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Genetic regulation of metal ion homeostasis in *Staphylococcus aureus*

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Abstract

The acquisition of metal ions and the proper maturation of holo-metalloproteins are essential processes for all organisms. However, metal ion homeostasis is a double-edged sword. A cytosolic accumulation of metal ions can lead to mismetallation of proteins and cell death. Therefore, maintenance of proper concentrations of intracellular metals is essential for cell fitness and pathogenesis. *Staphylococcus aureus*, like all bacterial pathogens, uses transcriptional metalloregulator proteins to aid in the detection and the genetic response to changes in metal ion concentrations. Herein, we review the mechanisms by which *S. aureus* senses and responds to alterations in the levels of cellular zinc, iron, heme, and copper. The interplay between metal ion sensing and metal-dependent expression of virulence factors is also discussed.

Keywords

Staphylococcus aureus; metal ion; metal homeostasis; metalloregulator; iron; manganese

Metal ion acquisition and usage

Transition metals, including iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), are essential **micronutrients** (see Glossary) in virtually all biological systems and play vital roles for living organisms, including bacterial pathogens. The acquisition of some metal ions is necessary for survival (i.e. Fe and Zn), while other metal ions are typically cytotoxic and are only adventitiously imported into the cell (i.e. Hg and Cu). Relatively high titers of certain metal ions, like Fe, are necessary for fitness. Others, like Mn, are only required in minute amounts. Importantly, unlike other kinds of essential nutrients, metal ions are not biochemically synthesized and cannot be degraded. In *Staphylococcus aureus*, the use of metal ion cofactors is required in a diverse array of biochemical processes including carbon transformations, nucleic acid and protein synthesis, DNA replication, regulation of virulence factor expression, and the metabolism of reactive oxidative species (ROS) [1–3].

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In response to infection, and to combat invading pathogens like *S. aureus*, vertebrate hosts restrict the availability of transition metals in a process known as **nutritional immunity** (reviewed in [4]). A number of studies have identified the **S100 family** of proteins as metal chelators that can inhibit bacterial growth by competing for metal ion nutrients (reviewed in [5]). Within *S. aureus* abscesses, neutrophils release calprotectin (S100A8 and S100A9) to inhibit growth of *S. aureus* by limiting its access to divalent metals, including Zn^{2+} , Mn^{2+} , Fe^{2+} , and Cu^{2+} [6–8]. Other S100 family members include psoriasin (S100A7), which was demonstrated to ligate Zn^{2+} and prevent the growth of bacterial pathogens *in vitro* and on human skin [9], as well as calgranulin C (S100A12), which is a Zn^{2+} - and Cu^{2+} -binding neutrophil protein [10–12]. Staphylococcal access to plasma-associated Fe is also restricted by the host via the secretion of lactoferrin and transferrin, which chelate free iron ions, as well as hemopexin and haptoglobin, which sequester **heme** and hemoglobin, respectively (reviewed in [13]). The combined effect of these molecules lowers the concentration of metal nutrients available to pathogens.

To oppose host strategies of metal ion limitation, *S. aureus* synthesizes small molecule **metallophores** and proteins which promote the acquisition of metal ions from the environment. *S. aureus* produces two **siderophores** (staphyloferrin A and staphyloferrin B), which bind to free iron with high affinity and compete with iron-sequestering host proteins like lactoferrin and transferrin [14–17]. Iron ions bound to staphyloferrin A and B are imported by the HtsABC and SirABC transporters, respectively [17–19]. During infection, *S. aureus* produces **staphylopine** to compete with the host for Ni, Co, and Zn [20, 21], as well as hemolysins to rupture red blood cell membranes, resulting in hemoglobin release. Hemoglobin is subsequently degraded to heme and free iron via a series of import and processing steps mediated by the staphylococcal Isd system (reviewed in [22]).

Although metals are essential for viability and growth, cytosolic accumulation can result in poisoning. An excess of iron or copper ions can be harmful in aerobic environments due to their roles as catalysts in **Fenton reaction** chemistry, which leads to the production of damaging oxygen radicals [23, 24]. Transition metals can also be toxic due to their binding to adventitious sites on apo-proteins (known as **mismetallation**), which can result in protein inactivation or other unintended allosteric effects [25].

