7

Trace Metals in Host–Microbe Interactions

The Microbe Perspective

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Abstract

Metals play a central role in the outcome of host–pathogen interactions. Microbes must acquire metals for metabolic processes, with nearly a half of all enzymes requiring a metal cofactor for function, yet microbes can be poisoned by metals. The host innate immune defenses are thought to exploit these vulnerabilities to protect against invading pathogens, whereas microbes can respond by employing multiple strategies to maintain their metal homeostasis. An understanding of these microbial strategies combined with knowledge of the diverse metal challenges faced by different microbes in the various host niches could inform the development of much needed new approaches for combating infectious diseases. This chapter summarizes extensive discussions on the interplay of metal ions in host–microbe interactions, from the microbial perspective. The focus is on five key areas, highlighted as requiring a greater understanding: (a) how we define and determine metal availability, (b) the different levels and sources of metals available to microbes in different niches within the host, (c) the effect of the metal status of a pathogen, as derived from its prior environment, on its ability to establish an infection or the severity of disease, (d) the interplay between metals and the microbiota, and (e) how metal restriction and metal oversupply can kill or inhibit the growth of microbes. This chapter provides an overview of current understanding in these areas and raises a number of important open questions in need of future research.

Introduction

Our discussions addressed the relationship between the metal status of different host niches and the capabilities of different microbes (pathogens and commensals) within these niches to compete for metals and avoid metal poisoning in determining disease outcomes. The best understood microbial systems belong to the bacteria and fungi; hence these organisms formed the basis of the discussions. Four background papers to this Forum informed and contributed greatly to our discussions: Lemire et al. (Chapter 2), Bachmann and Weiser (Chapter 3), Loutet et al. (Chapter 4), and Imlay (Chapter 5).

Metal Availability

How do we define and determine metal availability in different environments, both within and outside microbial cells? In approaching this question, we focused primarily on intracellular metal availability and how metal-requiring proteins acquire their correct metal cofactors (Figure 7.1).

Metal Availability outside Microbial Cells

Hosts are suspected of manipulating metal availability in ways that stymie or support the growth of pathogenic and commensal microbes. This concept is difficult to approach and evaluate, because metal availability remains poorly defined and understood. What we consider as bioavailable metal to a pathogen in the host environment may, to some extent, differ depending on the particular pathogen and the array of metal uptake systems and receptors that they possess and express; microbes frequently employ multiple uptake systems, particularly for iron (Fe), to meet their metabolic needs. In addition, the bioavailability of a particular metal within a specific niche is dependent not only on the total metal content, but also on other factors: metal speciation (including oxidation state, mineralization, and the presence of metal-binding ligands), pH, and oxygen levels. The ability of a cell to acquire a given metal may also depend on the concentration of competing metals for expressed transport systems. In terms of trying to address the metal requirements of microbes, there are difficulties in carrying out growth experiments in metal-restricted conditions. The use of chelators to restrict a single metal in microbial growth experiments can be problematic as few chelators are available that are highly specific for a particular metal, and for microbial growth experiments in complex media, alternative practical options are not yet available. A direct assay of the availability of a given metal is not easily accomplished and may be better defined by analyzing the rate at which a given organism is able to accumulate the metal.

Microbial metal-responsive transcription factors act to integrate the expression of cellular metal uptake, export, and sequestration systems with both their

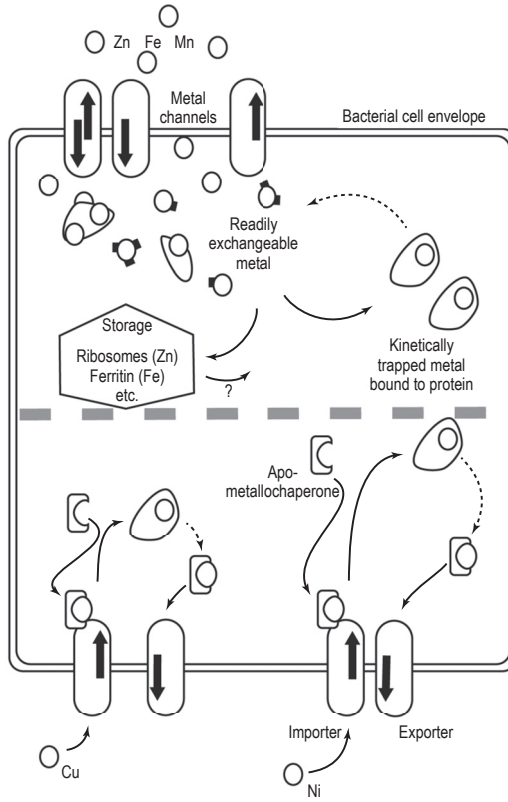

Figure 7.1 A model for the control of metal availability inside bacteria. Metal channels function to import and export various nutritional metals (circles). These include outer membrane/cell-surface receptors, inner membrane transporters, and transporters that span the entire cell envelope. A readily exchangeable pool of metals (including zinc, iron, and manganese) is thought to exist that is bound to proteins, small metabolites, and membrane surfaces. These metals can be kinetically trapped when bound to some proteins (dashed lines). Metals can also be sequestered for storage (e.g., zinc and iron are stored in ribosomes and ferritin, respectively), which may contribute to the metal buffering capacity of the cell. The metallation status of Zn, Fe, and Mn proteins is thought to be determined by the thermodynamic equilibrium with the readily exchangeable metal pool. For copper and nickel, protein metallochaperones may circumvent thermodynamic competition by delivering cognate metals from importers to their designated proteins (both in the cytoplasm and cell envelope). Excess metal or metal released from protein turnover may also be transported by metallochaperones for efflux.

metabolic needs and environmental metal availability. Metal-sequestering proteins can store metals and allow for their controlled release in cells, whereas transmembrane metal transporters control metal ion uptake into and efflux out of microbial cells. The lack of passive permeation of metal ions across cell membranes means that the active uptake and efflux of metal ions by the transmembrane transporters ultimately determines the intracellular metal levels.

Metal Availability inside Microbial Cells

There is still uncertainty as to how proteins acquire the correct metals. Although we currently have tools (e.g., inductively coupled plasma mass spectrometry) that can easily measure the total metal content in microbes (see Matusch et al., this volume), it remains unclear as to what form this metal is in and to what extent it is accessible to metal-requiring proteins. The field has not yet even reached consensus on the speciation of metals in cells and how to define the metal available for binding to high-affinity protein sites in cells.

Another consideration is that metals tend to associate to metal-binding sites of proteins according to the Irving-Williams series. Hence the relative availability of different metals in a cell is likely to determine which metal species associate with a given protein. For example, the concentration and speciation of the Zn and Fe pools within a cell may be controlled to favor Fe binding to an Fe-requiring protein, even though the affinity of the protein for zinc may be higher than that for iron. Alternatively, selection could occur at a kinetic level using a specific metal shuttle (or metallochaperone), thus overcoming thermodynamic constraints. The extent of the involvement of metal shuttles in assisting proteins in acquiring their correct metal cofactors, however, remains a subject of debate.

