Published in final edited form as:

Curr Opin Microbiol. 2012 February ; 15(1): 10–14. doi:10.1016/j.mib.2011.11.004.

The impact of metal sequestration on *Staphylococcus aureus* metabolism

Neal D. Hammer and Eric P. Skaar^{*}

Summary of recent advances

The Gram-positive pathogen *Staphylococcus aureus* poses a serious risk to public health due to its prevalence as a commensal organism, its ability to cause a multitude of diseases, and the increasing incidence of antibiotic resistant strains. *S. aureus* infects diverse niches within vertebrates despite being challenged by a robust immune response. The host-pathogen confrontation occurs in an environment nearly devoid of metals that are essential for bacterial proliferation. *S. aureus* is able to flourish in these conditions and often causes significant morbidity and mortality. This review highlights current themes pertaining to the process of host-mediated metal sequestration known as “nutritional immunity”, *S. aureus* metal acquisition strategies, and how proliferating within a metal restricted environment impacts bacterial physiology.

Introduction

Staphylococcus aureus is a leading cause of global morbidity and mortality [1-3]. This extracellular pathogen innocuously colonizes the anterior nares of one-third of the world’s population and is commonly associated with commensal colonization of the skin [4,5]. Vertebrates decrease the infectious capacity of invading microbes by limiting the availability of essential nutrients in a process known as nutritional immunity [6]. Once *S. aureus* breaches the epithelium, it survives within the bloodstream and disseminates to the peripheral organs despite limited access to nutrient metals [7,8]. A pathological hallmark of *S. aureus* infection is the formation of tissue lesions called abscesses (Fig. 1). Abscesses represent the extensive accumulation of neutrophils that surround invading staphylococci in an attempt to confine bacterial proliferation. In a mouse model of systemic infection, *S. aureus* colonizes peripheral tissues 1 to 3 hours post infection but abscess formation in the kidneys, heart and liver is not observed until 4 days post infection. This mimics the pathology of human disease and highlights the ability of *S. aureus* to colonize diverse niches within the host [7].

Neutrophils restrict the growth of *S. aureus* by creating an environment devoid of available iron, manganese, and zinc. These metals are required cofactors for metabolic processes that facilitate staphylococcal growth [9]. Despite restricted access to these metals, *S. aureus* can cause life threatening disease. Understanding the mechanisms by which *S. aureus* survives

© 2011 Elsevier Ltd. All rights reserved.

^{*}To whom correspondence should be addressed. Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, 1161 21st Ave., South, A5102 MCN, Nashville, TN 37232-2363, (615) 343-0002 PH., (615) 343-8492 Fax, eric.skaar@vanderbilt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

within the metal-depleted environment of the host will provide an enhanced understanding of metabolic processes utilized by this pathogen during infection and will lead to new avenues for combating staphylococcal disease. Guided by this perspective, this review will (i) define the mechanisms by which vertebrates sequester iron, manganese, and zinc, (ii) describe the strategies *S. aureus* utilizes to overcome metal sequestration, and (iii) present ideas on how nutritional immunity may impact *S. aureus* physiology during infection.

Vertebrates restrict access to essential metals

Iron, manganese, and zinc are transition metals that act as cofactors for enzymes involved in many cellular processes including DNA synthesis, respiration, and defense against reactive oxygen and nitrogen species [10,11]. The absence of iron, manganese, or zinc severely impedes staphylococcal growth *in vivo* and *in vitro* [9,12]. Greater than 90% of iron in vertebrates is found within host cells, rendering it unavailable to *S. aureus* [13]. The intracellular localization of iron represents the first barrier of nutritional immunity that must be circumvented by extracellular pathogens. Additionally, host proteins that sequester iron, manganese, and zinc provide another barrier to *S. aureus* proliferation.

The host restricts the amount of available iron in the serum through the iron-binding activity of the glycoproteins lactoferrin and transferrin (Fig. 2A). However, the majority of iron (75 to 80%) is complexed to the tetrapyrrole ring of heme [13]. Most heme-iron is bound by hemoglobin that functions to transport oxygen to peripheral tissues within circulating erythrocytes (Fig. 2A). Hemoglobin is the most abundant heme-binding protein in vertebrates, making it an attractive iron source for pathogens that are able to liberate it from erythrocytes [14]. In the event of erythrocyte lysis, the host proteins hemopexin and haptoglobin sequester the liberated heme and hemoglobin, respectively, providing another barrier to heme acquisition [15,16]. *S. aureus* utilizes a multifaceted approach that targets transferrin and hemoglobin in an effort to satisfy its requirement for nutrient iron (Fig. 2B).