In *S. aureus* and other organisms, metal-dependent regulatory proteins (**metalloregulators**) work to maintain metal homeostasis by sensing the bioavailability of metal ion(s) and controlling the transcription of genes involved in metal ion import, storage, distribution, and efflux. These metal sensing mechanisms typically involve reversible interaction of the regulator protein with one or more specific metal ions, which alters the affinity of the regulatory protein for specific DNA sequences located in target promoter operons. Alterations in the occupancy of the metalloregulator on the operator modulates transcription of target genes. In the case of **two-component regulatory systems**, stimulation of a histidine kinase (via association with a ligand and/or a change in physiological state) modulates its kinase or phosphatase activity, thereby altering the phosphorylation status of the response regulator (reviewed in [26, 27]). The phospho-state of the response regulator determines its affinity for promoter DNA sequences, and ultimately regulates transcription of downstream target genes. Altogether, these processes sustain the appropriate levels of

transition metal ions and enable *S. aureus* to adapt to changing conditions within the host environment and/or shifting metabolic demands.

In this review, we describe several of the key metalloregulators involved in the genetic regulation of metal ion homeostasis in *S. aureus*. We highlight fundamental and recent developments in the understanding of their functions and mechanisms. We also discuss the crosstalk between genetic regulation of metal ion homeostasis and virulence factor production.

The Fur family

The ferric uptake regulator (Fur) family of metalloregulators are widespread in the *Staphylococci*, as well as other Gram-negative and Gram-positive bacteria [28]. There are three paralogs of the Fur family found in *S. aureus*: Fur (zinc uptake regulator), and PerR (peroxide-sensing regulator) [29]. Fur family regulators are homodimers consisting of two structural and functional domains: a N-terminal winged-helix DNA binding domain, and a C-terminal dimerization domain (Figure 1A). Each monomer contains binding sites for two or three metal ions. The key metal sensing site is typically located near the hinge region that links the DNA binding and dimerization domains. Metal ion binding to this site bridges both domains, causing a structural change that repositions the two DNA binding domains in a caliper-like conformation compatible with DNA binding [30, 31]. A non-regulatory metal binding site includes a structural Zn^{2+} that is required for protein folding and dimerization [32, 33]. Some Fur family members contain a third metal binding site of unclear function [34, 35]. The metal binding specificity of Fur family members is thought to be dictated by both the affinity of the binding site ligands for the cognate metal(s) and the relative availability of each metal ion [36].

As the namesake member of the Fur family, the physiological roles and mechanisms of Fur as an Fe-dependent transcriptional repressor are well-established. In iron-replete conditions, Fur is primarily associated with Fe^{2+} and bound to a conserved operator DNA sequence (**Fur box**) in the promoter region upstream of target genes [37, 38]. When bound to the Fur box, Fur often partially overlaps the -35 and/or -10 initiation sites, thereby occluding RNA polymerase binding and repressing gene transcription (Figure 1B) [39]. Upon a decrease in cellular iron levels, Fe^{2+} is released from Fur, triggering dissociation of the Fur dimer from the Fur box, and subsequent binding and transcription initiation by RNA polymerase (Figure 1B) [39]. Recent work has demonstrated in *Escherichia coli* that Fur also responds to dioxygen tension (via oxidation of intracellular Fe^{2+} to Fe^{3+}) and that transcription of the Fur regulon is activated under anaerobic growth conditions [40, 41]. While it has been established in *S. aureus* that Fur responds to media iron concentrations, it is currently unclear if it also responds to alterations in intracellular redox status.