What Is the Nature of the Pool of Exchangeable Metal inside Cells?

In our discussion of the possible nature of the pool of cellular metals available to bind to proteins, it was noted that within cells, metal ions are bound by both high-affinity ligands (such as metalloproteins) and low-affinity ligands (including small molecules and nonspecific binding to macromolecules). However, the field has not yet agreed upon the best way to describe the cellular concentration of metal ions "available" to bind to high-affinity ligands within proteins. Semantically, we dislike the term "free" metal, as it connotes metal bound to nothing but water (fully hydrated metal), which is unlikely to exist within cells. There was, however, general agreement that "available" metals are likely bound to metabolites and adventitious biomolecule surfaces. These metals are likely to be labile: through ligand-exchange reactions, they are accessible to the active metal-binding sites of enzymes. The term "readily exchangeable" metal was put forward as a term to reflect that the ability of metals to populate a binding site is modulated by competing ligands within the cell. Thus, the exchangeable metal pool is buffered by rapid equilibration with other ligands. The extent to which a particular metal is readily exchangeable may differ for different metals. Metals for which we have known metallochaperones (copper [Cu] and nickel [Ni]) should be considered as the least readily exchangeable, whereas manganese (Mn), zinc (Zn), and iron may be considered the most readily exchangeable.

The question then arises: Which molecules are the primary ligands for the pool of exchangeable metal? This is important for modeling metal availability in cells. It is not known if cells modulate the metal-binding capacity of this ligand pool, by increasing or decreasing the concentration of a predominant liganding metabolite, to control the amount or identities of the metals that are available to enzymes. It is likely that the ligands include free amino acids (cysteine, histidine, glutamate) and glutathione; however, they could also include functional groups on the surfaces of membranes, nucleic acids, and proteins. A further consideration is the role and buffering capacity of dedicated metal stores (such as bacterial ferritins) or, for eukaryotic fungal pathogens, vacuoles that have general metal storage capacity or specialized functions (e.g., "zincosomes"; Devirgiliis et al. 2004).

In addition to not knowing the major components of the weak ligand pool in cells, we do not know the size of this weak ligand pool. The kinetics of Zn dissociation from some proteins is faster than would be predicted from simple dissociation rate constants, suggesting that some small molecules facilitate the extraction of metals. It probably follows that these ligands speed the flow of metals between low- and high-affinity binding sites, allowing thermodynamic equilibration to be more rapidly attained. In principle, the size and nature of the weak ligand pool could influence how quickly and specifically various metals populate authentic binding sites in enzymes.

In summary, the nature of the ligands involved in such an important feature of metal homeostasis remains largely unknown. In addition, the importance of this pool of metal ions in allowing pathogens to survive under low metal conditions is still not understood. There are some methods that could be used to evaluate the exchangeable metal pools. Currently, small-molecule fluorescent probes are useful for monitoring calcium and Cu fluxes in cells (Dodani et al. 2011; Davidson and Duchen 2012); a similar approach could potentially be used to monitor other metals in cells, although the probes would need to be in thermodynamic equilibrium (this is often not true for regulators, which may kinetically trap metals). An estimate of the readily exchangeable pool of zinc in *Escherichia coli* has been made by the expression of engineered fluorescent carbonic anhydrase proteins with defined Zn affinity (Wang et al. 2012a). Such an approach might be generalized to other metal pools (e.g., iron and manganese) as well as other microbial species. Protein-based metal sensors are also useful as they can be tagged, but again, their rate of metal exchange must be considered. Another approach would be to use X-ray fluorescence microprobe (XRM) imaging (Fahrni 2007). However, this technique measures only the metal identity and amount; it does not provide information about the metal ligand. For eukaryotic cells, fluorescent ligands and XRM imaging can, however, provide information about the metal compartmentalization in organelles. For a summary of techniques available for the analysis of metals in biological samples, see Matusch et al. (this volume).

What Are the Native Metals Bound to Enzymes, and How Does One Figure This Out?

In the published literature, there is substantial uncertainty, and even mis-identification, of the metals that populate specific enzymes. This arises from metal exchange *in vitro*. Mis-identification can lead to a failure to recognize the dependence of certain enzymes and pathways upon the availability of a certain metal. For example, native metal ions may be released from proteins upon cell lysis, followed by binding of an incorrect metal ion. This mechanism is particularly problematic for iron and manganese, which are often replaced by zinc upon cell lysis. Retention of redox sensitive metals, such as ferrous iron, by proteins can frequently be improved by cell lysis under anoxic conditions. Ideally, we would like to identify the metals bound to proteins in live cells. A potential method of doing this would be to use mass spectrometry to identify protein metalloforms *in vivo* (So et al. 2013). However, currently there is a problem of sensitivity: it is not possible to get resolution at the subcellular level, and it is difficult to obtain enough material to measure single metal-protein complexes.

Do Metalloforms of an Enzyme Differ Depending on Circumstance?

Some proteins actually have multiple metalloforms *in vivo*. These metalloforms are part of a response to the availability of the metals from a habitat and cellular stores. Less clear is the frequency with which a secondary metalloform actually supplies sufficient enzyme activity *in vivo*. Examples of successful Mn substitution for iron exist (Anjem and Imlay 2012); however, in other cases, full activity is met instead by the expression of a Mn-specific isozyme (Compan and Touati 1993; Cotruvo and Stubbe 2011). Similarly, zinc is capable of replacing nonredox ferrous iron, at least in some cases (and vice versa). For metalloproteins harboring structural metal sites, isozyme substitution can release a metal for general use. An example is Zn release from the substitution of ribosomal proteins (Nanamiya et al. 2004). Many metalloproteins are, however, highly metal specific; the loss of availability of the cognate metal results in loss of activity to the cell. For example, proteins with iron-sulfur (Fe-S) centers and heme are Fe specific, and a lack of iron requires adaption of metabolism. In addition to liberating iron by metal substitution, as described above, microbes may employ an Fe-sparing response that reduces the expression of pathways requiring heme or Fe-S proteins. Example pathways that are repressed are the TCA cycle and sections of the respiratory chain (Masse et al. 2005).

In addition to simply restricting microbial access to essential metals, a hostile host may be able to poison microbes by forcing protein mis-metallation. The microbial metal homeostasis adaptation mechanisms have the potential to mute the possible effect. As such, knowledge of these adaption mechanisms

may point to an understanding of how a combination of host metal sequestering and supply systems can be used to control microbes.

Do Thermodynamics of Binding Sites Control the Metallation Status?

