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Recent developments in copper and zinc homeostasis in bacterial pathogens

Joseph J Braymer and David P Giedroc

Copper and zinc homeostasis systems in pathogenic bacteria are required to resist host efforts to manipulate the availability and toxicity of these metal ions. Central to this microbial adaptive response is the involvement of metal-trafficking and metal-sensing proteins that ultimately exercise control of metal speciation in the cell. Cu-specific and Zn-specific metalloregulatory proteins regulate the transcription of metal-responsive genes while metallochaperones and related proteins ensure that these metals are appropriately buffered by the intracellular milieu and delivered to correct intracellular targets. In this review, we summarize recent findings on how bacterial pathogens mount a metal-specific response to derail host efforts to win the 'fight over metals.'

Addresses
Department of Chemistry, Indiana University, Bloomington, IN 47405-7102, USA

Corresponding author: Giedroc, David P (giedroc@indiana.edu)

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Metal ions at the host–pathogen interface

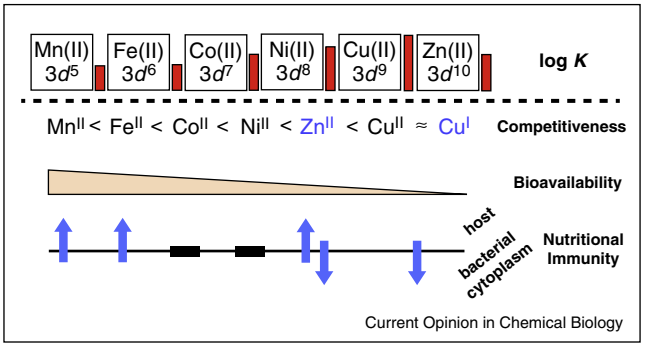
Strict control of the homeostasis of transition metal ions is essential to all forms of life. The cellular balance of metal ions is orchestrated by proteins and small molecules, and when cellular physiology is disrupted by aberrant metal metabolism, human disease can occur [1]. This need for cellular control of metal homeostasis is exploited by the innate immune system during a bacterial infection. Here, the host attempts to restrict the availability of essential nutrients in a process generally termed nutritional immunity [2,3•,4] while inundating the bacterial cell with a wide range of toxic insults, including low pH, reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive chlorine species (RCS), and hydrolases [5,6]. A key aspect of this assault is an extensive perturbation of the availability of the four major transition metals required by the bacterium: iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). In contrast to host processes that attempt to limit a pathogen's access to Fe and Mn, recent work reveals that high Cu concentrations are used to kill microbial invaders, particularly intracellular pathogens (Figure 1) [7,8]. For Zn, the work taken collectively supports a role for both host-mediated toxicity [4,7] and sequestration [3•,9] as a means to restrict pathogen viability upon host infection. Although these microbial defense mechanisms disrupt transition metal homeostasis of most bacteria [3•,4,9], successful pathogens have evolved mechanisms of adaptation to these perturbations [4,10,11].

Molecular basis of Cu(I) and Zn(II) toxicity

Cu and Zn speciation is defined by the types of ligands encountered in the cell [12,13], while metal specificity is collectively dictated by metal coordination number and geometry, the rates of exchange in and out of metal complexes, and redox state (valence) [14]. On the basis of the binding affinities of selected targeting, trafficking, and metal-sensing proteins [12,15,16], Cu(I) is thought to be buffered by a typical cell in the attomolar range, while Zn(II) is buffered in the nanomolar [17•] to picomolar [16] range; however, these values may vary for different bacteria. As a rule of thumb, chelate binding affinities for Cu(II) and Zn(II) are generally higher than for earlier first-row divalent transition metals for a given ligand, a trend known as the Irving–Williams series for divalent ions (Figure 1) [18]. The bioavailability of Cu and Zn is therefore generally low and inversely proportional to competitiveness relative to other first-row metals, which dictates that their availability in cells be tightly regulated. Further, the major redox state of copper is monovalent Cu(I) in the bacterial cytoplasm due to the low reduction potential maintained by low-molecular-weight thiols relative to the Cu(II)/Cu(I) redox couple (−0.22 V and +0.15 V, respectively, relative to the normal hydrogen electrode) [13]. The sulfur-containing amino acids cysteine and methionine play important roles as soft bases that readily coordinate the soft acid Cu(I). These properties make unregulated Cu(I) highly toxic as evidenced by the ability of Cu(I) to mediate disassembly of iron-sulfur (Fe-S) clusters leading to dysfunctional cellular metabolism [19,20]. The vulnerability of Fe-S clusters to Cu(I) remains to be validated as a general mechanism of Cu toxicity in other bacteria, particularly those that lack significant Fe-S cluster-containing proteins in their metallomes [21]. Our molecular-level understanding of intracellular zinc toxicity is far less clear, although a model invoking mismetallation of metalloenzymes through competition is a reasonable, albeit largely untested one [22].