In addition to restricting iron availability, the host also limits the availability of manganese and zinc (Fig. 2A). The host protein calprotectin inhibits the growth of *S. aureus* by sequestering manganese and zinc [9]. Calprotectin is a major protein constituent of the neutrophil cytoplasm and is found within abscesses at concentrations in excess of 1 mg/ml [17,18]. The observation that a ring of necrotic neutrophils surround the staphylococci within the abscess suggests that calprotectin is released from the neutrophils as a result of cell lysis [7]. Following intravenous challenge with *S. aureus*, calprotectin-deficient mice exhibit increased bacterial loads in the liver underscoring the importance of this host protein to protection against infection [9]. Although manganese levels are increased within abscesses of calprotectin-deficient animals compared to their wildtype counterparts, zinc levels are not changed suggesting a calprotectin-independent mechanism for zinc sequestration within abscesses. *S. aureus* is able to persist within tissue abscesses despite host-mediated metal chelation [7]. Given the importance of manganese and zinc to staphylococcal metabolism, it is likely that *S. aureus* is able to outcompete the host for these metals; however, the mechanisms by which manganese and zinc are acquired during infection are only beginning to emerge [19].

S. aureus metal acquisition strategies

The iron-limited environment of the host triggers the expression of *S. aureus* iron acquisition systems through alleviation of Fur-mediated transcriptional repression [20,21]. Fur represses the transcription of genes involved in iron acquisition when *S. aureus* is grown in iron-replete conditions. Transcription is activated when *S. aureus* encounters an iron-deplete environment resulting in the expression of the staphylococcal iron acquisition systems. These systems target the most abundant sources of iron, the iron-containing host

proteins hemoglobin and transferrin. *S. aureus* obtains iron by importing host heme directly or by stealing iron from transferrin through the activity of iron-chelating siderophores.

The first step in staphylococcal heme-iron acquisition is the release of hemoglobin from erythrocytes through hemolysin-mediated erythrocyte lysis [22]. Upon release, hemoglobin is captured by the *S. aureus* iron-regulated surface determinant system (*isd*). The Isd system catalyzes the extraction of heme from hemoglobin and the passage of heme through the cell wall. The transfer of heme from the *S. aureus* hemoglobin receptor through the bacterial cell wall is mediated by proteins containing NEAT domains [23]. An *isd*-encoded ABC transporter facilitates the import of heme into the cytoplasm (Fig. 2B). Once in the cytoplasm, heme is degraded by *isd*-encoded heme oxygenases, facilitating the release of iron [24,25]. Heme is the preferred iron-source of *S. aureus* [26] and the importance of the *isd* system to pathogenesis is best exemplified by studies revealing that the staphylococcal hemoglobin receptor IsdB is required for full virulence [27]. IsdB is also required for renal abscess formation five days post infection [7]. These facts underscore the significance of *isd*-mediated heme-iron acquisition to *S. aureus* pathogenesis. Notably, the essentiality of iron has ensured that *S. aureus* has evolved additional mechanisms for acquiring this nutrient during infection.

Staphyloferrin A and staphyloferrin B are siderophores produced by *S. aureus* that function to scavenge iron in serum (Fig. 2B). The cognate lipoprotein receptors and associated import systems for staphyloferrin A and B are well characterized and have been reviewed elsewhere [20,28]. *S. aureus* strains unable to produce or utilize either staphyloferrin A or staphyloferrin B are attenuated in their ability to cause disease [29,30]. These siderophores provide an alternative source of iron for *S. aureus* if the bacteria are unable to acquire heme during infection. This approach to iron acquisition affords *S. aureus* the flexibility to meet the challenges of the iron-limiting environment of the host.

The transcriptional repressors MntR and Zur allow *S. aureus* to respond to manganese and zinc deplete environments, respectively, and regulate the expression of high affinity metal transporters [19,31]. In the absence of manganese and zinc, MntR- and Zur-mediated transcriptional repression is alleviated and the manganese and zinc transport systems are activated. The importance of the manganese transporter MntABC is evident when *S. aureus* is grown in the presence of calprotectin (Fig. 2B). Strains inactivated for *mntA* and *mntB* and hence are unable to transport manganese, are more sensitive to the effects of calprotectin than wildtype *S. aureus* [9]. The contribution of the Mnt system to staphylococcal pathogenesis is beginning to be resolved [9,19], but given the significance of manganese and zinc to *S. aureus* growth it is likely that the full arsenal of manganese and zinc acquisition pathways have yet to be uncovered.