The Fur regulon in *S. aureus* consists of genes involved in iron import and acquisition, including the siderophore transport system (*sir*) and the ferrichrome uptake operon (*fhu*) [42, 43]. A *S. aureus* fur mutant alters the expression of genes coding for enzymes utilized in central metabolism suggesting that Fur-dependent regulation extends beyond genes utilized for iron acquisition [44]. This includes decreasing flux through the TCA cycle, which is

reliant on Fe-dependent enzymes, and increasing glycolytic flux, resulting in increased fermentation and decreased respiration [44]. A *fur* mutant also has altered production of excreted proteins (exoproteins) [2]. Many of these exoproteins are involved in pathogenesis, including cytotoxins (LukD, LukE, Hla). The *fur* mutant has increased virulence in murine models of pneumonia and skin abscess [2, 42]. It remains to be determined if these phenotypes are the result of direct Fur regulation or defective Fe homeostasis.

In some bacteria, Fur can function as a transcriptional activator via RNA polymerase recruitment, anti-repressor activity, and transcriptional regulation of small, non-coding RNAs (reviewed in [45]). In *B. subtilis*, it was recently shown that Fur is a transcriptional activator for the PerR-repressed *pfeT* iron efflux pump [46]. It is not yet clear if Fur also activates gene expression in *S. aureus*. However, a number of loci (i.e. aconitase, *acnA*) have decreased expression in a *fur* mutant, supporting the possibility. There is also evidence of direct regulation by apo-Fur in *Helicobacter pylori* [47–49], although it is currently unclear whether this property of Fur is conserved in *S. aureus*.

Zur is a Zn-dependent metalloregulator [29]. Most biochemical studies of Zur have been conducted in *B. subtilis*. Like Fur, Zur forms a dimer and contains multiple metal binding sites, which include a structural Zn^{2+} site and two regulatory metal sensing Zn^{2+} sites [50]. Interestingly, the two metal sensing sites have different Zn-binding affinities ($\sim 10^{13}$ and $\sim 10^{12} \text{ M}^{-1}$, respectively) [50]. This observation indicates that a Zur dimer binds Zn with negative cooperativity, which allows Zur to sense a wider range of cellular Zn concentrations and gradually derepress target operons [50, 51]. It also reveals a role for Zur in buffering cellular Zn levels at very low concentrations, which likely prevents mismetallation of other Fe- or Mn-binding proteins that could bind Zn^{2+} with greater affinity than their cognate metals based on the relative positions of each ion within the **Irving-Williams series** [50]. In *S. aureus*, intracellular levels of free Zn are normally buffered at levels below those of Fe and Mn [35].

During Zn-replete conditions in *S. aureus*, Zur represses the expression of *mreAB*, which encodes for Zn importers [29]. However, a *zur* mutant does not display increased Zn sensitivity, suggesting that other mechanisms exist to protect *S. aureus* from cytosolic Zn accumulation [29]. Zur has also been shown to regulate transcription of the Zn metallochaperone ZigA [52]. It was recently reported that the *cnt* locus is dual regulated by Zur and Fur [53]. The *cnt* operon (previously named *oppI*) is comprised of three gene clusters which function in the synthesis, transport, and export of staphylopin [21]. Analysis of these gene clusters found that the promoter regions contained bona fide Fur- and Zur-boxes, which are occupied by Fur and Zur, respectively, during metal-replete conditions to repress *cnt* transcription [53]. These observations describe a previously unknown link between Zur and staphylopin-dependent metal transport, which plays an important role in virulence and infection outcomes [54].

PerR is the third Fur family member present in *S. aureus*. First characterized in *B. subtilis*, PerR is a metal-dependent peroxide sensor that regulates inducible peroxide-defense genes [55]. PerR contains two metal-binding sites: a structural Zn^{2+} site and a regulatory metal binding site that coordinates either Fe^{2+} or Mn^{2+} [56]. In the presence of low intracellular

