Molecular Mechanisms of *Staphylococcus aureus* Iron Acquisition

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Abstract

The unique redox potential of iron makes it an ideal cofactor in diverse biochemical reactions. Iron is therefore vital for the growth and proliferation of nearly all organisms, including pathogenic bacteria. Vertebrates sequester excess iron within proteins in order to alleviate toxicity and restrict the amount of free iron available for invading pathogens. Restricting the growth of infectious microorganisms by sequestering essential nutrients is referred to as nutritional immunity. In order to circumvent nutritional immunity, bacterial pathogens have evolved elegant systems that allow for the acquisition of iron during infection. The gram-positive extracellular pathogen *Staphylococcus aureus* is a commensal organism that can cause severe disease when it gains access to underlying tissues. Iron acquisition is required for *S. aureus* colonization and subsequent pathogenesis. Herein we review the strategies *S. aureus* employs to obtain iron through the production of siderophores and the consumption of host heme.

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INTRODUCTION

MRSA: methicillin-resistant *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive nonmotile coccus that is clinically distinguishable by a golden hue and the ability to clot vertebrate blood. *S. aureus* is often found as a part of the skin microflora and innocuously colonizes the nares of one-third of the world's population (42, 55). Upon breaching the epithelium, this extracellular pathogen can cause severe ailments, including bacteremia, pneumonia, osteomyelitis, endocarditis, and septic shock (40). Moreover, *S. aureus* develops resistance to antibiotics at a remarkable pace, presenting a significant clinical challenge. Methicillin-resistant *S. aureus* (MRSA) has recently become a serious problem in the clinical setting, highlighted by the fact that mortality due to MRSA infection has surpassed HIV-associated mortality in the United States (11). The decreasing efficacy of available antibiotics underscores the need to increase our understanding of the fundamental processes that promote *S. aureus* pathogenesis, as these processes could represent targets for novel therapeutics.

In the late 1800s, Alexander Ogston discovered *S. aureus* cocci in pus isolated from an abscess (1), establishing the formation of tissue abscesses as a pathological hallmark of *S. aureus* infection. Within the abscess, *S. aureus* is confronted with a robust host immune response and an environment devoid of essential nutrients (13, 17). Nutrient iron is required for *S. aureus* growth and persistence within abscesses and hence must be acquired during infection (13, 71, 79, 81). Most vertebrate iron is utilized as a cofactor in biochemical reactions that occur intracellularly. This intracellular pool of iron is generally not available to extracellular pathogens such as *S. aureus*. Moreover, the amount of free iron found within the serum is negligible, as it is usually complexed to high-affinity iron-binding proteins. This process of iron sequestration by the host, also referred to as nutritional immunity, inhibits the growth of invading microorganisms (9, 83). In response to this severe iron limitation, *S. aureus* has evolved sophisticated strategies to obtain iron required to proliferate within vertebrates. This review seeks to provide a comprehensive analysis of the pathways *S. aureus* utilizes to obtain iron during infection.

IRON IS SEQUESTERED WITHIN VERTEBRATES

S. aureus is a commensal organism that can inflict life-threatening damage upon its host if it is able to gain access to underlying tissues. The ability of *S. aureus* to colonize nearly every major vertebrate organ underscores the considerable public health threat posed by this organism. *S. aureus* is the number one cause of heart and skin infections, the number one cause of soft tissue infections, the leading cause of hospital-acquired infections, and a primary cause of bacterial pneumonia (7, 9, 28, 35, 47, 83). Each organ presents a unique challenge to colonization. Factors such as oxygen tension, organ-specific immune responses, and the availability of nutrients influence the outcome of staphylococcal pathogenesis.

Iron acquisition has been referred to as the "critical determinant" deciding the outcome of the host-pathogen interaction (83). Greater than 90% of the iron in mammals resides intracellularly and is therefore not a viable source of iron for extracellular pathogens unless it can be liberated from host cells (24). Extracellular iron is bound by high-affinity iron-binding proteins such as transferrin, found in the serum, and lactoferrin, found in the lymph and mucosal secretions. These glycoproteins have a high affinity for free iron. *S. aureus* indirectly steals iron from lactoferrin or transferrin through the production of siderophores. Siderophores are secreted small molecules that have an extremely high affinity for iron and outcompete host iron-binding proteins. Siderophore-iron complexes are recognized by cognate receptors on the bacterial surface, permitting the theft of iron from lactoferrin or transferrin.

