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Metalloregulation of Gram-positive pathogen physiology

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Abstract

Due to the unique redox potential of transition metals, many of these elements serve important roles as cofactors in numerous enzymes. However, the reactive nature of metal becomes an intracellular threat when these ions are present in excess. Therefore, all organisms require mechanisms for sensing small fluctuations in metal levels to maintain a controlled balance of uptake, efflux, and sequestration. The ability to sense metal ion concentration is especially important for the survival of pathogenic bacteria because host organisms can both restrict access to essential metals from invading pathogens and utilize the innate toxicity of certain metals for bacterial killing. Host-induced metal ion fluctuations must be rapidly sensed by pathogenic bacteria so that they can activate metal transport systems, alter their physiology to accommodate differences in metal concentrations, and regulate the expression of virulence factors.

Introduction

Metal starvation signals the up-regulation of acquisition systems [1] while metal excess activates the expression of efflux pumps and other proteins involved in metal resistance [2*]. Additionally, numerous physiological processes in bacteria are controlled in response to fluctuations in ion concentrations, resulting in decreased expression of metal-dependent enzymes upon metal deprivation [3]. This review will highlight recent advances in iron- and copper-responsive metalloregulation in a number of Gram-positive pathogens. The ability to sense fluctuations in these metals is of particular importance to pathogens because host organisms restrict iron availability [4,5] and induce copper toxicity [6**,7**].

Iron metalloregulation: A pathogen's response to host-induced metal restriction

Vertebrate hosts restrict access to essential nutrients from invading pathogens in a phenomenon known as "nutritional immunity" [4]. Iron sequestration by the host is the archetypal form of nutritional immunity. The mechanisms utilized by the vertebrate host to restrict iron-access to pathogens include a physiological pH that results in low iron solubility, intracellular localization of iron, and storage of iron within iron-binding proteins. The net result of these mechanisms creates an environment virtually devoid of free iron with greater than 90% of iron residing within host cells [1,5].

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Highly efficient iron acquisition machinery is essential to the survival of invading pathogens in order to support the function of key iron-dependent enzymatic processes [1]. Many proteins of the respiratory chain possess iron-sulfur clusters, peroxidases and cytochromes utilize the iron-containing molecule heme as a prosthetic group, and other enzymes such as ribonucleotide reductase require iron to catalyze their reactions [8]. While iron is important to the survival of almost all organisms, iron excess produces extreme toxicity due to the ability of this element to catalyze the Fenton reaction, leading to the generation of hydroxyl radicals that damage biological molecules [9]. Therefore, intracellular iron levels are tightly monitored through the action of metalloregulatory proteins.

Numerous families of metalloregulatory proteins have been discovered and characterized over the past few decades. Metalloregulatory families capable of sensing intracellular iron levels include the *f*erric *u*ptake *r*egulator (Fur) and the *d*iphtheria *tox*in *r*epressor (DtxR) [2*]. Fur family proteins are typically utilized for iron-sensing in low-G+C Gram-positive microorganisms, and DtxR regulators are more often found in high-G+C Gram-positive bacteria [10]. Some pathogens such as *Mycobacterium tuberculosis* possess both Fur and DtxR homologues [11]. Both of these metalloregulators bind to DNA operator sites upon association with their cognate metal ligands. Fur and DtxR proteins typically function as repressors, shutting down gene expression upon DNA binding [2*]. However, in a few instances, these metalloregulators have been found to activate the expression of certain genes [12,13].

Fur and DtxR control the expression of numerous gene categories in response to iron starvation including iron acquisition systems [10]. In addition to regulating iron uptake, the DtxR family proteins induce the expression of virulence factors in response to metal deprivation, including the diphtheria toxin of *Corynebacterium diphtheriae* for which this metalloregulatory protein class is named [14]. While Fur also regulates virulence [15*,16], of particular note is the ability of this protein family to redirect central metabolism from respiration to fermentation in order to alter cellular iron requirements [17,3]. Specific iron-containing respiration enzymes that are repressed in response to iron-starvation include succinate dehydrogenase, aconitate hydratase, and fumarate hydratase. The repression of iron-dependent enzymes upon iron-deprivation has been termed the “iron-sparing response” [3,18].

Because iron-deprivation conditions are directly relevant to the host environment, several recent analyses of transcriptomic and/or proteomic profiles under iron-limiting conditions were performed to identify iron-responsive genes in *Staphylococcus aureus* [17], *Mycobacterium tuberculosis* [19], *Streptococcus pneumoniae* [20], *Bacillus anthracis* [21], and *Listeria monocytogenes* [22]. These studies revealed Fur- and/or DtxR-regulated genes as well as iron-responsive genes that may be controlled independently of these known iron-sensing metalloregulators.