The metallation status of many Zn and Fe proteins is likely determined by thermodynamic equilibration with the readily exchangeable metal pools in cells. However, the metal status of some proteins likely reflects the kinetic trapping of a metal rather than its thermodynamic equilibration. The trapping of zinc in a Mn periplasmic binding protein provides one example (McDevitt et al. 2011). These differences in the exchange rates of metals may lead to differences in de-metallation behavior. For example, the loss of a metal from a habitat may lead to de-metallation of thermodynamically determined proteins (e.g., some simple Fe and Zn enzymes) by exchange with the cellular ligand pool, but not proteins with a kinetically trapped metal ion (e.g., superoxide dismutase, SOD). This also raises the prospect that the spectrum of under-metallated proteins would differ, depending on whether a protein was de-metallated during metal starvation (SOD would retain its metal) or synthesized during starvation (SOD would not have acquired its metal). It is possible that a variation in Zn affinities of native Zn proteins could constitute a prioritization system under Zn starvation that has been evolutionarily selected. In addition, differences may be seen in the Zn affinities among proteins, if a protein is able to use an alternative metal during Zn deficiency. It is not clear if all proteins are fully metallated under metal-sufficient conditions; the retention of apoenzymes may contribute to the buffering capacity of a cell and may allow the cell to respond to variations in metal content and identity.

What Is the Involvement of Metal Shuttles (Metal Ligands and Proteins)?

Protein metallochaperones direct metals to preferred client proteins and circumvent thermodynamic competition. At present, data indicate that nickel and copper are often delivered this way; characterized examples include Cu metallochaperones for Cu, Zn-SOD and caa3-type cytochrome oxidase, and Ni metallochaperones for hydrogenase and urease (Kaluarachchi et al. 2010; Robinson and Winge 2010; Osman et al. 2013). It was noted, though, that *Caenorhabditis elegans* delivers copper to Cu/Zn-SOD without using a metallochaperone (Jensen and Culotta 2005). There are no compelling data to say that iron, zinc, and manganese are handled by metallochaperones, and it appears likely that either there are no protein-based metallochaperones or that redundant systems exist. The role of metallochaperones in protein metallation is an important question, because it may limit the extent to which cellular metal imbalances can lead to protein mis-metallation. Where metal selectivity is dependent on the relative metal concentrations, metal imbalances will lead to mis-metallation. Nickel and copper are delivered to a relative handful of

proteins, and so the evolution of a metallochaperone system that includes a receiver domain on client proteins may have been easier. Metallochaperones for iron, zinc, and manganese might also be disfavored if cells have evolved to substitute one metal for another under conditions in which the primary metal is unavailable; a strict metallochaperone system might not allow that latitude.

Summary

Not all determinants of metal selectivity in cells are yet known. These determinants vary for each metal ion and may change depending on the nutritional environment and pathogen. Currently there are two extreme paradigms for metal selectivity (Figure 7.1). The first stipulates that a metal ion is transported into the cell and immediately transferred to a metallochaperone protein, which delivers the metal to a specific metalloprotein. Most likely, copper and nickel follow this type of pathway in many, but not all, organisms. For metal ions that activate a small number of proteins, these metallochaperone-dependent pathways likely occur. In contrast, other metal ions, such as zinc and iron, do not frequently have identified, dedicated metallochaperone proteins. The metal selectivity in cells for these ions is proposed to depend primarily on the metal affinity of the metalloprotein and the cellular "readily exchangeable" metal concentration, which is determined by both the total metal content and the buffering capacity of the cell. Metal-responsive regulators likely sense this metal pool to modulate the expression of metal homeostatic systems according to cellular needs. These systems control the readily exchangeable metal concentration. In many cases, neither the metal affinity of the proteins nor the readily exchangeable metal concentration in a cell is known, which makes estimation of the native metal ion difficult. Furthermore, the readily exchangeable metal ion concentration in the cell likely varies with metal nutrition and, possibly, cellular metabolites that function as metal buffers. We do not know whether microbes actively manage metal pool speciation to optimize the availability of the appropriate metal for macromolecular incorporation. Under changing metal loads, the metal(s) bound to metalloproteins may also vary. In some cases, the enzymes retain activity with the substituted metal ion, suggesting that this mechanism may potentially be used to address metal scarcity and metal overload. However, the importance of this mechanism during infections is not known and may vary for different microbes.

Different Levels and Sources of Metals in Different Microbial Niches within the Host

Here we focused on the different potential metal sources available to microbes at distinct locations within the host. At the heart of this discussion was the opinion that the metal sources available to a particular pathogen will vary

depending on a variety of factors, including its particular niche within the host, its route of entry, array of metal uptake and storage systems, and strategies for survival within the host.

Diversity of Ecological Niches for Microbes in the Vertebrate Host

The vertebrate host represents a diverse array of ecological niches for colonizing microbes that vary, for example, in the level of available oxygen, nutrients, metals, metal-binding ligands, and pH. In regard to the availability of metal ions for microbes in these different sites, a primary consideration is the degree to which the availability is due to external sources versus a dependence on host-derived metal ions. For example, microbes that inhabit the gastrointestinal tract may predominantly acquire metal ions provided from the intake of food, whereas microbes that inhabit the skin or upper respiratory tract are primarily dependent on host-derived metal ions. Within the gastrointestinal tract there will also be substantial differences in the nature of the available metal ions in the food, due to the various stages of processing: exposure to acid pH in the stomach, exposure to proteases, and exposure to microbes and microbial products in the lower gut. Metal ions in the form of salts present in food (included as additives) or provided in the form of supplements are modified by the acidic environment of the stomach and influence the subsequent utilization by microbes and uptake by the host.

Although the major differences between environments in different host systems and subdivisions are fairly obvious, the degree to which various "microniches" might exist or be created by specific host–pathogen interactions and influence metal availability is less known. Different microbes that can colonize the same region have a different repertoire of metal ion acquisition strategies, and it is not readily apparent whether this is a reflection of inhabiting a distinct niche, accessing different compartments, or a result of microbial interactions. For instance, it is unclear why transferrin receptors are present on *Neisseria meningitidis* and a subset of the "commensal" *Neisseria* species, whereas other *Neisseria* possess a distinct and different repertoire of Fe acquisition strategies (Marri et al. 2010).

Sources and Forms of Metal Ions in Different Niches

For niches in which the host is the primary source of metal ions, there is a paucity of information regarding the ultimate source or form of available metal ions. For instance, on the mucosal surface of the upper respiratory tract there are no well-described mechanisms for provision of metal ions to the microbes that inhabit that environment. It is generally presumed that glandular cells secrete lactoferrin onto the mucosal surface, but it is normally in the apo form; thus it will not provide a source of iron but will influence the availability and form of any iron that is present. Similarly, the secretion of lipocalin 2 (siderocalin) will

capture Fe-siderophore complexes on the mucosal surface but may not represent provision of metal ions to that environment. The secretions from glandular cells contain mucins, which are rich in cysteines, and other constituents that would be capable of complexing metal ions; however, the metal ion content of the secretions has not yet been determined.