In addition to being bound by proteins, iron is also complexed to the tetrapyrrole ring of heme. Heme represents 80% of iron within the host and is the preferred iron source of *S. aureus* (71). The most abundant hemoprotein within vertebrates is hemoglobin, which binds four molecules of heme and is contained within circulating erythrocytes. In order to access this rich source of iron, *S. aureus* lyses erythrocytes through the secretion of hemolysins, resulting in the liberation of hemoglobin. The host counters the displacement of hemoglobin through the action of the high-affinity hemoglobin-binding protein haptoglobin. Haptoglobin is plentiful within the serum and becomes more abundant during inflammation (56). The hemoglobin-haptoglobin complex is one of the strongest noncovalent interactions reported in serum, ensuring that the complex does not dissociate until the proteins and heme are recycled in the liver (37). Despite the strength of the hemoglobin-haptoglobin complex, *S. aureus* is capable of binding this complex and utilizing it as a source of iron (79).

Damage to erythrocytes also results in the release of free heme from liberated hemoglobin. To prevent bacterial access to this iron source, hemopexin binds heme with high affinity and traffics it to the liver, where it is endocytosed by hepatocytes and cleared from the serum (77). This interaction favors the host because *S. aureus* cannot use the hemopexin-heme complex as a source of iron (79). In vitro studies have validated heme, hemoglobin, and haptoglobin-hemoglobin as sources of iron that support *S. aureus* proliferation. Scanning electron microscopy has established that erythrocytes are present within abscesses, confirming that *S. aureus* has abundant access to this critical nutrient source during tissue colonization (13). Iron homeostasis in the host is strictly regulated to ensure that iron is available for essential biochemical reactions while preventing iron-associated toxicity and bacterial growth. Animal models have confirmed that disrupting host iron homeostasis increases the severity of *S. aureus* infections (31). Moreover, *S. aureus* infections in the healthcare setting are often associated with patients that have alterations in their iron status. Liver transplantation can result in iron overload, as the liver is the primary source of iron in the human body (24). The mortality rate in liver transplant patients that suffer from *S. aureus* bacteremia is over 80% (69). It is clear that disruptions in iron homeostasis not only are detrimental to the host, but also lead to increased *S. aureus* disease due to

Siderophore: low-molecular-weight compounds that bind iron with an extremely high affinity

Heme: a redox-active molecule composed of a tetrapyrrole ring encircling an iron atom

Hemoglobin: oxygen-transporting protein found within vertebrate erythrocytes that binds four heme molecules

the promotion of bacterial replication during infection. The following sections provide a detailed overview of our current understanding of the mechanisms *S. aureus* employs to acquire iron during infection.

Fur: a transcriptional regulator that responds to iron concentrations

NIS: nonribosomal peptide synthetase independent pathway

S. AUREUS RESPONDS TO IRON STARVATION THROUGH THE ACTIVITY OF THE TRANSCRIPTIONAL REGULATOR FUR

S. aureus responds to the iron-restricted environment of the host by dramatically altering its protein expression profile. This change in protein expression is mediated by the iron-dependent *f*erric *u*ptake *r*egulator (*fur*) (29, 79). In the presence of iron, Fur binds a consensus DNA sequence known as the *fur* box, found upstream of *fur*-regulated genes. When iron becomes limiting, Fur is released from the DNA, alleviating Fur-mediated transcriptional repression (5). Transcriptional repression of the Fur regulon occurs when *S. aureus* is iron replete and derepression occurs when *S. aureus* is iron starved. Hence, *S. aureus fur*-deficient strains exhibit a gene expression profile that mirrors that of iron-starved organisms. In vivo imaging of *S. aureus*–infected mice has demonstrated that Fur-regulated genes are expressed in heart and kidneys abscesses, suggesting that staphylococci are starved for iron in these organs (60, 63). A comparison of cytoplasmic protein profiles between wild-type *S. aureus* and an isogenic *fur* mutant found 20 staphylococcal proteins that are more abundant in the absence of Fur, suggesting that these proteins are negatively regulated by Fur (29). In addition to iron acquisition systems, this analysis revealed an increase in glycolytic and fermentative enzymes, indicating that *S. aureus* modulates its metabolism in order to adapt to the iron-starved environment of the host. Under iron-limiting conditions, *S. aureus* increases fermentative metabolism, resulting in the production of lactate. Secretion of lactate lowers the pH of the microenvironment and the affinity of transferrin for iron (29). These data demonstrate that *S. aureus* alters the host environment in a way that promotes the release of iron from host proteins, presumably increasing iron availability.