concentrations of peroxide, oxidation of PerR is catalyzed by the bound Fe^{2+} ion, which leads to the incorporation of an oxygen atom into nearby histidine ligand(s) within the PerR metal binding site [33, 57]. Oxidation induces a conformational change in PerR, resulting in decreased affinity for its DNA operator sequence and derepression of target gene transcription [58]. The *S. aureus* PerR regulon includes genes involved in ROS metabolism (*katA*, *ahpCF*, *trxB*) and iron regulation and storage (*fur*, *bcp*, *ftn*), revealing a key connection between peroxide stress response and Fe homeostasis [42, 59–61]. In *S. aureus*, PerR is hypersensitive to peroxide and the oxidized Fe^{3+} bound form predominates under normal growth conditions [62]. Work in *B. subtilis* demonstrated that most target regulons are repressed by both the Mn and Fe forms of PerR, but some genes (including *fur* and *perR* itself) are repressed only when PerR is bound to Mn^{2+} [63]. In these cases, PerR functions as a sensor of the cellular Fe/Mn ratio. It is unclear what determines the molecular basis for this differential regulation, or if a similar role for PerR is conserved in *S. aureus*.

Heme sensor system (HssRS)

The concentration of free iron in host tissues is maintained at low concentrations that is likely unable to support the growth of *S. aureus* [64]. Nonetheless, host tissues are rich in other forms of iron, such as heme, which serves as a key cofactor for hemoproteins found in human blood. Heme is thought to be the preferred Fe source for *S. aureus* [65]. To satisfy iron nutrient requirements, *S. aureus* utilizes the iron-regulated surface determinant system (Isd) to acquire, traffic, import, and release of iron ions from heme [22] (see Figure 2). Additionally, it has been proposed that a second locus, known as the heme transport system (*htsABC*), is also involved in heme import [65, 66]. Several studies have indicated that Hts is a transporter of staphyloferrin A [18, 19]. However, it remains unclear whether Hts also directly imports heme, and if so, what are the mechanism(s) of transport. Expression of both the *isd* and *hts* operons is under the transcriptional control of Fur [67, 68]. During infection, the Isd and Hts systems are integral to staphylococcal virulence and pathogenesis [65, 69].

At high concentrations heme can be a toxic molecule capable of generating ROS and cellular damage [70]. To modulate intracellular heme levels and to protect from the toxic side effects of heme, *S. aureus* encodes for heme detoxification machinery, which is regulated by the heme sensor system, HssRS [71]. HssRS is a two-component regulatory system comprised of a membrane-localized histidine kinase (HssS) and a cytoplasmic response regulator with a DNA-binding domain (HssR) [72] (Figure 2). The generally accepted model for HssRS function is consistent with other two-component systems, in which HssS recognizes a heme-associated stimulus, triggering the phosphorylation of HssR [72]. Phosphorylation increases the binding affinity of HssR for target DNA sequences and stimulates their transcription [72]. HssRS may represent one of only a few described two-component regulatory systems that responds to hemoglobin, a molecular marker of vertebrate tissue [73–75].

Upon stimulation, HssRS increases transcription of the heme-regulated ABC transporter HrtAB [71, 72] (Figure 2). *S. aureus* strains lacking either *hrtA* or *hrtB* fail to adapt to heme toxicity [71]. Perhaps counterintuitively, in murine abscess models of infection an *S. aureus* *hrtA* mutant strain exhibited increased virulence in liver tissue [71]. It is suggested that this phenotype arises from a dramatic increase in expression and secretion of

immunomodulatory effectors observed in the *hrtA* mutant, which resulted in inhibited recruitment of host neutrophils to the site of infection [71].

The nature of the signal that is directly sensed by the HssS histidine kinase has remained elusive. It has been speculated that HssS may sense heme by direct binding. However, bioinformatic-based approaches do not reveal a potential heme-binding domain in HssS, given that the predicted sensing domain of HssS shares little sequence similarity to known heme-binding proteins and does not contain conserved ligands typically engaged in iron coordination [4, 72]. One possibility is that rather than binding heme directly, HssS may sense one of the toxic effects that heme has on the cell, such as the buildup up heme metabolites which arise from the breakdown of heme by *S. aureus* [72]. Similarly, it remains unclear whether HrtAB directly exports heme molecules and/or potentially toxic heme metabolic byproduct(s) [76]. It is unclear if HssRS has other targets beyond *hrtAB* [72].