The presence of surface receptors on microbes for Fe-containing host proteins or protein complexes and the dependence of some microbial species on these receptors for survival in the host suggest that either these host proteins (transferrin, hemoglobin, hemoglobin-haptoglobin, heme-hemopexin, ferritin) are somehow transported to the mucosal surface, or that the microbes which possess these receptors are capable of accessing or residing in the submucosal space to access these Fe sources. Episodic bleeding or damage and replacement of mucosal epithelial cells are possible sources for these proteins, but whether this would maintain an adequate supply of metal ions is unclear. The recent observation that *Helicobacter pylori* modulates the activity of stomach epithelial cells to bring Fe-loaded transferrin from the submucosal surface to the epithelial surface (Tan et al. 2011) is a demonstration of how microbes could play an active role in accessing host-derived metal ions and influence the form of available metal. Clearly this is an area that requires further investigation, to determine the degree to which this type of phenomenon may impact on the availability and the form of metal ions in various niches in the host.

In the gastrointestinal tract, the form and availability of metal ions will depend substantially on dietary intake (see Ackland et al., this volume). For instance, diets rich in meat will provide a ready source of heme iron that would be a preferred source of iron for both microbes and the host alike. However, the composition of the diet can also reduce the availability of metal ions by altering the form; for example, phytates in grain-rich diets are known to inhibit the uptake of Zn ions (Lim et al. 2013). The form and availability of metal ions can be further modulated through the interplay between microbes and their host. For example, the release of the enterobactin class of siderophores by microbes to complex available ferric iron derived from the lumen of the gastrointestinal tract can be countered by the secretion of host lipocalin 2 (Flo et al. 2004).

The Intracellular Environment

The intracellular environment of host cells has a different spectrum of metal sources for pathogens to acquire (Figure 7.2). Furthermore, metal levels will vary between the host cell cytoplasm and the various intracellular compartments. Metal availability to intracellular microbes will depend on the lifestyle of the pathogen (e.g., extra- or intra-phagosomal) and their various strategies to propagate within the intracellular niches; for example, an ability to manipulate host cell Fe homeostasis to increase Fe availability (Cassat and Skaar 2013). The majority of the host heme and hemoproteins are intracellular. In addition, ferrous iron predominates in the cytoplasm. Still the host cell cytoplasm is

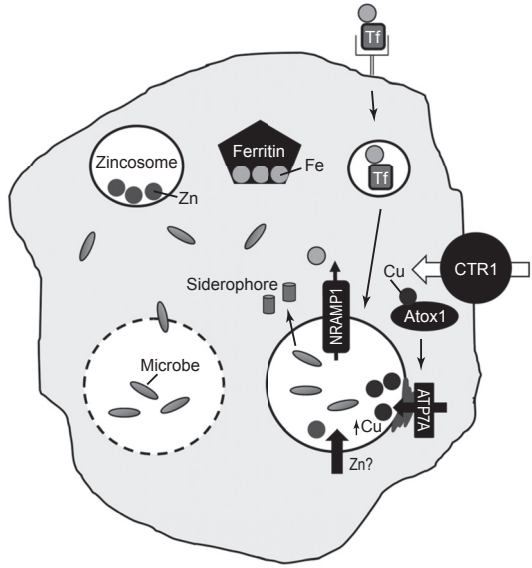

Figure 7.2 Overview of metal levels and sources for intracellular pathogens. Iron-loaded transferrin (Tf) enters cells via receptor-mediated endocytosis. Iron is stored in the cytoplasm bound to transferrin, and zinc can be stored in specialized vesicles called zincosomes. NRAMP1 localizes to the phagosmal membrane of infected macrophages, where it is thought to pump metals (iron, zinc, and manganese) out of the phagosome to starve microbes of these metals. In contrast, Cu influx into macrophage phagosomes is associated with increased microbicidal activity and a requirement for microbial Cu-resistance systems. Activation of macrophages causes increased levels of the CTR1 Cu importer and localization of the ATP7A Cu pump from the *trans*-Golgi network to phagosomal membranes, with ATOX1 acting as a Cu metallochaperone to shuttle copper to ATP7A. Zinc may also be pumped into phagosomes as an antimicrobial defense against certain pathogens. Within phagosomes, some microbes (e.g., *Mycobacterium tuberculosis*) can have access to recycling endosomes and thus gain access to iron from transferrin. Intracellular pathogens can also produce siderophores which, for *M. tuberculosis*, can diffuse out of phagosomes and access cytoplasmic sources of iron. Other pathogens (e.g., *Listeria monocytogenes*) can gain access to these iron sources by escaping from the phagosome and entering the host cell cytoplasm. Some pathogens may also manipulate host cell metal homeostasis to their advantage.

sensed as an Fe-limited environment by microbial cells, because inorganic iron is bound in ferritin by other proteins and small molecular weight compounds. The status of iron and other metals is different within phagosomes. NRAMP1 (SLCIIA1) is thought to efflux iron, zinc, and manganese from phagosomes, making them more metal deficient (Cellier et al. 2007). Controversy remains, however, about the direction of transport of these metals. It has been suggested that the pH of the vacuolar compartment may determine the direction of transport of this proton-driven pump (Jabado et al. 2000). Furthermore, the

phagosomal pH may be critical for the speciation of the metal ions in this compartment.

Whereas metal limitation is a primary strategy, it appears that the influx of some metals into microbe-containing phagosomes may be an important component of macrophage antimicrobial defenses. Certainly for copper, several different lines of research have shown that pathogens are exposed to high Cu levels in macrophage phagosomes, and Cu detoxification systems are required for their survival within this compartment (White et al. 2009; Osman et al. 2010; Ward et al. 2010; Achard et al. 2012; Ding et al. 2013; Shi et al. 2014). It has also been suggested that zinc may be pumped into phagosomes as an antimicrobial defense against *M. tuberculosis* (Botella et al. 2011). Although elevated Zn levels are detected within mycobacterial-containing phagosomes, it is possible that Zn influx allows acidification of the vacuolar compartment which leads to killing, rather than there being a direct effect of Zn toxicity on the microbe.

Microbial Strategies for Obtaining Metals

Microbes possess numerous strategies for obtaining metals, and these are discussed in detail in the background papers (see Lemire et al., Bachmann and Weiser, Loutet et al., and Imlay, all this volume). Notably, the production of siderophores is a widely used strategy for obtaining iron, and some pathogens may share the types of siderophore produced. Some organisms produce multiple siderophores with differing roles. Notably, *Klebsiella pneumoniae* produces four types of siderophores, and the phenotypes for the siderophore mutants are distinct with respect to their ability to cause respiratory tract infections and remove iron from transferrin (Bachman et al. 2011, 2012). The production and/or use of multiple siderophores (endogenous or produced by other microbes) can also provide pathogens with a strategy to circumvent the host's attempt to prevent microbial Fe uptake. The production of lipocalin 2 by the host sequesters some siderophores, thereby preventing Fe acquisition by these siderophores. However, while lipocalin 2 binds to enterobactin and other phenolate-type siderophores, some bacteria, such as *Salmonella enterica* and *K. pneumoniae*, glucosylate enterobactin to produce salmochelin that is not bound by lipocalin 2 (Raffatellu et al. 2009). In addition, some bacteria can produce siderophores, such as pyochelin and yersiniabactin, that are not recognized by lipocalin 2 (Bachman et al. 2011; Cornelis and Dingemans 2013).