Fur regulates the expression of staphylococcal virulence factors involved in attachment to host cells, biofilm formation, and manipulation of host wound healing (3, 12, 36, 38). Fur also regulates the expression of secreted cytolytic and immunomodulatory toxins, which play a profound role in decreasing the host immune response to favor bacterial survival (54). In *fur* mutants the cytolytic toxins are more abundantly expressed, whereas immunomodulatory toxins are decreased compared to those in wild-type cells. This altered exoprotein profile leads to an increase in susceptibility to neutrophil killing and, consequently, reduced virulence in a murine pneumonia model of infection (78). These findings highlight the importance of Fur to staphylococcal pathogenesis.

STAPHYLOCOCCUS AUREUS SIDEROPHORE PRODUCTION

Extracellular iron complexed to host proteins, such as transferrin and lactoferrin, are targets for the iron-scavenging activity of siderophores. Siderophores are small molecules that are secreted by bacteria and have an exceptionally high affinity for iron. *S. aureus* produces two distinct siderophores, staphyloferrin A and staphyloferrin B, that share many properties (**Figure 1*a***). Bacterial siderophore biosynthesis proceeds through two different pathways, the nonribosomal peptide synthetase pathway and the nonribosomal peptide synthetase independent pathway (NIS). Both staphyloferrin A and staphyloferrin B are synthesized via the NIS pathway and are part of the carboxylate family of siderophores. The genes involved in the biosynthesis and import of both staphyloferrin A and staphyloferrin B are regulated by Fur and are therefore maximally expressed in iron-limiting environments (29, 45). These siderophores have been synthesized in vitro and the chemically synthesized siderophores restore the iron-scavenging defects of mutants unable to produce the respective siderophore.

The presence of a third *S. aureus* siderophore, aureochelin, has been suggested but this molecule has not been characterized (19). Supernatants isolated from mutants unable to synthesize both staphyloferrin A and staphyloferrin B are unable to support the growth of wild-type *S. aureus* in an iron-depleted environment (6). This finding highlights the importance of staphyloferrin A and staphyloferrin B to *S. aureus* iron acquisition and calls into question the relevance and/or existence of aureochelin. In vitro studies have demonstrated that siderophore-mediated iron acquisition is the dominant mechanism by which *S. aureus* steals iron from transferrin, suggesting that *S. aureus* does not produce a transferrin receptor involved in iron acquisition (58).

STAPHYLOFERRIN A

The importance of staphyloferrin A to *S. aureus* iron acquisition was demonstrated by the discovery that mutants unable to produce staphyloferrin B sustain limited growth in serum (6). A bioinformatic screen looking for homologs of known NIS synthetases in *S. aureus* identified the genes that encode for staphyloferrin A production, the *sfnABCD* operon (6, 18). Genetic and biochemical approaches subsequently confirmed the assignment of *sfnABCD* as encoding for staphyloferrin A synthesis (6, 18). Inactivation of *sfnABCD* does not attenuate growth in serum, but inactivation of *sfnABCD* in a mutant unable to produce staphyloferrin B severely impairs *S. aureus* growth in serum (6). Biochemical experiments revealed that staphyloferrin A has a mass of 479 Da and is composed of a molecule of D-ornithine that links together two molecules of citrate via amide bonds (41). Mixing citrate, D-ornithine, ATP, SnfB, and SnfD is sufficient for the in vitro synthesis of staphyloferrin A (18). Consistent with the structure of staphyloferrin A, addition of D-ornithine to *S. aureus* growth media results in the increased production of staphyloferrin A (51).

ABC transporters specific for siderophores are often found in close proximity to the genes responsible for siderophore production. Genes encoding the ABC transporter HtsABC are found adjacent to the *sfn* operon; however, HtsBC has been reported to transport heme (**Figure 1*a***). In a series of elegant genetic experiments, Beasley et al. (6) determined that the HtsABC transporter is also required for staphyloferrin A import (**Figure 1*a***). These results suggest that HtsABC might be a promiscuous system involved in the transport of multiple iron sources.