Copper-sensitive operon regulator (CsoR)

Unlike the other metal ions discussed herein, copper is not thought to be an essential micronutrient in *S. aureus*. The only predicted Cu-utilizing enzyme in *S. aureus* is the QoxABCD *aa3* menaquinol terminal oxidase, which functions redundantly with a second, Cu-independent cytochrome *bd* terminal oxidase, CydAB [77]. Therefore, copper is not essential for dioxygen respiration in *S. aureus* during growth under standard laboratory conditions. However, a *qox* mutant strain is defective in liver colonization of a murine infection model, suggesting that Cu acquisition and/or utilization could be an important process in the host [78].

Upon entering *S. aureus*, intracellular reductants, including low-molecular weight thiols, reduce Cu^{2+} to Cu^{1+} . Intracellular Cu^{1+} can be cytotoxic if concentrations are not carefully regulated [79]. The copper-sensitive operon regulator (CsoR) is a Cu^{1+} -responsive regulator of copper homeostasis in *S. aureus* (see Figure 3) [80]. As a member of the larger CsoR/RcnR family of bacterial transcriptional repressors, CsoR forms a tetrameric structure and uses a WXYZ amino acid fingerprint to coordinate copper ions [81]. There is not a structure of a CsoR family regulator in complex with DNA; however, biophysical studies suggest that two CsoR proteins bind symmetrically per operator sequence [81, 82]. Cu^{1+} binding by CsoR is predicted to induce a conformational change which compacts the protein structure and allosterically inhibits DNA binding, causing release of operator DNA [82, 83].

The CsoR regulon in *S. aureus* consists of the copper-sensitive operon *copAZ*, which contains genes encoding proteins involved in Cu^{1+} copper efflux (CopA) and cytosolic buffering and scavenging (CopZ) [80, 84] (Figure 3). Recently, it was discovered that in addition to *copAZ*, CsoR regulates the *copBL* (*copB* has also been referred to as *copX*) and *copBmco* operons, found on mobile DNA, which provide hypertolerance to Cu in some *S. aureus* strains [79, 84–86]. Genetic evidence suggests that *copB* encodes a second Cu^{1+} exporter that is functionally redundant with CopA, while *copL* encodes for a membrane-bound, surface-exposed, Cu^{1+} -binding lipoprotein [79]. It is proposed that *copL* functions to prevent copper uptake by binding extracellular copper ions and preventing them from entering or re-entering the cell after export by CopA or CopB [79] (Figure 3). In some *S.*

aureus strains, CsoR also regulates the transcription of *mco*, which encodes a predicted Cu-dependent multicopper oxidase, although the precise function(s) of Mco remains unknown [87]. *S. aureus* strains lacking the *copAB* and *copBmco* operons have decreased survival in phagocytes [86]. These findings highlight the importance of proper Cu homeostasis for pathogenesis. Moreover, *copBL* is the only genetic operon shared between the ACME and COMER transposable elements, which were acquired by the USA300 and USA300-LV clones, respectively [88]. These strains have contributed clonal epidemics of community acquired *S. aureus* in North American (USA300) and South American (USA300-LV) countries [88]. Shared *copBL* acquisition suggests that the hypertolerance to Cu may have helped facilitate the spread of these clones.

Manganese transport regulator (MntR)

Manganese is an essential micronutrient in *S. aureus* where it is utilized as a cofactor of superoxide dismutase and plays a key role in defending against the oxidative burst of phagocytes during infection [1]. Two staphylococcal systems are used to import Mn ions from the environment: the MntABC ABC-type transporter and the MntH Nramp family transporter [89] (see Figure 4). During pathogenesis, both the MntABC and the MntH systems compete with calprotectin to acquire Mn in tissue abscesses [90].