An analysis of the *S. aureus* proteome under conditions of iron deprivation and upon deletion of the *fur* gene revealed global changes in gene expression and an overall switch from respiration to fermentation [17]. This study demonstrated that this metabolic change not only alters the biological iron requirement of this organism, but also produces an accumulation of acid end products which alter the local pH and allow for iron release from host transferrin.

The transcriptomic analyses of *S. pneumoniae*, *B. anthracis*, and *L. monocytogenes* undergoing iron-starvation reveal similar iron-sparing responses in which iron-dependent proteins are down-regulated [20,21,22]. These studies also uncovered additional, unexpected physiological responses to iron-deprivation. For example, *M. tuberculosis* undergoes

significant changes in lipid content [19] and protein turnover rate [23] in response to fluctuations in iron levels.

In addition to altering physiologic processes, bacterial pathogens also modulate production of virulence factors upon changes in iron availability. For example, biofilm formation occurs in response to iron limitation through Fur-dependent and Fur-independent mechanisms in *S. aureus* [24,25]. Similarly, biofilm formation proteins of *S. pneumoniae* are also up-regulated upon iron-deprivation [20]. The transcriptomic studies of *B. anthracis* revealed numerous iron-regulated virulence factors including several putative internalin genes that are important during infection [21]. The Fur protein of *S. aureus* controls the expression of global regulators such as Sae, Agr, and Rot which are known to regulate the expression of virulence factors [16]. Therefore, virulence gene expression in this organism is particularly responsive to iron levels. Deletion of the *S. aureus f*ur gene results in overall reduced virulence in a mouse pneumonia model, underscoring the importance of iron-sensing in the host environment [15*]. Many of the gene categories and physiological processes regulated in response to fluctuations of intracellular iron levels in Gram-positive bacteria are highlighted in Figure 1.

Copper metalloregulation: A pathogen's response to host-induced metal toxicity

Like iron, the reversible oxidation of copper contributes to its usefulness as an enzymatic cofactor as well as its toxicity due to the fact that copper generates reactive oxygen species by participating in Fenton-like chemistry [26]. Additionally, copper induces thiol oxidation, leading to sulfhydryl depletion [26,27**]. The final component of copper toxicity can be attributed to the fact that this element is highly competitive for binding sites in metalloproteins [8,2*]. Trace amounts of uncomplexed copper will displace the correct metal cofactors from essential enzymes [28]; therefore, bacteria must maintain an intracellular environment that is virtually devoid of free copper.

Recent studies demonstrated that macrophages have evolved the ability to harness the bactericidal activity of copper [6**]. Macrophages traffic a copper-specific P_1 type ATPase, ATP7A, from the Golgi apparatus to phagosomal compartments upon stimulation with proinflammatory agents in order to mediate bacterial killing. New data also suggest that other host environmental niches may be rich in copper. For example, respiratory pathogens such as *S. pneumoniae* and *M. tuberculosis* activate a copper stress response in the nasopharynx and the lungs of mice [7**,29]. Therefore, the ability to sense copper fluctuations is critical for pathogenic organisms.

A number of metalloregulatory protein families are capable of sensing fluctuations in intracellular copper levels including ArsR-SmtB, CsoR, and CopY. These metalloregulators control gene expression via de-repression in which metal-binding results in dissociation of the transcriptional regulator from the DNA strand and subsequently allows gene transcription [2*]. In addition to these traditional metalloregulators, two-component systems capable of sensing extracellular copper concentration have been identified in a few bacteria [30,31].

The metalloregulatory mechanisms utilized for copper-sensing within Gram-positive pathogens are diverse. CsoR family copper sensors have been shown to be important in *M. tuberculosis* [29,32*], *S. aureus* [33**,34], and *L. monocytogenes* [35]. Copper-sensing is accomplished through the action of a CopY transcriptional repressor in *S. pneumoniae* [7**], *Enterococcus hirae* [36], and *Enterococcus faecalis* [37]. Finally, *Corynebacterium glutamicum*, a model organism for studying processes in pathogenic actinomycetes, utilizes

a two-component system known as CopRS for sensing extracellular copper levels [31]. CopRS is highly conserved in the pathogenic bacterium *C. diphtheriae*.