Siderophores are a particularly versatile mechanism for acquiring iron in a variety of different niches. Accordingly, they are prevalent in microbes that reside in the gastrointestinal tract and that are transmitted by the fecal-oral route. Although siderophores are a widespread mechanism for obtaining iron, there are multiple additional strategies. These include transferrin, lactoferrin, ferritin, and hemoglobin receptors as well as heme, ferrous, and ferric Fe transporters. Notably, most of the receptors for the host Fe-binding proteins or protein

complexes (transferrin, lactoferrin, hemoglobin-haptoglobin, and heme-hemopexin) are host specific and have only been found in microbes that inhabit the upper respiratory tract or genitourinary tract. Pathogens which propagate within a specific niche in the host tend to have and use the transport systems that are effective within that niche. *Bordetella* use siderophore and heme uptake systems during different stages of upper respiratory tract infections, and there appears to be sequential expression of these systems based on environmental signals (Brickman and Armstrong 2009). In contrast, a study with *Yersinia enterocolitica*, which monitored transcriptional expression of the yersiniabactin system and a heme transporter, found different levels of expression in different organs (blood, liver, spleen); however, both systems seemed to be responding coordinately in these niches (Jacobi et al. 2001). In *Y. pestis,* yersiniabactin is essential in the initial lymphatic spread and/or growth in the regional lymph node following a flea bite. However, after gaining access to the bloodstream, this siderophore is irrelevant to subsequent Fe acquisition and growth. In pneumonic infections, a yersiniabactin mutant is attenuated but still lethal at higher infectious doses. However, the disease shifts from growth in the lungs and pneumonic disease to a septicemic disease (Fetherston et al. 2010; Lee-Lewis and Anderson 2010). The Yfe/Sit and Feo ferrous transporters are not critical during pneumonic infection but are important during bubonic plague. It is not clear whether two additional *Y. pestis* ferrous Fe transporters play a role in the lungs, or whether ferrous Fe sources are irrelevant in the lungs (Fetherston et al. 2012). *In vitro* studies have not shown a role for bacterial heme transporters for intracellular growth in host cells; instead some bacteria use a combination of siderophore and ferrous Fe uptake systems for intracellular Fe acquisition (Runyen-Janecky et al. 2003; Perry et al. 2007).

In contrast to Fe/heme uptake systems, identified bacterial Zn and Mn transporters show much less diversity. They primarily consist of ABC-type transporters, such as ZnuABC for zinc and SitABCD/YfeABCD for manganese, as well as the NRAMP-family Mn transporter, MntH (Hood and Skaar 2012). A number of studies with different pathogens have shown loss of virulence when the Zn or Mn transporters are disrupted, supporting the notion that the host is also limiting for these metals (Kehl-Fie and Skaar 2010). Mutation of the two known Mn transporters (Yfe and MntH) in *Y. pestis* causes a loss of virulence in the mouse model of bubonic plague but not in the pneumonic plague model (Perry et al. 2012), again suggesting that niches have different metal availabilities. The neutrophil-derived protein calprotectin (as well as other S100 family proteins), which chelates manganese and zinc, is one mechanism for withdrawing these metals from the sites of extracellular infections. It is likely that other Mn- and Zn-sequestering mechanisms also exist. Whereas most metal-chelating compounds may primarily act to sequester metals away from microbes, they also have the potential to serve as a source of metals for microbes capable of accessing the metals from them. As such, they may provide some pathogens a competitive advantage; for example. *S. enterica* serovar Typhimurium is able

to utilize calprotectin-bound zinc which enables it to outcompete the gut microbiota to colonize the gut and invade (Liu et al. 2012). As a similar strategy, the pathogenic fungus *Candida albicans* secretes its own "zincophore" protein to bind and obtain zinc from the environment (Citiulo et al. 2012).

The question arises as to whether pathogens differ from commensals in having a greater capacity to compete for metals by possessing a greater array of metal uptake pathways. We concluded that there is no evidence to support this; more detailed studies need to be undertaken, including cataloguing the array of systems used by pathogens and commensals in different niches. However, assigning organisms as commensals or pathogens can also be complex. Importantly, when examining metal uptake systems in microbes, multiorganism systems should be considered, as microbes rarely exist in isolation within the host. Furthermore, the extent to which variations in the total metal content of different organ systems (e.g., between the genitourinary tract, upper respiratory tract, and gastrointestinal tract) is influenced by the composition of the microbial communities at these locations and their array of metal acquisition strategies should also be considered.

Summary

Whereas we accept that there are likely differences in the available sources of metals to microbes in different locations of the host, beyond the gut we largely do not know what these are: neither the metal levels nor the variety of metal-binding ligands. Direct measurements of metal availability are challenging due to limits of detection as well as difficulties in sampling the various niches, particularly for studies with humans. The use of model hosts (mammalian as well as others, such as zebrafish and *C. elegans*) offer advantages as they permit controlled studies into the effects of diet and other factors on metal sources. A potential strategy to identify and compare the various metal sources within the different host niches would be to catalog (e.g., using metagenomics) the different metal uptake systems in the microbes that naturally inhabit them, rather than focusing on pathogens that are often only in transit through these environments. Knowledge of the substrates for identified metal uptake systems may yield clues as to what is being accessed. (Meta-)transcriptomic approaches can be used to monitor the temporal (and spatial) expression of metal import and export systems; however, the question arises as to whether their expression is being driven by microbial needs or their exposure to host factors in a particular niche. It would be useful to combine knowledge of expression studies of host metal transporters with those of microbial metal-handling systems, which could also be combined with measurements of total metal levels in tissues (e.g., by laser ablation ICP-MS). It also may be useful to compare germ-free versus nongerm-free animals to gain an idea of the effect of resident microbes on metal availability.

Effect of the Metal Status of a Pathogen, as Derived from its Prior Environment, on its Ability to Establish an Infection or the Severity of Disease

Does the previous environment of a pathogenic microbe (e.g., is it replete or deplete for certain metals) have an effect on its transmission to a host and disease outcome. In other words, can microbes be primed for entry into a host by their metal status?

Does Preinfection Metal Exposure Impact on Infectivity and Disease Outcome?