Transcription of *mntABC* is regulated by the DtxR family metalloregulator MntR [89] (Figure 4). MntR shares a similar structural architecture to other metalloregulators, consisting of an N-terminal HTH-motif DNA-binding domain and a C-terminal dimerization domain, with the metal sensing site located between the two domains [91]. The metal binding site of each MntR monomer can occupy two metal ions. The coordination geometry determines the selective response of MntR to its cognate metals; larger metal ions (Mn^{2+} and Cd^{2+}) form a binuclear complex with MntR and are activated, while smaller metal ions (Fe^{2+} , Co^{2+} , and Zn^{2+}) do not fully occupy the site resulting in low activity [92, 93]. The interdomain region of MntR is flexible and can adopt a range of orientations relative to the dimerization domain. Upon Mn^{2+} or Cd^{2+} binding, the linker region is rigidified, which restricts the conformation of the protein and is thought to promote DNA binding [94]. There is not yet a structure of MntR in complex with DNA.

The promoters of both *mntABC* and *mntH* contain proposed MntR box sequences that are binding sites recognized by MntR. In *B. subtilis*, MntR acts a repressor of *mntABC* transcription and an activator of *mntH* transcription [95]. There are conflicting studies on the role of manganese and MntR in *mntH* transcription in *S. aureus*. While one report noted Mn-dependent regulation of *mntH* transcription, others have found little to no effect ([89, 90] and Al-Tameemi *et al.* under review). These seemingly discordant observations may be the result of differing culture conditions or different genetic backgrounds.

The gene encoding the RsaC small non-coding RNA is co-transcribed with *mntABC* and regulated by MntR [96]. RsaC interacts with the superoxide dismutase (*sodA*) transcript to inhibit its expression [96]. By decreasing *sodA* expression, RsaC prevents the synthesis of Mn^{2+} -dependent SodA when Mn is limiting. RsaC also interacts with the *sufC* transcript which encodes an iron-sulfur cluster synthesis enzyme, as well as *zur*, *znuB*, and *znuC* that

function in Zn homeostasis [96, 97]. These findings suggest potential crosstalk between Mn, Zn, and Fe homeostasis. *mntABC* transcription is also negatively regulated by PerR [89].

Like other metals, excess cytosolic Mn is cytotoxic. Until recently, the mechanism(s) of Mn efflux in *S. aureus* were unclear. In the presence of high Mn, *S. aureus* expresses the cation diffusion facilitator family transporter MntE to remove excess cytosolic Mn [98] (Figure 4). Upregulation of MntE was shown to be dependent on the presence of MntR, but it remains unknown if MntR directly regulates *mntE* transcription [98]. An *S. aureus* strain lacking MntE exhibits accumulated intracellular Mn, but decreased levels of intracellular Fe, highlighting the interplay between Fe and Mn homeostasis [98]. The presence of MntR and MntE were also required for full virulence of *S. aureus* in murine models of infection, indicating the importance of controlling intracellular Mn levels for pathogenesis [98].

Concluding Remarks

The acquisition of metal ions and prevention of cytosolic metal ion imbalance are essential processes for all microbes. Here, we have discussed how these processes are controlled by a set of metalloregulators that carefully monitor and respond to alterations in the concentrations of cytosolic metal ion pools in *S. aureus*. These responses vary from the increased import of necessary metals to the removal of unwanted, cytotoxic ions or small molecules. Recently, researchers have also highlighted the roles of these metalloregulators in controlling the transcription of efflux pumps that help restore metal ion homeostasis when titers exceed ideal levels [79, 98]. The study of metal ion homeostasis in *S. aureus* continues to gain attention because of four key discoveries: (1) hosts protect themselves through nutritional immunity by sequestering specific metal ions [6], (2) hosts use Cu ions to aid bacterial killing in the phagolysosome [99], (3) *S. aureus* uses metal detoxification genes, including those encoded on mobile DNA, to circumvent the human immune system [85, 97], and (4) metal ion acquisition and homeostasis are promising antimicrobial targets [3]. There is also renewed interest in the roles of metalloregulators in controlling the expression of *S. aureus* genes that function in pathogenetic actions including toxin production and biofilm formation. Future studies will uncover if these phenotypes are through direct regulation or an indirect consequence of altered cytosolic metal ion pools. As the threat of antibiotic resistant *S. aureus* increases, metal ion acquisition and homeostasis remain viable targets for antimicrobial development.