Because various niches within the host environment contain toxic levels of copper, the ability to sequester and export this metal is particularly important in pathogenic bacteria. *S. aureus* [38,33**], *L. monocytogenes* [35], *S. pneumoniae* [7**] and *M. tuberculosis* [39,40] each encode copper metallochaperones and efflux pumps in order to counter the toxic effects of copper. Deletion of copper detoxification systems results in decreased virulence in *S. pneumoniae* and *M. tuberculosis* [7**,39,40]. Interestingly, deletion of an important copper efflux pump in *L. monocytogenes* does not impact its virulence in a mouse model, indicating that not all host niches are equally copper-rich [35]. Since copper excess is directly relevant to the conditions experienced within the host environment, global transcriptomic responses to copper toxicity have been analyzed for *M. tuberculosis* [41], *S. aureus* [27**], *E. faecalis* [37], and *C. glutamicum* [31]. All of these analyses reveal up-regulation of efflux and sequestration systems upon copper exposure but other copper-regulated genes vary from organism to organism.

The copper-stress response of *M. tuberculosis* involves a set of 30 genes, many of which function to protect against oxidative stress. This finding indicates that the major component of copper toxicity in *M. tuberculosis* is cellular damage caused by the generation of reactive oxygen species. Other copper responsive genes of *M. tuberculosis* include putative transcriptional regulators of various metalloregulatory families that may represent uncharacterized copper sensors within this organism [41]. Studies have demonstrated that copper sensing is accomplished through at least two CsoR family regulators in *M. tuberculosis*, underscoring the importance of copper-sensing in bacterial pathogens [32*].

The *S. aureus* global response to copper is similar to that of *M. tuberculosis*, including genes involved in copper efflux, protection against oxidative stress, and the misfolded protein response. Interestingly, *S. aureus* also decreases the expression of global virulence regulators *sae* and *agr* upon copper exposure. These virulence regulators control biofilm formation and experimental evidence shows that the presence of copper reduces biofilm development [27**]. This observation provides an explanation for the effectiveness of copper surfaced catheters against the formation of *S. aureus* biofilms [27**,42].

The copper stimulon of *S. pneumoniae* differs from that of *M. tuberculosis* and *S. aureus* in that expression of genes involved in oxidative stress and misfolded protein responses is not altered. This indicates that the source of copper toxicity is not the same for all Gram-positive pathogens. Instead, the copper stress response of *S. pneumoniae* includes expression of virulence factors such as StrH [7**]. StrH is an *N*-acetylglucosaminidase important for the evasion of opsonization and neutrophil-mediated killing [43].

Copper-regulated genes of *C. glutamicum* are also of particular interest because this organism responds to copper excess by up-regulating its cytochrome *c* maturation processes [31]. This response is significant because cytochrome *c* is a copper-dependent enzyme. This observation is proof-of-principal that bacteria not only alter central metabolic pathways in order to accommodate metal deprivation but also regulate metabolic processes to take advantage of metal availability. Many of the major gene categories regulated in response to copper excess in various Gram-positive bacteria are highlighted in Figure 2.

Conclusions

In addition to iron- and copper-metalloregulation, numerous recent advances have been made in studies focused on manganese [44], magnesium [45,46], and zinc [47,48] homeostasis in Gram-positive microorganisms. These studies are of particular importance in

the field of pathogenesis because the concentrations of each of these metals are tightly controlled within the vertebrate host [4,49,50,51,52]. Metal ion homeostasis is important for all bacteria. However, the host environment represents a battlefield in which metal ion fluctuations are utilized as a tool for killing invading pathogens. Therefore, maintenance of metal homeostasis represents a particular challenge to pathogenic organisms. Studies focusing on metal ion homeostasis in Gram-positive pathogens are uncovering metal-regulated virulence factors and metabolic processes that are critical for the survival of invading microorganisms and may ultimately yield novel drug targets.

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Highlights

- Pathogenic organisms encounter metal stresses in the host environment.
- Pathogens alter their physiology to accommodate iron restriction within the host.
- Copper excess within the host induces multiple forms of toxicity in bacteria.