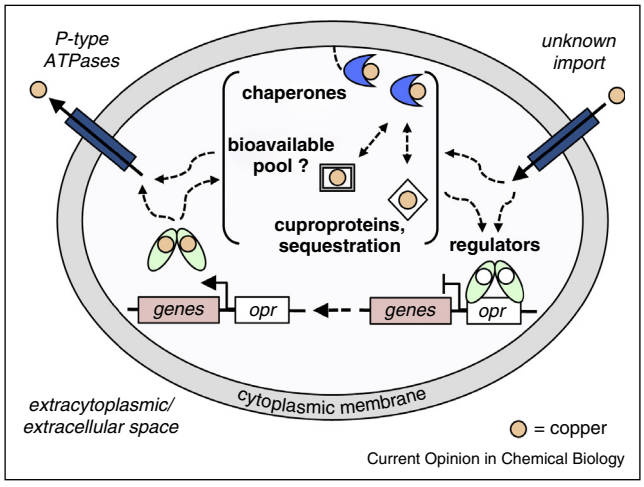
A second potential impact of copper toxicity is the chemistry of Cu(I) with host-mediated hydrogen peroxide

Figure 1



Bioinorganic chemistry at the host–pathogen interface. Cu(II) has the highest affinity for a given ligand compared to other first row transition metals as exemplified by the height of the *red* bars which depict the NIST approved log *K* values relative to the Cu(II)–aspartic acid complex [Cu(II)–aspartic acid, log *K* = 8.9]. This empirical relationship is described as the Irving–Williams series and relates to the relative competitiveness of first-row transition metals in a cellular environment [18]. Cu(I) predominates in the cytoplasm and like Zn(II), forms high-affinity complexes with softer acids (histidine, cysteine, methionine), and is therefore also considered a highly competitive metal. Bioavailability is inversely proportional to competitiveness and has been roughly approximated on the basis of the relative binding affinities of metal-dependent transcriptional regulators [16]. Metal-centric nutritional immunity is defined as the host's attempt to both sequester metal ions from cells (*upward-facing blue arrows*) and/or bombard the bacterial cytoplasm with metal-ion stress (*downward-facing blue arrows*). Roles of Co and Ni in nutritional immunity are not yet known (*black bars*).

Figure 2



Overview of copper sensing and trafficking within the bacterial cytoplasm. Copper enters the cytoplasm through largely unknown mechanisms. Copper speciation within the cell depends on the relative concentrations of Cu(I) bound to the bioavailable pool, for example, copper bound to low-molecular-weight thiols, cytoplasmic binding proteins, for example, MymT [28] and CutC [29]), chaperones, and Cu(I) sensors. The thermodynamics and kinetics of Cu(I) speciation remain incompletely understood and may be dictated by the concentrations at which copper homeostasis proteins become saturated. Importantly, Cu(I) overload must ultimately be sensed by Cu(I)-dependent metalloregulators (*light green* calipers) causing transcriptional derepression as a result of dissociation from the DNA operator-promoter region (*white* rectangle, opr) (or transcriptional activation) and expression of Cu(I) resistance genes (*pink* rectangle, labeled genes). It is these upregulated copper resistance proteins that ultimately function in Cu(I) resistance, either via sequestration or export through P-type ATPases.

(H_2O_2) or superoxide ($O_2^{-\bullet}$). Labile Fe(II) is accepted as a major source of intracellular oxidative damage in cells given its ability to heterolytically cleave H_2O_2 to form reactive hydroxyl radical $OH^\bullet$ and oxidized Fe(III); this process becomes catalytic in the presence of cellular reductants [23,24]. Although uncomplexed Cu redox cycles faster than Fe *in vitro* [24–26], the degree to which Cu(I)-catalyzed Fenton chemistry is relevant *in vivo* remains uncertain due to the lack of a comprehensive understanding of Cu(I)-ligand speciation in the cell and how Cu(I)-complexes are modulated by myriad toxic insults at the host–pathogen interface.

Cu sensing and trafficking

Many pathogens accumulate micromolar levels of cell-associated Cu [12,22] despite possessing a little or no clearly defined cytoplasmic need for the metal [27]. For Gram-negative bacteria, it is presumed that much of this Cu localizes to the periplasm and is bound to essential cuproproteins, although some pathogenic bacteria additionally harbor Cu(I) sequestration proteins in the cytoplasm [28,29]. Once inside the cell, bacterial copper chaperones generally represent a first line of defense against Cu(I) toxicity imparted by the host (Figure 2) [11]. The founding bacterial metallochaperone is *Bacillus subtilis* CopZ, which is structurally identical to Atx1 initially characterized in yeast (Figure 3a) [30]. CopZ adopts a ferredoxin-like fold where Cu(I) forms a *bis*-thiolato digonal coordination complex with a Cys-X-X-Cys loop sequence (where X is any amino acid) that is additionally capable of coordinating a small molecule from solvent. Metallochaperones buffer highly competitive Cu(I) to low levels in the cytoplasm [31] and shuttle Cu(I) to intracellular targets including cytoplasmic Cu(I) sensors and to Cu(I)-specific P-type ATPase effluxers with rapid exchange kinetics through an associative, ligand exchange mechanism that prevents release of Cu(I) into bulk solution (Figure 2) [10,32].

A new perspective on Cu(I) trafficking has been reported for the Gram-positive respiratory pathogen *Streptococcus pneumoniae* [33••]. This work reveals that the ancient cupredoxin fold [34] (Figure 3), known to play prominent roles in electron transfer and bacterial respiration, has been co-opted to function as a novel plasma membrane-anchored Cu(I) chaperone (CupA) that is capable of delivering Cu(I) to the N-terminal metal-binding domain (MBD) of the Cu(I)-effluxer CopA ($CopA^{MBD}$) [33••]. This Cu(I) transfer is thermodynamically favorable and