Glossary

Fenton reaction

the chemical process in which peroxide (H_2O_2) is converted to a hydroxyl radical ($\cdot\text{OH}$) via a metal ion catalyst, typically Fe^{2+}

Fur box

a conserved, inverted repeat sequence of DNA found in the promoter region of Fur target genes; the binding site of typical Fur occupation during iron-replete conditions (also note: MntR box, Per box, and Zur box, which are the sites of MntR, PerR, and Zur binding, respectively)

Heme

an iron-containing compound of the porphyrin class which functions as the cofactor for hemoglobin (within erythrocytes) and myoglobin (within myocytes)

Irving-Williams series

a reference order of the relative stabilities of complexes formed between ligands and divalent transition metals ($Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$)

Metallophore

an excreted soluble molecule that transiently binds to metal ions to aid in their sequestration and transport

Metalloregulator

a DNA-binding protein that alters the transcription of DNA to RNA in direct response to the availability of metal ion(s)

Micronutrient:

a vitamin or mineral that is required in trace amounts for the normal growth and development of an organism

Mismetallation

occurs when an inappropriate metal ion has occupied the metal binding site of a holo-metalloprotein

Nutritional immunity

the process during infection in which a host sequesters nutrients essential to bacterial fitness

S100 proteins

a family of human proteins produced by the host immune system that tightly bind to metal ions to aid nutritional immunity

Siderophore

a high-affinity, iron-chelating compound secreted by microorganisms that is used to sequester and transport iron ions from the environment into the cell

Staphylopine

a nicotinanamine-like metallophore produced by *S. aureus* that aids in the acquisition and transport of Co^{2+} , Zn^{2+} , and Ni^{2+}

Two-component regulatory system

a stimulus-response coupling mechanism used by prokaryotes to detect and adapt to changes in environmental conditions; consists of a membrane-localized sensor kinase and a DNA-binding cytosolic response regulator

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Highlights

- The Fe-responsive metalloregulator Fur controls expression of virulence factors, including exoproteins, suggesting that the *S. aureus* Fur-dependent regulon extends beyond genes utilized in Fe homeostasis.
- Fur and Zur co-operate to regulate synthesis of staphylopine, a metallophore used to acquire metal ions by competing with host factors that provide nutritional immunity.
- The HssRS two-component regulatory system prevents heme intoxication by sensing cytosolic heme levels and increasing expression of HrtAB, which exports heme or heme metabolites.
- The Cu-responsive metalloregulator CsoR controls transcription of the *copAZ* Cu detoxification genes and the newly described *copBL* and *copBmco* operons that provide hypertolerance to Cu.
- The MntR metalloregulator preserves Mn homeostasis by regulating transcription of the Mn importer *mntABC*, as well as the expression of *mntE* to export excess cytosolic Mn.

Outstanding Questions

- Free cytosolic metal ions can be cytotoxic. Once a metal ion has entered a *S. aureus* cell, are there factors that act as intracellular metal ion buffers? Do small molecules, such as low molecular weight thiols, play a role in helping to buffer the cytosol and/or facilitate the donation of metal ions to metalloregulators [100]?
- The apo-form of the *E. coli* Fur metalloregulator was found to be proteolytically degraded upon the loss of its metal cofactor [101]. Are *S. aureus* metalloregulators recycled or degraded if they are not associated with DNA or metal ions?
- A large portion of intracellular Fe is used to synthesize iron-sulfur (FeS) clusters in *S. aureus* and other bacteria. What role(s) do metalloregulators have in regulating this process?
- In *E. coli*, Fur acts as an indirect oxygen sensor by sensing the balance between pools of Fe²⁺ and Fe³⁺. Does Fur use a similar mechanism in *S. aureus* to detect alterations in intracellular redox status?
- A number of loci (example: *acnA*) have decreased expression in an *S. aureus* *fur* mutant. Does Fur activate gene expression in *S. aureus*, and if so, what is the mechanism of activation? Does apo-Fur play a regulatory role in *S. aureus*?
- In *B. subtilis*, mRNA transcripts corresponding to more than twenty genes have significantly altered abundances in a *zur* mutant, and eighty direct Zur-binding sites have been identified [102]. What genes are regulated by Zur in *S. aureus*?
- PerR functions as a sensor of the cellular Fe/Mn ratio in *B. subtilis*. Is this role of PerR conserved in *S. aureus*?
- The HssSR two-component regulatory system responds to cytosolic heme accumulation. Does HssS directly bind heme, or is it activated by a heme-associated byproduct?
- The HtsABC transporter imports iron via transport of staphyloferrin A. Does the Hts system also play a direct role in heme transport, and if so, what is the mechanism?
- The CsoR metalloregulator aids in preventing intoxication by Cu ions. What is the mechanism(s) of copper toxicity in *S. aureus*?
- In *B. subtilis*, MntR acts a repressor of *mntABC* transcription and an activator of *mntH* transcription [95]. Does MntR play a role in *mntH* expression in *S. aureus*?