Metal availability, whether it is in the essential or toxic range, has a strong impact on the transcriptome and the metabolism of pathogenic microbes. As microbial pathogens change from one environment to the other during dissemination, metal status is retained, at least in the short term, thereby providing a rheostatic physiological parameter of potential importance in infection. As many virulence factors and large-scale metabolic pathways are either dependent on metals for their catalytic activities and/or are under physiological control of metal-sensing systems, microbial pathogens may exhibit very different phenotypes when confronting the host, depending on previous exposure to different metal concentrations. Furthermore, the impact of this prior metal exposure may vary across the different organ systems that impose different metal stresses. Alternatively, the host's nutritional immune strategies might be, at least initially, circumvented by a sufficiently large pool of metals, or metal detoxification systems, in the microbe. This effect is further aggravated by the presence of metal storage systems, such as bacterial ferritins or fungal vacuoles. After a period of metal surplus, starvation can likely be buffered during the early stages of an infection. This seems especially true for opportunistic commensal and environmental pathogens, as they are naturally exposed to metal species and concentrations that are very different from the host. With a sufficiently large pool of metals, growth may continue for several generations in the absence of external sources due to metal partitioning to daughter cells. These few to tens of divisions may be decisive in establishing an infection, or at least in allowing survival until new sources of metals are accessed. Opportunistic commensals may thus serve as a good, biologically relevant model for studying the effect of preinfection metal levels.

An example of an environmentally derived human pathogen is the fungus *Cryptoccocus neoformans*, which in the environment can exist on the leaves of trees and grasses or in bird guano, with low- or high-metal availability, respectively (Heitman et al. 2011). In the environment, the yeast or spore form is aerosolized and rendered available for entry into the lung, the initial site of infection in humans or other mammals. Successful colonization of the

lung allows *C. neoformans* to disseminate to the bloodstream and cross the blood-brain barrier, where it causes lethal meningitis, particularly in immune-compromized individuals, but also in seemingly immunocompetent subjects. Therefore, we need to consider how the ability of organisms (such as *C. neoformans* or other pathogenic microbes) to acquire metals or resist metals, at different locations in its life cycle, impacts on a successful primary infection in the lung or other primary infection site. When *C. neoformans* enters the lung during an infection, it is engulfed by alveolar macrophages, which, among other antimicrobial effectors, use Cu toxicity within the lumen of the phagosome as an antimicrobial weapon (Figure 7.2). For a leaf-derived infecting yeast form, the immediate stress arguably should be higher in this model, where the expectation is that the *C. neoformans* is somewhat Cu deficient and has not primed a Cu defense response, which in this case is strongly mediated by the Cu-inducible, Cu-sequestering metallothionein proteins (Ding et al. 2011, 2013). Successful colonization of the lung allows *C. neoformans* to escape from alveolar macrophage surveillance, disseminate to the circulation, cross the blood-brain barrier, and enter the brain where it causes lethal meningitis. In the central nervous system, *C. neoformans* produces melanin as a virulence factor, using the Cu-dependent enzyme laccase (Salas et al. 1996). This need for copper is further driven by well-characterized Cu-dependent Fe uptake permease-oxidase complexes, which serve as a major source of iron for Fe-S cluster biogenesis or as a direct catalytic cofactor for a variety of enzymes (Kronstad et al. 2013). Consequently, as an environmental opportunistic pathogen, *C. neoformans* finds itself transitioning from a Cu overload environment to an environment where Cu acquisition is essential to thrive, once again inverting the balance between external sources and metabolic need.

The acquisition of zinc by fungal and bacterial pathogens is dictated largely by high-affinity Zn importers at the plasma membrane, the transcription of which is induced under Zn deficiency and dampened by Zn satiety. In addition to the integral membrane Zn importers, starvation for zinc leads to the expression of an auxilliary Zn acquisition system, Pra1, in the fungal gastrointestinal tract commensal and pathogen *C. albicans* (Citiulo et al. 2012). Pra1 protein has roles in both Zn binding and immune modulation via direct interaction with the complement system. As zinc plays a critical role in immune competency, Pra1, which binds multiple Zn atoms, could in addition compromise immune function by sequestering zinc. As Pra1 expression is induced by Zn deficiency, Zn levels encountered by *C. albicans* prior to the transition to the pathogenic phase may therefore play a role in the early stages of systemic infection. Furthermore, as with most pathogenic microbes, when *C. albicans* experiences Fe starvation it undergoes a change in carbon metabolism, away from the heavily Fe-dependent TCA cycle and oxidative phosphorylation. Alternative pathways, such as the glyoxylate cycle in fungi, are likely to be more beneficial for survival to the antimicrobial insults in the glucose-poor environment of the phagosome during infection. In fact, *C. glabrata* depends

on xenosiderophores, produced by other fungi, to survive macrophage engulfment after being grown under conditions of low external iron, but it can become independent of siderophore-mediated Fe uptake in macrophages engineered to mimic human Fe-overload disease due to a dysfunctional Fe exporter ferroportin (Nevitt and Thiele 2011).

In bacterial pathogens, Fe starvation is known to induce the expression of a plethora of virulence-associated factors, such as hemolysins in *Staphylococcus aureus* (Schmitt et al. 2012). The 50% lethal dose (LD_{50}) of cells prestarved for iron is accordingly significantly lower. This poses the question of where these effects may be seen in natural infections, and how they can be correctly replicated in the laboratory. The precise nature of the changes likely depends on the type of transmission and, hence, environments encountered (e.g., directly from host to host, via vectors, or entering the host from the environment or a commensal stage). In *Y. pestis*, the pathogen is transmitted from the blood via a flea vector to a new host. Inside the insect gut, blood is digested, with significant changes in the concentration of available iron likely ensuing (Perry et al. 1993).

Most infection models are based on preculture conditions in the laboratory, which do not mimic the natural habitat or source from which the infecting organisms may derive metals in nature. Thus far, data strongly indicate that in addition to other factors (e.g., temperature, pH, and carbon sources), the metal supply of precultured pathogens should be taken into account. This will lead to infection experiments that better simulate the events in nature. As data or estimates on naturally occurring preinfection metal concentrations are not widely available, it would be informative to test the effect of biologically relevant metal concentrations on the outcome of laboratory infection experiments.

For pathogens that spend time in the external environment, mechanisms must exist to allow metal transport to support basic metabolic needs for survival. This challenge may be compounded by potential requirements for elaborating degradative enzymes to liberate environmental metal sources so that the metals are available to serve as substrates for cell-surface metal import mechanisms. The absence of these mechanisms, or of metal substrates for import, demand the elaboration of mechanisms to mobilize metals from intracellular stores such as bacterial ferritin or, in the case of fungal pathogens, from the lumen of vacuoles for copper, iron, or zinc via dedicated transporters. The latter could serve as a buffer against vastly changing metal concentration, but data on the role of these buffers (and their capacity) in infections are for the most part still lacking. Furthermore, several mechanisms are feasible for the pathogen to avoid other detrimental effects of differing pre- and peri-infection metal concentrations. With respect to metal toxicity, ligand-inactivated transporters may help in avoiding metal overload after a switch from relative starvation to metal surplus. The notion is that metal concentrations in microbial populations are generally Gaussian-distributed and may allow subpopulations with appropriate metal homeostasis mechanisms to cope with their previous environment. They

may, therefore, achieve "correct" metal concentrations, which allow them to progress more easily with a productive infection.