The crystal structure of HtsA, the lipoprotein receptor of staphyloferrin A, supports its involvement in siderophore transport (6, 33). This structure represents the first siderophore receptor to be structurally characterized from a gram-positive bacterium. The conformational change that highlights HtsA staphyloferrin A binding leads to an unprecedented ligand entrapment (33). Given the structural features of HtsA–staphyloferrin A binding, it is unlikely that heme is a ligand for HtsA. This suggests that an alternative lipoprotein acts as a receptor for heme transport through HtsBC, although this supposition remains to be experimentally verified.

STAPHYLOFERRIN B

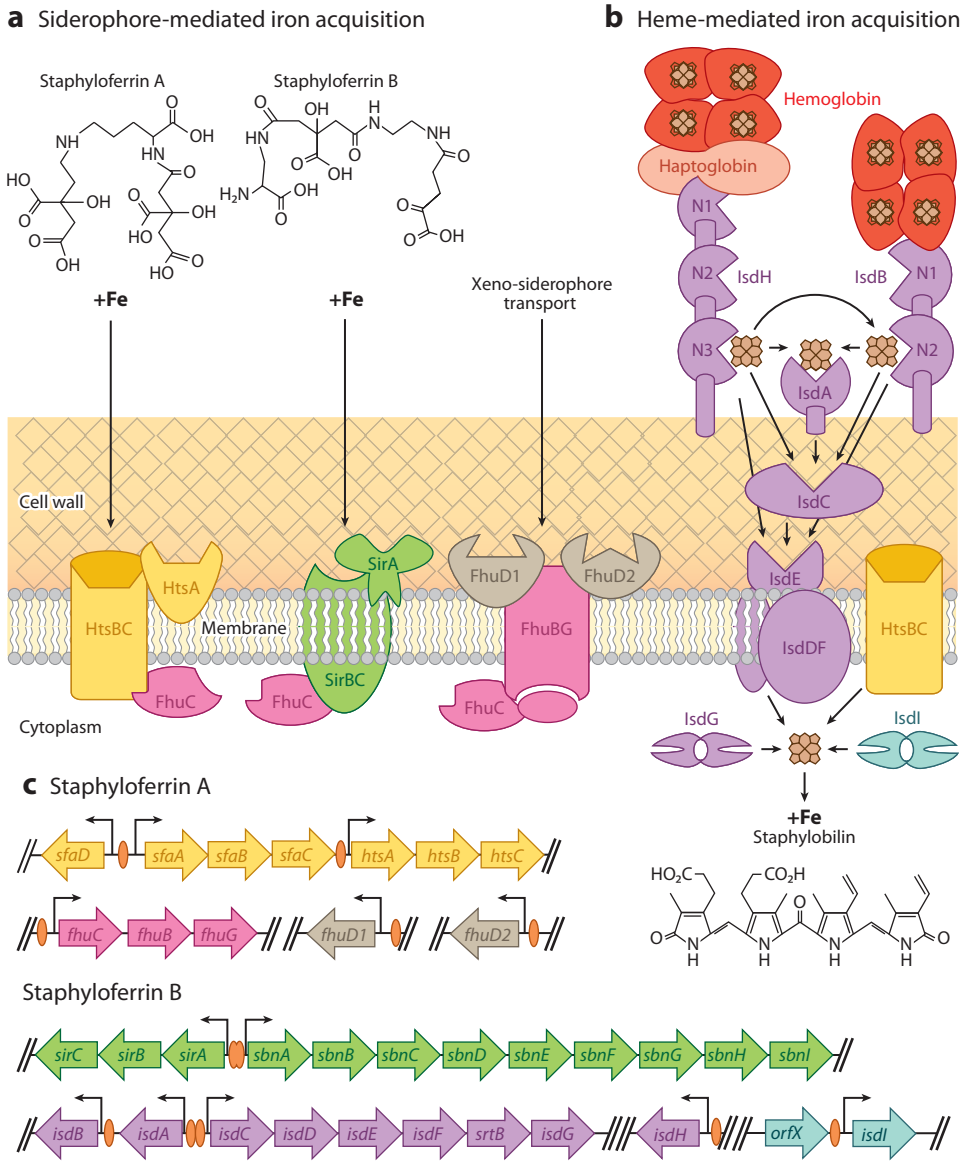
The structure of staphyloferrin B is distinct from that of staphyloferrin A (**Figure 1*a***). Staphyloferrin B is an α-hydroxycarboxylate siderophore composed of L-2,3-diaminopropionic acid (Dap), 1,2-diaminoethane (Dae), and α-ketoglutaric acid, and it has a molecular mass of 448 Da. The synthesis of staphyloferrin B is encoded by the *sbnABCDEFGHI* operon (14). In vitro synthesis of staphyloferrin B requires only the NIS synthetases SbnC, SnbE, and SbnF and the decarboxylase SbnH. These proteins synthesize staphyloferrin B when mixed with ATP, magnesium, Dap, citrate, Dae, and α-ketoglutaric acid (14). Inactivation of *sbnE* leads to a decrease in bacterial load in the kidneys of systemically infected mice, underscoring the importance of staphyloferrin B–mediated iron acquisition to pathogenesis (20). Staphyloferrin