The impact of nutritional immunity on *S. aureus* physiology

Bacterial metal acquisition has been referred to as the critical determinant for the outcome of infection [6]. However, little is known about the role of acquired metals in staphylococcal physiology during infection. Elucidating the protein recipients of host-derived iron, manganese, and zinc will uncover the metabolic pathways that are active during *S. aureus* pathogenesis. A clue to the identity of these proteins comes from a unique feature of the metals: distinctive redox potentials which make them ideal cofactors for enzymes involved in transferring electrons between molecules. The transfer of electrons is critical to *S. aureus* physiology during infection for at least two reasons: (i) the enzymatic reduction of reactive oxygen and nitrogen species detoxifies these poisonous molecules and (ii) the transfer of electrons is essential for the generation of a proton motive force (PMF) that increases energy production through respiration (Fig. 2C). As many a 30% of bacterial proteins require a

metal cofactor, so it is likely that other metabolic processes will be affected by host-mediated metal sequestration [10,32,33].

The host immune response to bacterial infection includes the generation of the effector molecules superoxide and nitric oxide. Bacterial pathogens detoxify these molecules through the action of superoxide dismutase, catalase, and flavohemoglobin [19,34,35]. The genome of *S. aureus* encodes homologues of these metal-dependent enzymes and the importance of superoxide dismutase and flavohemoglobin to *S. aureus* pathogenicity has been established experimentally [34,36,37]. The sequestration of manganese by calprotectin increases the sensitivity of *S. aureus* to superoxide stress during infection. This is due to the reduced activity of the staphylococcal manganese-dependent superoxide dismutases, SodA and SodM [37]. Inactivation of *sodA* and *sodM* leads to reduced bacterial loads in the livers of systemically infected mice and in the abscesses of subcutaneously-infected mice, highlighting the importance of these enzymes to bacterial survival within the host [36,37].

Compared to other bacteria, *S. aureus* exhibits increased resistance to the effects of nitric oxide in part due to the expression of flavohemoglobin, which is encoded by the *hmp* gene (Fig. 2C). Flavohemoglobin mutants are less virulent in the systemic mouse model of infection [34]. Interestingly, flavohemoglobins typically require heme as a cofactor [38]. The heme requirement of *S. aureus* flavohemoglobin remains to be experimentally verified, but the fact that *S. aureus* can synthesize heme *de novo* is consistent with the possibility that Hmp is a hemoprotein. This *de novo* synthesis of heme requires iron and the gene products from the *hemAXCDBL*, *hemEHY*, *hemN*, and *hemZ* loci [39, for review see 40]. In addition to being used as a potential cofactor for flavohemoglobin, heme is an essential cofactor for *S. aureus* respiratory metabolism. The finding that inactivation of the heme-dependent cytochrome subunit *qoxB* leads to a significant reduction of bacterial loads in the liver emphasizes the importance of respiratory metabolism during staphylococcal infection [41]. The ability to both synthesize and acquire heme likely affords *S. aureus* and many other pathogenic bacteria the metabolic flexibility to overcome the challenges of colonizing diverse niches within the vertebrate host.

It is unknown how *isd*-mediated heme acquisition coordinates with heme synthesis pathways to maximize survival of *S. aureus* during infection. The iron status of the bacterium likely determines how exogenous heme is utilized. For example, when *S. aureus* encounters an environment where heme is the sole iron source, it is degraded to release iron. Alternatively, at sites of colonization where iron is available due to siderophore-mediated acquisition, exogenous heme might be more valuable as a cofactor. This contention is supported by *in vitro* experiments demonstrating that heme is segregated intact to the membrane when *S. aureus* is grown in the presence of alternative iron sources [26]. The significance of this finding is underscored by the fact that membrane-localized, heme-dependent cytochromes and reductases facilitate respiratory metabolism. These results suggest that *S. aureus* has the ability to alter exogenous heme trafficking in order to maximize respiration during infection.