To determine the nature and effect of the influence of previous metal exposure on infections, several experimental approaches are likely to be informative. For example, *in vivo* competition experiments with deletion or transposon mutant libraries of pathogens grown under different, defined metal preconditions could help to determine the necessary genetic setup that is pivotal to the outcome of the initial infection. The actual metal status of microbes re-isolated from the host after infection could be investigated; in combination with different preculture metal levels, this would give insight into whether or not high (or low) metal levels in the pathogen are kept within the host, so as to provide a selective advantage for growth and dissemination in the host during infection.

Summary

The ability to acquire or resist metals in a previous environment likely sets the stage for determining, to a significant extent, the fitness of a microbial pathogen for dissemination to additional tissues for virulence. Consequently, understanding the environments from which microbial pathogens are derived is an underappreciated, yet important area for investigation. Moreover, for microbial pathogens that possess multiple systems for metal acquisition, such as the use of ferrous Fe importers and siderophore-mediated Fe uptake, the selection of which mechanisms are used in specific environments likely provide a mechanism for optimally positioning a pathogen for initiating successful colonization of host tissues. Clearly the metal levels of the infecting pathogen influence the expression of many genes, including known virulence factors. However, in laboratory infection experiments, metal levels are not controlled for normally. They may not be simulative of the environment from which the pathogen normally derives (e.g., gut, skin, vector), or they may have little relevance to the levels encountered by the pathogen prior to the initial host infection event. Biological relevance of these experiments may therefore be limited in this respect. In addition, for different pathogens, the metal concentrations that precede infection, as well as their metal status, constitute an area for further investigation. With this information in hand, one could then consider if changes (within biologically relevant ranges) in specific prior metal exposure levels influence the course and/or outcome of an infection. Would different prior exposure levels alter the ability of pathogens to cope with severe changes in metal levels during the initial stages of infection, such as those occurring during an inflammatory response? Are these changes "predicted" by other external cues before the infection? Does prior metal exposure generally serve as a signal in itself for induction of metal-independent virulence factors?

The Interplay between Metals and the Microbiota

Here we focused primarily on the influence of the microbiota composition on metal availability in different body environments and vice versa. Related topics were also discussed in other groups, with some degree of overlap (see Rehder et al., Ackland et al., and Maret et al., this volume). The complexity of the different microbial communities that exist at different anatomical sites within the body has been highlighted by The Human Microbiome project, supported by the U.S. National Institutes of Health Common Fund (NIH HMP Working Group 2009).

Metals and the Microbiota

Evidence that a host's microbiota can contribute to human physiology and immune status has been generated from a range of studies. With regard to metals and the microbiota, future research should be aimed at examining (a) whether the microbiota influences the bioavailability of dietary metals and (b) whether dietary or environmental intake of metals influences the structure and function of the microbiota (see Ackland et al., this volume).

Some early work addressed the influence of the microbiota on tissue Fe distribution using germ-free mice (Donati et al. 1969). Thus, the above questions need to be revisited with state of the art sequencing, which provides rich analysis and definition of complex communities, along with new knowledge about how the microbiota influences development of the immune system (Arrieta and Finlay 2012), and susceptibility to colonization by pathogens (Buffie and Pamer 2013). Initial goals should be to discern correlations between the microbiota content and metal availability in the host and, *more importantly*, to determine the underlying mechanisms and consequences of those correlations. It is vital to get beyond simply counting numbers and extend any findings to uncover mechanisms that relate to the microbiota. This would likely include investigations into the meta-transcriptomics and metabolic output of these complex communities in relation to metal homeostasis.

There are already studies that show specific effects of metals in modulating microbial communities and the role of the host in the competition for these micronutrients. For example, the expression of a high-affinity ABC transporter for Zn uptake by *Campylobacter jejuni* is required for its survival in chicken intestines in the presence of normal microbiota, but not when chickens are reared under germ-free conditions with a more restricted microbiota (Gielda and DiRita 2012). The ability to survive was associated with the presence of numerous Zn-binding proteins in the intestines of conventional chicks, which were absent from limited-microbiota chicks. This study demonstrates that the microbiota in the gastrointestinal tract stimulates a reduction in Zn availability and, as such, restricts the growth of bacteria that lack the ability to compete using high-affinity transporters. In this regard, human polymorphisms in Zip4

affect Zn uptake in the small bowel (Lichten and Cousins 2009), but whether or not this is exacerbated or otherwise influenced by the microbiota is unclear. A comprehensive picture of how host and microbial factors in the gut affect metal dynamics in the host is lacking (NIH HMP Working Group 2009). Given the significance of the upper intestine on metal uptake in the body, research on how the microbiota might contribute to this process should focus on the small bowel inhabitants, without ignoring those of the colon and even feces, where much microbiota work related to pathogenesis of infection has been focused.

The influence of any correlations that are discovered on the susceptibility to, or outcome of, infection should be assessed in human populations and model systems to ensure that both clinical correlations and underlying mechanisms are addressed. The burden of metal deficiencies in particular populations (e.g., Zn deficiencies in Pakistan and elsewhere) is a well-recognized issue (Akhtar 2013; Bhutta et al. 2013), and these populations would serve logically as the basis for human-centered research on any correlations with the microbiota. Laboratory models such as the mouse or zebrafish will ultimately be important for understanding basic mechanisms of how diet and microbiota interact. Veterinary research on growth-promoting use of Zn oxide includes, for example, investigations into its effects on stimulating beneficial microbes within the pig ileum (Vahjen et al. 2011). Whether or not this correlates to changes in susceptibility to infection has yet to be determined, but such research might provide data for hypotheses to test in human populations.

Some skin pathologies arise from metal deficiencies (Ackland and Michalczyk 2006), and Zn replacement in otherwise deficient populations has had rapid therapeutic effect on respiratory symptoms, in addition to diarrheal symptoms (Prasad 2013). Thus, research into relationships between the microbiota and metal homeostasis, and their potential effects on infectious disease, should also consider the skin and respiratory microbiota in addition to gut microbiota. It is likely that metal acquisition and utilization by these communities relies on access to host sources, whereas the microbes in the gut could additionally obtain these micronutrients directly from the diet.

A further consideration is the status of the colonized host surface. Inflammatory states will affect the availability of metals, the influx of host defense factors that are dependent on metals, and the composition of the microbiota and metal utilization by these populations. In general, acquisition of metals has been studied in pathogens, or opportunistic pathogens, which are adept at handling the inflammatory states they encounter in the host during disease. Our understanding of metal dynamics in organisms in a strict commensal relationship with their host, and especially complex communities of commensal organisms, is far less complete. Based on estimates of the size and complexity of the microbiota, this population has the potential to act as a significant reservoir/requirement for total metal species associated with a host.