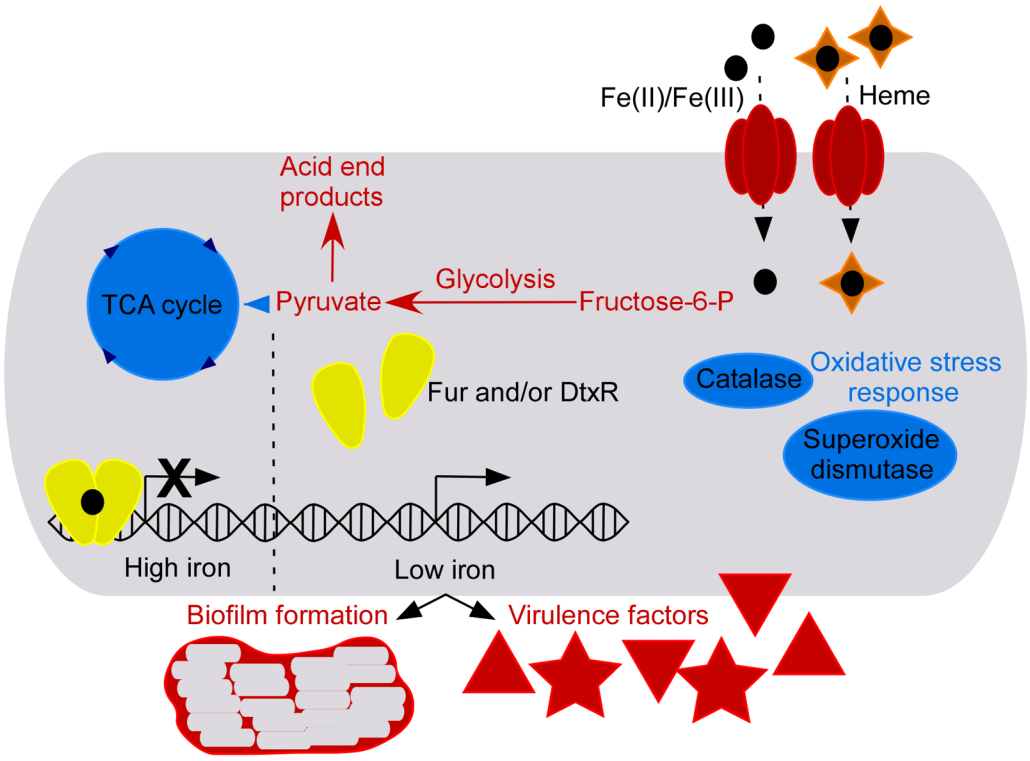

Figure 1. Iron metalloregulation in Gram-positive pathogens

Fur/DtxR iron-sensors are highlighted in yellow. The figure displays their most common function as iron-responsive repressors; however, these proteins can also act as activators in certain instances. Various physiological processes and proteins known to be regulated in response to iron fluctuations in one or more of the organisms highlighted in this review are depicted. Red coloration indicates that these proteins or processes are up-regulated under iron limitation while blue indicates repression in low iron. Black circles represent iron atoms and orange diamonds are heme.

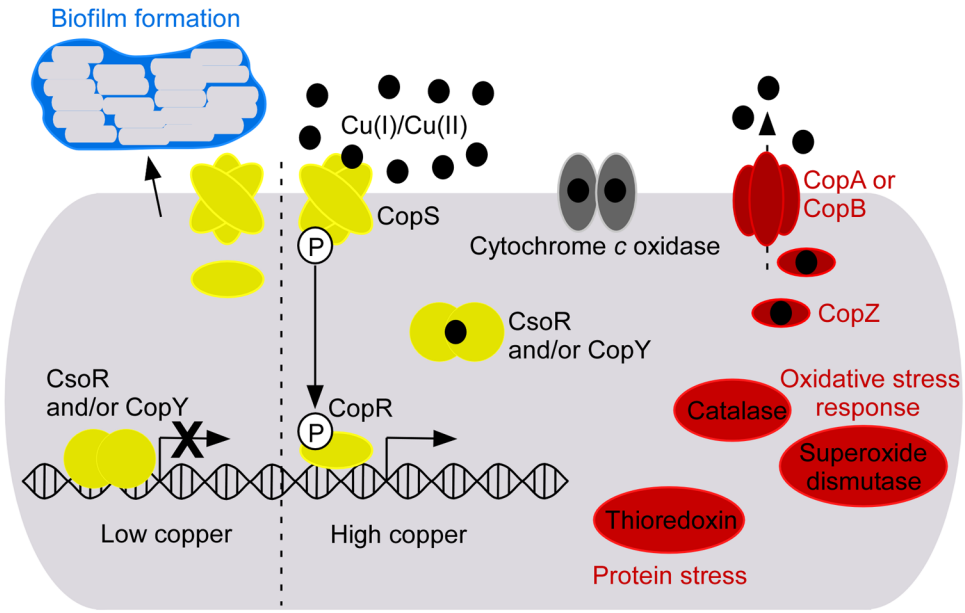

Figure 2. Copper metalloregulation in Gram-positive pathogens

CsoR, CopY, and CopRS copper-sensors are highlighted in yellow. Various proteins known to be regulated in response to copper excess in one or more of the organisms mentioned in this review are depicted. Red coloration indicates that these proteins are up-regulated by copper excess. Blue coloration indicates repression in high copper. Cytochrome *c* oxidase has been given a gray coloration because this protein is up-regulated by copper excess in *C. glutamicum* [31] but it is repressed by copper in *M. tuberculosis* [41]. Black circles represent copper atoms.