Staphylococci that cannot respire are forced to produce energy through fermentation, which reduces both growth and hemolysin production [42,43]. However, fermentative enzymes such as alcohol dehydrogenases also require metal cofactors [42], supporting the possibility that the fermentative pathways of *S. aureus* are affected by the availability of metals during infection. Recent analysis of protein databases estimates that one third of all proteins require a metal cofactor [44-46]. Clearly, there is much to be learned regarding how alterations in metal availability impact *S. aureus* physiology during infection.

Concluding remarks

The transition metals iron, manganese, and zinc are vital for the proliferation of bacterial pathogens such as *S. aureus*. Vertebrates exploit this requirement through metal sequestration as a means to control bacterial growth. *S. aureus* has evolved strategies to overcome this nutritional immunity and is capable of causing significant morbidity and mortality. Given the importance of metals to *S. aureus* proliferation during infection, the pathways dedicated to metal acquisition and the protein recipients of these metals are appealing targets for therapeutic intervention. Inhibiting these pathways has the potential to restrict the capacity of *S. aureus* to generate energy and defend itself against reactive oxygen and nitrogen species. This possibility is particularly attractive considering *S. aureus* has the capability to resist all currently available antimicrobials.

References

1. Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA*. 2005; 293:3012–3021. [PubMed: 15972563]
2. Kleven RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*. 2007; 298:1763–1771. [PubMed: 17940231]
3. McCaig LF, McDonald LC, Mandal S, Jernigan DB. *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. *Emerg Infect Dis*. 2006; 12:1715–1723. [PubMed: 17283622]
4. Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, et al. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. *J Infect Dis*. 2006; 193:172–179. [PubMed: 16362880]
5. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med*. 1998; 339:520–532. [PubMed: 9709046]
6. Weinberg ED. Iron and susceptibility to infectious disease. *Science*. 1974; 184:952–956. [PubMed: 4596821]
7. Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. *FASEB J*. 2009; 23:3393–3404. [PubMed: 19525403] An elegant study that demonstrates the heme scavenging factor IsdB, among other staphylococcal proteins, is required for persistence and abscess formation in the murine model of infection.
8. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005; 3:948–958. [PubMed: 16322743]
9. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, Anderson KL, Dattilo BM, Dunman PM, Gerads R, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science*. 2008; 319:962–965. [PubMed: 18276893]
10. Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM. Metal ions in biological catalysis: from enzyme databases to general principles. *J Biol Inorg Chem*. 2008; 13:1205–1218. [PubMed: 18604568]
11. Bullen, JJ.; Griffiths, E. *Iron and Infection: Molecular, Physiological and Clinical Aspects*. John Wiley and Sons; New York: 1999.
12. Pishchany G, McCoy AL, Torres VJ, Krause JC, Crowe JE Jr, Fabry ME, Skaar EP. Specificity for human hemoglobin enhances *Staphylococcus aureus* infection. *Cell Host Microbe*. 2010; 8:544–550. [PubMed: 21147468]
13. Drabkin D. Metabolism of the Hemin Chromoproteins. *Physiological Reviews*. 1951; 31:345–431. [PubMed: 14891376]
14. Crichton, R. *Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences*. Vol. 2. John Wiley & Sons, Ltd.; West Sussex, England: 2001.