Dap: diaminopropionic acid

Dae: 1,2-diaminoethane

B import is mediated by the *s*taphylococcal *i*ron *r*egulated transporter (*sirABC*) (21). SirBC is predicted to be the membrane permease that supports the transport of staphyloferrin B into the cytoplasm. SirA is the lipoprotein receptor component of the staphyloferrin B import system, and SirA undergoes, in a manner analogous to HtsA (32), a conformational change leading to the enclosure of staphyloferrin B in the binding pocket. The fact that staphyloferrin A and staphyloferrin B bind their cognate lipoprotein receptors with dissociation constants in the low nanomolar range underscores the exquisite evolution of this organism to obtain iron from its host during infection.

As staphyloferrin A and B are likely the only siderophores produced by *S. aureus*, it is possible that these molecules perform unique roles during infection. Staphyloferrin A and B may scavenge iron from different host proteins



or reach maximal iron-binding capacity at different sites of colonization. Alternatively, the metabolic pathways that supply the precursors for the biosynthesis of each siderophore may be favored at specific sites of colonization. Future work will refine the role of each siderophore during infection.

TRANSPORT OF SIDEROPHORES ACROSS THE STAPHYLOCOCCAL MEMBRANE

Transport of molecules across the membrane requires energy, and therefore ABC transporters are typically associated with an ATPase. One unusual feature of *sirABC* and *htsABC* is that neither operon encodes a putative ATPase (72). The *f*erric *h*ydroxymate *u*ptake operon *fhuCBG* encodes a putative ATPase named *fhuC* (also referred to as *fhuA*). FhuC is the ATPase necessary for transport of staphyloferrin A and staphyloferrin B (6, 72). This finding was established by the observation that growth of an *fhuCBG* mutant is inhibited in an iron-depleted environment and cannot be restored by the addition of supernatants containing either staphyloferrin A or staphyloferrin B. Growth of this mutant can be complemented by *fhuC* provided in *trans* and supplemented with supernatant containing either staphyloferrin A or staphyloferrin B (6). Therefore, inhibiting FhuC could have debilitating effects on the ability of *S. aureus* to procure iron during infection, establishing FhuC as a promising therapeutic target.

Despite the fact that *S. aureus* has not been demonstrated to produce hydroxymate-type siderophores, this organism has the capacity to utilize these siderophores as a source of iron. The ability to scavenge hydroxymate siderophores produced by other bacteria, also known as xenosiderophores, may allow *S. aureus* to establish residence among the microbiome. The uptake of xenosiderophores by *S. aureus* is also dependent upon *fhuCBG* (65). The lipoprotein receptors FhuD1 and FhuD2 are required for *fhuCBG*-mediated xenosiderophore uptake (**Figure 1*a***). The two genes encoding FhuD1 and FhuD2 (*fhuD1* and *fhuD2*) are located within distinct loci in the *S. aureus* genome (**Figure 1*c***). Unlike SirA and HtsA, FhuD1 and FhuD2 undergo only a modest conformational change upon siderophore binding (66, 67). This feature of the lipoprotein receptors probably reduces the affinity for any one siderophore but facilitates a broad-spectrum binding ability for many xenosiderophores. *S. aureus* can therefore outcompete other bacteria by stealing xenosiderophores while retaining staphyloferrin A– and staphyloferrin B–mediated iron uptake. This feature likely contributes to the observation that *S. aureus* is a significant clinical problem for patients that have been

