evolution of an epidemic African clade of *Salmonella*. *Proc. Natl. Acad. Sci. U. S. A.* 115, E2614–E2623