15. Hwang PK, Greer J. Interaction between hemoglobin subunits in the hemoglobin. haptoglobin complex. *J Biol Chem.* 1980; 255:3038–3041. [PubMed: 7358726]
16. Tolosano E, Altruda F. Hemopexin: structure, function, and regulation. *DNA Cell Biol.* 2002; 21:297–306. [PubMed: 12042069]
17. Gebhardt C, Riehl A, Durchdewald M, Nemeth J, Furstenberger G, Muller-Decker K, Enk A, Arnold B, Bierhaus A, Nawroth PP, et al. RAGE signaling sustains inflammation and promotes tumor development. *J Exp Med.* 2008; 205:275–285. [PubMed: 18208974]
18. Clohessy PA, Golden BE. Calprotectin-mediated zinc chelation as a biostatic mechanism in host defence. *Scand J Immunol.* 1995; 42:551–556. [PubMed: 7481561]
19. Horsburgh MJ, Wharton SJ, Cox AG, Ingham E, Peacock S, Foster SJ. MntR modulates expression of the PerR regulon and superoxide resistance in *Staphylococcus aureus* through control of manganese uptake. *Mol Microbiol.* 2002; 44:1269–1286. [PubMed: 12028379]
20. Beasley FC, Heinrichs DE. Siderophore-mediated iron acquisition in the staphylococci. *J Inorg Biochem.* 2010; 104:282–288. [PubMed: 19850350]
21. Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science.* 2003; 299:906–909. [PubMed: 12574635]
22. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev.* 2000; 13:16–34. table of contents. [PubMed: 10627489]
23. Muryoi N, Tiedmann MT, Pluym M, Cheung J, Heinrichs DE, Stillman MJ. Demonstration of the iron-regulated surface determinant (Isd) heme transfer pathway in *Staphylococcus aureus*. *J Biol Chem.* 2008; 283:28125–28136. [PubMed: 18676371]
24. Reniere ML, Skaar EP. *Staphylococcus aureus* haem oxygenases are differentially regulated by iron and haem. *Mol Microbiol.* 2008; 69:1304–1315. [PubMed: 18643935]
25. Skaar EP, Gaspar AH, Schneewind O. IsdG and IsdI, heme-degrading enzymes in the cytoplasm of *Staphylococcus aureus*. *J Biol Chem.* 2004; 279:436–443. [PubMed: 14570922]
26. Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O. Iron-source preference of *Staphylococcus aureus* infections. *Science.* 2004; 305:1626–1628. [PubMed: 15361626]
27. Torres V, Pishchany G, Humayun M, Schneewind O, Skaar E. *Staphylococcus aureus* IsdB is a hemoglobin receptor required for heme iron utilization. *J Bacteriol.* 2006; 188:8421–8429. [PubMed: 17041042]
28. Hammer ND, Skaar EP. Molecular Mechanisms of *Staphylococcus aureus* Iron Acquisition. *Annu Rev Microbiol.* 2011
- *29. Beasley FC, Marolda CL, Cheung J, Buac S, Heinrichs DE. *Staphylococcus aureus* transporters Hts, Sir, and Sst capture iron liberated from human transferrin by Staphyloferrin A, Staphyloferrin B, and catecholamine stress hormones, respectively, and contribute to virulence. *Infect Immun.* 2011; 79:2345–2355. [PubMed: 21402762] This study establishes the importance of siderophore biosynthesis and transport to *S. aureus* pathogenesis.
30. Dale SE, Doherty-Kirby A, Lajoie G, Heinrichs DE. Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*: identification and characterization of genes involved in production of a siderophore. *Infect Immun.* 2004; 72:29–37. [PubMed: 14688077]
31. Lindsay JA, Foster SJ. *zur*: a Zn(2+)-responsive regulatory element of *Staphylococcus aureus*. *Microbiology.* 2001; 147:1259–1266. [PubMed: 11320129]
32. Kehl-Fie TE, Skaar EP. Nutritional immunity beyond iron: a role for manganese and zinc. *Curr Opin Chem Biol.* 2009
33. Somerville GA, Proctor RA. At the crossroads of bacterial metabolism and virulence factor synthesis in Staphylococci. *Microbiol Mol Biol Rev.* 2009; 73:233–248. [PubMed: 19487727]
34. Richardson AR, Dunman PM, Fang FC. The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. *Mol Microbiol.* 2006; 61:927–939. [PubMed: 16859493]
35. Mandell GL. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. In vitro and in vivo studies with emphasis on staphylococcal--leukocyte interaction. *J Clin Invest.* 1975; 55:561–566. [PubMed: 1117067]