Figure 1

A model of the *Staphylococcus aureus* iron acquisition pathways. (*a*) *S. aureus* produces two siderophores, staphyloferrin A and staphyloferrin B. Staphyloferrin A import is mediated by the HtsA lipoprotein and HtsBC permease. The SirA lipoprotein is the receptor for staphyloferrin B, and the SirBC permease mediates the translocation of staphyloferrin B across the membrane. *S. aureus* imports xenosiderophores produced by other bacteria through the binding activity of FhuD1 and FhuD2 receptor lipoproteins and the FhuBG permease. The energy needed for siderophore uptake is provided by the FhuC ATPase. (*b*) Heme acquisition is mediated by the Isd system. IsdH binds the hemoglobin-haptoglobin complex and IsdB binds hemoglobin. Heme is passed through the NEAr iron Transporter (NEAT) domains of IsdH (N1-N3), IsdB (N1-N2), IsdA, and IsdC. Heme can also be passed from IsdH or IsdB directly to the IsdE heme-receptor lipoprotein. Heme transport across the membrane occurs through either the IsdDF or the HtsBC permeases. Once in the cytoplasm heme is a substrate for the heme-degrading enzymes IsdG and IsdI. *S. aureus* degradation of heme leads to the release of iron and the production of staphylobilin. (*c*) Genetic loci involved in *S. aureus* iron acquisition pathways. The *sfa* operon encodes the genes required for staphyloferrin A biosynthesis, and the *sbn* operon encodes the genes required for staphyloferrin B synthesis. Promoter regions containing a consensus *fur* box are indicated by an orange oval. Genes are not drawn to scale.

Peptidoglycan: outer layer of gram-positive bacteria composed of peptides and glycan molecules

Sortase: a class of enzymes that covalently attaches proteins to the gram-positive cell wall

Iron-regulated surface determinant (Isd) system: Fur-regulated bacterial proteins responsible for the binding, import, and release of iron from host heme

administered siderophore-based therapy to treat iron-overload disease. For example, *S. aureus* is the leading cause of bacterial infection in thalassemic patients that have been treated with the siderophore desferrioxamine to reduce iron overload after a blood transfusion (8, 62). Because FhuD1 and FhuD2 increase the variety of iron sources that can be utilized by *S. aureus*, inhibiting these receptors could reduce the competitive advantage of *S. aureus* in bacterial communities and decrease the degree to which this pathogen is found as part of the normal flora. Considering that most hospital-acquired staphylococcal infections originate from the patient's normal flora, this decolonization strategy will have significant clinical impact (82).

HEME UPTAKE IN STAPHYLOCOCCI

Heme represents the primary reservoir of iron within vertebrates, and *S. aureus* can fulfill its iron requirement by obtaining iron from heme (24). In fact, *S. aureus* preferentially imports and utilizes heme-iron when grown in the presence of both transferrin and heme or hemoglobin, suggesting that heme-iron is the preferred source of iron during infection (71, 79). Hemoglobin is the most abundant hemoprotein in vertebrates, making this protein an attractive source of iron for *S. aureus*. The ability to lyse erythrocytes through the secretion of hemolysins promotes the release of hemoglobin from this intracellular reservoir. In this regard, *S. aureus* can utilize intact erythrocytes as the sole iron source in vitro (79). These experiments suggest that *S. aureus* encodes systems dedicated to the acquisition of heme-iron bound to erythrocyte hemoglobin.

STAPHYLOCOCCUS AUREUS HEME AND HEMOGLOBIN RECEPTORS

Gram-positive bacteria are shielded from the environment by a thick cell wall composed of the disaccharide *N*-acetylmuramic acid-(β1-4)-*N*-acetylglucosamine (MurNAc-GlcNAc) and the cell wall tetrapeptide (L-Ala-D-isoGln-L-Lys-D-Ala). Chains of MurNAc-GlcNAc are bound to the cell wall peptide via an amide bond between MurNAc and the L-Ala. In *S. aureus*, the glycan chains and the cell wall tetrapeptide are cross-linked via a pentaglycine crossbridge, producing a continuous layer of peptidoglycan (23, 30). This network of peptidoglycan protects the cytoplasmic membrane from environmental insult and is the region of the bacterium that directly interfaces with the host. Sortase A (SrtA) is a transpeptidase that covalently anchors proteins to the cell wall of *S. aureus* (48–50). SrtA substrates are characterized by a hydrophobic domain, followed by an LPXTG motif and a charged tail located at the C terminus of the protein. Surface-localized proteins are linked to the cell wall via SrtA-mediated cleavage of the LPXTG domain between the threonine and glycine, and via a subsequent transpeptidation between the modified LPXTG domain and the glycine peptide of an immature peptidoglycan subunit (for a review of *S. aureus* SrtA see Reference 53). *S. aureus srtA* mutants are severely attenuated in a murine abscess model of infection (13, 48).