Microbial systems for sensing and acquiring iron, zinc, and manganese appear to be highly conserved. Transcriptome and integrative metagenome and

metabolome studies of the microbiota are therefore likely to be informative about whether a host environment is metal replete or deplete.

Summary

Metals appear to affect the size and composition of the microbiota. There is a growing appreciation for how alterations in the microbiota affect various infectious and noninfectious disease states. As these studies define specific microbial species and factors that impact disease, it will be important to define the role of metals in influencing these effects of the microbiota.

How Metal Restriction and Oversupply Can Kill or Inhibit the Growth of Microbes

What are the mechanisms by which exposure of microbes to metal excess or metal deficiency can inhibit microbial growth or cause death? When considering this question, we need to keep in mind that excess of one metal may also induce deficiency of a different metal. Hence the interplay between different metals is important.

How Do Metals Poison Microbial Cells?

In our discussions of the effects of metal restriction and metal oversupply, we considered the effects on a model organism, *E. coli*, so as to lay out the type of information that allows one to focus on this topic.

The Effects of Metal Restriction

E. coli growth fails upon Fe restriction due to loss of heme and Fe-S cluster synthesis. Manganese is used only as a substitute for iron when the latter is unavailable; thus Mn restriction is consequential only in that circumstance. Zinc is much more broadly used, yet restriction has been difficult to study due to trace Zn contamination of culture systems. Copper activates very few enzymes, and these are useful but dispensable (at least in artificial culture); thus restriction is relatively benign. However, it is possible that in certain environments these enzymes become critical; examples are Cu/Zn-SOD to protect against high extracellular reactive oxygen species (Craig and Slauch 2009). In contrast to *E. coli*, other bacteria may rely more heavily on manganese (e.g., *Bradyrhizobium japonicum*; Hohle and O'Brian 2012) and/or less on iron (e.g., *Borrelia burgdorferi*; Posey and Gherardini 2000). Collectively, the impact of metal restriction likely varies from organism to organism, according to how (or if) the organism uses a particular metal. For an individual organism, this may also depend on its immediate environment.

How an Overabundance of Intracellular Metals Impedes Cell Growth

Excess iron leads to Fenton chemistry that generates hydroxyl radicals; its major effect is to accelerate mutagenesis. The level of damage, however, does not rise to the point of growth inhibition, let alone death. In *E. coli*, excess manganese blocks heme synthesis by competitively inhibiting ferrochelatase. Since the requirement for heme arises from its use in cytochrome oxidase, Mn overloading blocks growth under oxic, but not anoxic, conditions. Iron can be outcompeted by excess zinc in Fe-using nonredox enzymes. Copper poisons solvent-exposed Fe-S clusters. Additional mechanisms may also exist but have not been elaborated. The targets in *E. coli* are, however, not universal: lactic acid bacteria, for example, lack both heme and Fe-S clusters and are therefore resistant to manganese and possibly copper. Thus, some metals damage narrow subclasses of enzymes, and organisms which lack these may prove substantially resistant. More research needs to be done to understand the mechanisms of metal intoxication of various pathogens. Although *E. coli* has provided invaluable information, it is not a universal model.

The Effect of Metal Import and Export Systems

It is notable that genetic mutations in metal import and export systems are often needed to establish metal restriction and metal overloading. Both metal restriction and overloading are common experiences for many microbes. Many biological habitats are metal limited. Conversely, when a metal-limited bacterium moves from a metal-poor habitat to a metal-rich environment, the cytoplasmic metal levels are likely to overshoot, because the extant import systems are not allosterically controlled. This is quickly corrected by the induced synthesis of export pumps, some of which are encoded by plasmids that enable growth in metal-rich habitats (Gutierrez-Barranquero et al. 2013) simultaneously with the repression of import pumps. Transient overshooting is not a problem for the microbe, since the poisoning mechanisms are readily reversed when metal levels fall. Inhibition of heme synthesis and of Fe enzymes is relieved when manganese and zinc dissociate from the enzymes that they are inhibiting; as Cu levels decline, the damaged Fe-S clusters are repaired. Thus, for overloading to have an important effect on bacterial populations, the metal stress would have to be persistent and of a severity that cannot be corrected by homeostatic responses.

Metal importers with surface-exposed metal-binding ligands may be more vulnerable to metal poisoning than cytoplasmic enzymes. The cytoplasm has an abundance of low molecular weight metabolites, such as cysteine, that continuously facilitate the extraction of incorrect metals from inhibited enzymes. However, such compounds are less likely to be present in the periplasm or the extracellular environment to provide similar protection to extra-cytoplasmic metal-binding sites. Thus, for example, the inhibition of a Mn importer by

zinc may not easily be reversed; irreversible inhibition of the *Streptococcus pneumoniae* ABC-type Mn importer by Zn binding has recently been reported (Counago et al. 2013). The impact of fluctuations in the bioavailability of one metal can also depend on a second metal, if both are able to perform equivalent functions. For example, because Mn import can correct for Fe deficiency, the absence of both metals can be synergistic. Conversely, since excess zinc competes for the Fe sites of enzymes, excess zinc can exacerbate Fe deficiency.

Exposure to Metal Surfaces

Solid metallic copper, Cu(0), or silver is able to kill many/most microbes rapidly through surface contact. This property has been exploited in the design of antimicrobial surfaces (e.g., door handles, Cu pipes, incubator linings, catheters), which reduce the incidence of nosocomial infection. The effect seems dissimilar to what solubilized copper can achieve, but the underlying molecular mechanism of metallic Cu toxicity is currently unknown.

Nonnutritional Metals

Nonnutritional metals have been used to treat microbial infection. In the pre-antibiotic era, toxic compounds such as mercurials and arsenicals were used to treat infections such as syphilis. More recently, silver has been exploited for its antimicrobial properties, for example, in wound dressings (Mijnendonckx et al. 2013). Gallium has also been explored as an antibacterial agent, with some success demonstrated in treating *Pseudomonas aeruginosa* lung infections (Halwani et al. 2008). Metallotherapeutic agents are now being explored to treat parasitic infections, including using ruthenium, rhodium, and gold derivatives of chloroquine as antimalarials (Navarro and Visbal, this volume). However, the antimicrobial effects of these metals are largely unknown.

Summary

Since the effects of metals are directed toward the activities of enzymes, metal availability has the greatest impact on growing organisms that must maintain high metabolic fluxes. Actively growing organisms are more sensitive to iron, which can catalyze Fenton chemistry on hydrogen peroxide, a by-product of metabolism. Static biofilms, for example, are less likely to be affected. It appears that the vulnerability of microbes to both metal limitation and metal toxicity is exploited as part of the host's antimicrobial arsenal. Whereas the effects of metal overloading and underloading are usually bacteriostatic, in a host this might be good enough, since nongrowing organisms can then be cleared by the host's immune defenses. For some metals, such as arsenic, the toxic effects are still unknown, and further research is needed to understand these mechanisms.

Knowledge of how metals kill cells can be used to design more optimal metal-based antimicrobial treatments.

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