Figure 1: Structure and activation mechanism of Fur metalloregulators.

(A) Fur metalloregulators are dimers. Each monomer consists of an N-terminal DNA-binding domain (tan) and a C-terminal dimerization domain (green), which are connected by a hinge region (purple). There are typically two metal binding sites per monomer: the Fe²⁺ sensing site (orange) is coordinated by residues in the hinge region, and the non-regulatory Zn²⁺ site (blue) is located within the dimerization domain. In the apo-state (left), the conformations of the two monomers differ. Upon metal and DNA binding (right), Fur adapts a caliper-like conformation with a two-fold axis of symmetry (PDB IDs 4RB0 and 4RB1) [30]. (B) *Left:* When free iron is limiting, Fe²⁺ is released from Fur, which triggers dissociation of the apo-Fur dimer from the Fur box, and subsequent binding and transcription initiation by RNA polymerase. *Right:* During iron-replete conditions, Fur binds Fe²⁺ and associates with the inverted repeat Fur box within the promoter of the target gene. When bound to the Fur box, the Fur dimer occludes the -35 and/or -10 initiation sites, which inhibits RNA polymerase binding and represses gene transcription.

Figure 2: Heme sensing and signaling in *S. aureus*.

(1) Heme is imported into the cytoplasm via the Isd system. It has been proposed that Hts, which has an established role in siderophore import, may also play a role in heme transport. (2) The HssS histidine kinase is activated by a heme-associated stimulus. Whether HssS directly senses heme or a byproduct of heme metabolism remains unknown. (3) Activation of HssS results in autophosphorylation of HssS and transphosphorylation of the HssR response regulator. Phosphorylated HssR binds to the promoter sequence of *hrtAB* and stimulates its transcription via recruitment of RNA polymerase. (4) Heme, or a heme-associated byproduct, is effluxed by the HrtAB exporter.

Figure 3: Model for copper ion homeostasis in *S. aureus* USA300.

The CsoR transcriptional regulator binds intracellular Cu^{1+} , leading to derepression of the *copAZ* and *copBL* operons. *copBL* (*copB* has also referred to as *copX*) is encoded on the ACME mobile genetic element. Cu^{1+} is effluxed via the CopA and CopB exporters. Extracellular copper binds to CopL lipoprotein on the surface of the cell, where it is prevented from entering or re-entering the cytoplasm. Intracellular copper ions are scavenged and buffered by the CopZ metallochaperone, which can traffic the Cu to CopA. The mechanism(s) of copper import remain unknown.

Figure 4: Model for manganese ion homeostasis in *S. aureus*.

Mn²⁺ ions are imported into the cell by the MntABC and MntH transporters. The MntR transcriptional regulator binds intracellular manganese and represses transcription of *mntABC*. It is unclear if transcription of *mntH* is Mn-dependent and/or regulated by MntR. To prevent cytotoxicity, excess Mn is effluxed by MntE. While upregulation of *mntE* is dependent on the presence of MntR, it is not known if this effect is a result of direct or indirect regulation.