The thickness of the cell wall presents a considerable barrier to the import of nutrients such as heme-iron. Heme was the first molecule for which a mechanism of import across the gram-positive cell wall was elucidated. Much of what is currently known about heme import was initiated by a genome-wide search for proteins homologous to SrtA. This search uncovered a second sortase encoded within *S. aureus* named sortase B (SrtB). SrtB is also a transpeptidase; however, this enzyme anchors proteins that contain an NPTQN motif in place of LPXTG. The genomic region surrounding *srtB* contains several features suggestive of a heme utilization system that has since been named the *i*ron regulated *s*urface *d*eterminant (Isd) system. The *S. aureus* Isd system is encoded within five operons: *isdA*, *isdB*, *isdCDEFsrtBisdG*, *isdH*, and *orfXisdI* (**Figure 1*c***). A consensus *fur* box is located upstream of each of these operons, and therefore all these genes are iron regulated (48, 63). IsdA, IsdB, and IsdH contain one

LPXTG motif each and hence are covalently anchored to the cell wall by SrtA. IsdC contains an NPQTN motif and is anchored to the cell wall by SrtB. IsdDEF is a membrane-localized ABC transporter complex, suggesting a role in transporting iron-containing molecules across the cytoplasmic membrane (50). IsdA, IsdB, IsdC, IsdD, IsdE, IsdG, and IsdI bind heme (50, 70). Heme uptake into *S. aureus* requires SrtA, SrtB, IsdA, and IsdE (50). The current model for Isd-mediated heme import proposes that IsdA, IsdB, and IsdH are surface-exposed hemoprotein receptors that pass heme to IsdC. IsdC then transports heme through the cell wall to the membrane-localized IsdDEF ABC transport system (**Figure 1*b***).

The transfer of heme between the Isd heme receptors to IsdE has recently been described in vitro. IsdA, IsdB, IsdH, and IsdC contain near iron transporter (NEAT) domains. NEAT domains are conserved stretches of 125 amino acids found within proteins that neighbor putative iron transporters (2). Each NEAT domain binds one heme molecule within a groove that coordinates binding to the heme-iron via a tyrosine residue (34, 68). IsdA and IsdC contain one NEAT domain each, whereas IsdB contains two NEAT domains. Heme is transferred unidirectionally from NEAT domain 2 of IsdB to the NEAT domains of either IsdA or IsdC. Heme is also transferred in a unidirectional manner from the NEAT domain of IsdA to the NEAT domain of IsdC (46). Transfer of heme through the cell wall–localized Isd proteins IsdA, IsdB, or IsdC to the membrane-localized heme receptor IsdE was confirmed in vitro using magnetic circular dichroism and electrospray ionization mass spectrometry. The NEAT domain of IsdC can transfer heme to IsdE. Heme is not transferred between IsdA and IsdE (52). These in vitro studies provide a structural basis for heme binding and transport through the cell wall that can be used to develop competitive inhibitors that disrupt this process.

In vivo heme is complexed to proteins. Hemoglobin represents an abundant reservoir of heme that is targeted by the *S. aureus* hemoglobin receptor IsdB (79). The hemoglobin-haptoglobin complex is also a viable source of iron for *S. aureus* and this complex is bound by IsdH (25, 27, 79) (**Figure 1*b***). IsdH contains three NEAT domains (25). Structural analysis of the IsdH NEAT domains revealed that NEAT domain 1 binds both hemoglobin and haptoglobin. NEAT domain 2 binds hemoglobin with nanomolar affinities and NEAT domain 3 weakly binds heme. Heme can be transferred in a bidirectional fashion from NEAT domain 3 of IsdH to NEAT domain 2 of IsdB, and in a unidirectional fashion to the NEAT domains of IsdA, IsdC, and IsdE (52, 85).These results suggest a model whereby IsdH strips heme from the hemoglobin-haptoglobin complex through the sequential activity of the NEAT domains and transfers heme to IsdB, IsdA, IsdC, or IsdE (**Figure 1*b***) (26, 59).