36. Karavolos MH, Horsburgh MJ, Ingham E, Foster SJ. Role and regulation of the superoxide dismutases of *Staphylococcus aureus*. Microbiology. 2003; 149:2749–2758. [PubMed: 14523108]
- ** 37. Kehl-Fie TE, Chitayat S, Hood MI, Damo S, Restrepo N, Garcia C, Munro KA, Chazin WJ, Skaar EP. Nutrient metal sequestration by calprotectin inhibits bacterial superoxide defense, enhancing neutrophil killing of *Staphylococcus aureus*. Cell Host Microbe. 2011; 10:158–164. [PubMed: 21843872] This study demonstrates host-mediated *in vivo* inhibition of manganese-dependent bacterial processes.
38. Ilari A, Bonamore A, Farina A, Johnson KA, Boffi A. The X-ray structure of ferric *Escherichia coli* flavohemoglobin reveals an unexpected geometry of the distal heme pocket. J Biol Chem. 2002; 277:23725–23732. [PubMed: 11964402]
39. Kafala B, Sasarman A. Isolation of the *Staphylococcus aureus* *hemCDBL* gene cluster coding for early steps in heme biosynthesis. Gene. 1997; 199:231–239. [PubMed: 9358061]
40. Anzaldi LL, Skaar EP. Overcoming the Heme Paradox: Heme Toxicity and Tolerance in Bacterial Pathogens. Infect. Immun. 2010; 78:4977–4989. [PubMed: 20679437]
- *41. Lan L, Cheng A, Dunman PM, Missiakas D, He C. Golden pigment production and virulence gene expression are affected by metabolisms in *Staphylococcus aureus*. J Bacteriol. 2010; 192:3068–3077. [PubMed: 20400547] This work establishes the importance of the cytochrome *aa₃* subunit, *qoxB*, to infection.
42. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol. 2006; 4:295–305. [PubMed: 16541137]
43. Schlag S, Fuchs S, Nerz C, Gaupp R, Engelmann S, Liebeke M, Lalk M, Hecker M, Gotz F. Characterization of the oxygen-responsive NreABC regulon of *Staphylococcus aureus*. J Bacteriol. 2008; 190:7847–7858. [PubMed: 18820014]
44. Vallee BL, Auld DS. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry. 1990; 29:5647–5659. [PubMed: 2200508]
45. Waldron KJ, Rutherford JC, Ford D, Robinson NJ. Metalloproteins and metal sensing. Nature. 2009; 460:823–830. [PubMed: 19675642]
46. Waldron KJ, Robinson NJ. How do bacterial cells ensure that metalloproteins get the correct metal? Nat Rev Microbiol. 2009; 7:25–35. [PubMed: 19079350]

Highlights

> The vertebrate host sequesters metals in an effort to reduce bacterial growth. > Metal depletion reduces bacterial growth. > *S. aureus* utilizes a multifaceted approach to overcome this metal sequestration. > The staphylococcal pathways that receive host-metals are unknown. > *S. aureus* utilizes host metals to maximize survival during pathogenesis.

Figure 1. *Staphylococcus aureus* proliferates within the metal limited environment of tissues abscesses to cause disease

The host response to *S. aureus* involves the extensive recruitment of neutrophils to the site of infection, leading to the formation of a tissue abscess. Lactoferrin (Lf) and Calprotectin (CP) are produced by neutrophils and severely limit the availability of iron and manganese to staphylococci. In addition, serum transferrin (Tf) further reduces the amount of iron within the host environment. Nonetheless, *S. aureus* has evolved metal acquisition systems that promote pathogenesis and disease.

Figure 2. The struggle for metals during staphylococcal infection

(A) The host proteins that sequester metals and participate in nutritional immunity. Hemoglobin is the major reservoir of heme-iron in vertebrates and is targeted by staphylococci in order to fulfill its requirement for iron. Haptoglobin and hemopexin bind hemoglobin and heme, respectively, and provide an additional barrier to staphylococcal heme-iron acquisition. The iron-binding glycoproteins transferrin (Tf) and lactoferrin (Lf) provide another obstacle to *S. aureus* iron (Fe^{+2}) acquisition. The host protein calprotectin (CP) also sequesters Mn^{+2} (manganese) and Zn^{+2} (zinc) in an effort to restrict staphylococcal growth. (B) The metal acquisition systems of *S. aureus*. *S. aureus* circumvents nutritional immunity by two primary mechanisms. One mechanism involves heme-iron acquisition through the Isd system. Alternatively, staphyloferrin A (Sa) and staphyloferrin B (Sb) scavenge iron from host iron-containing proteins such as Tf and Lf. The MntABC system competes with CP for manganese in an effort to support the proliferation of *S. aureus*. (C) Metals are essential cofactors in many bacterial metabolic processes. Nutritional immunity limits bacterial growth by disrupting metabolic pathways necessary for bacterial proliferation. The functions of flavohemoglobin (Fh), superoxide dismutases and catalase contribute to the ability of *S. aureus* to withstand host-generated nitric oxide or superoxide. These proteins require metal cofactors and will have reduced function when *S. aureus* is unable to procure iron or manganese. The ability to generate ATP via respiration (PMF) will also be disrupted by a lack of metals.