NEAT: near iron transporter

The in vitro experiments defining heme transfer via the NEAT domains of IsdH, IsdB, and IsdA imply that these proteins interact in such a way that allows for the transfer of heme between the proteins in vivo. Indeed IsdA and IsdB are colocalized to the cell surface in an iron-dependent fashion (60). Mild iron starvation results in the visualization of discrete IsdA and IsdB foci, whereas more severe iron starvation leads to the nearly circumferential distribution of both proteins. Recent findings suggest that subcellular protein localization in gram-positive cocci can be dictated by a YSIRK/GS motif encoded within the N terminus of the protein (10, 22). The signal sequence of IsdB contains a YSIRK/GS motif, whereas IsdA does not. Visualization of newly synthesized IsdB using gold-labeled IsdB antibodies and electron microscopy revealed that IsdB is found specifically localized to the sites of cell division. IsdA localization is more ubiquitous but a significant portion of IsdA is also found at the site of cell division (60). Despite the more uniform nature of IsdA localization, its contact with IsdB has been detected using immunoprecipitation, highlighting the strength of this interaction. Hemoglobin binding to the cell surface also colocalizes with IsdB at discrete iron-regulated foci. In keeping with the role of

IsdB and IsdH as hemoprotein receptors, inactivation of *isdB* or *isdH* reduces hemoglobin binding to *S. aureus*. In fact, mutating *isdH* or *isdA* in an *isdB* background severely abrogates the hemoglobin-binding activity of the cell. These results highlight the cooperative nature of hemoglobin binding between these proteins and provide support for the in vitro observations that heme can be transferred among IsdH, IsdB, and IsdA.

HEME TRANSPORT INTO THE CYTOPLASM

Compared to our understanding of the transport of heme through the cell wall, much less is known about the molecular details involved in transporting heme across the membrane. The current model of IsdDEF function is that the IsdE lipoprotein receives heme from IsdC. IsdE then passes heme to IsdF, the ABC permease, which transports heme through the membrane using energy provided by the ATP-hydrolyzing activity of IsdD. This model is supported by experiments showing that IsdE and IsdD bind heme and apo-IsdE receives heme from the NEAT domain of IsdC (34, 50, 52). However, inactivation of *isdDEF* impairs, but does not abolish, *S. aureus* growth on heme as the sole iron source. This implies that an additional membrane transporter(s) imports heme-iron. A homology search for alternative ABC-type iron transporters revealed a previously uncharacterized *fur*-regulated transport system named *hts* (*h*eme *t*ransport *s*ystem). The *htsABC* system, like *isdDEF*, comprises a membrane-associated lipoprotein (HtsA) and two putative ABC transporters (HtsB and HtsC). HtsB and HtsC have significant homology to HmuU, the heme transport permease of *Corynebacterium diphtheriae*. Inactivation of *htsB* and *htsC* reduces the ability of *S. aureus* to import heme-iron, which results in a decrease in bacterial burden in the liver and kidneys of mice infected with these mutants (71). The binding pocket of HtsA is a large basic patch of positively charged amino acids, making it difficult to envision that HtsA binds heme, a hydrophobic molecule (33). A plausible explanation for these two apparently conflicting findings is that HtsBC could interact with IsdE to transport heme and with HtsA to transport staphyloferrin A. HtsABC does not encode an ATPase, and FhuC has been implicated as the ATPase involved in siderophore transport (6, 65, 72). The energy needed for HtsBC to transport heme across the membrane is provided by an unknown protein. Inactivation of *isdE* together with *htsA* leads to a significant reduction in virulence, demonstrating the importance of these transporters to *S. aureus* survival in the host (47).

RELEASE OF IRON FROM HEME

In order to utilize heme as a source of iron, bacterial pathogens must have mechanisms of opening the macrocyclic conjunction of heme to release the iron atom. *S. aureus* encodes two cytoplasmic heme-degrading proteins within the Isd system: IsdG and IsdI. *isdG* is co-transcribed along with the other genes in the *isdCDEFsrtBisdG* operon (70). *isdI* is an intrachromosomal paralog of *isdG* that is also Fur regulated and encoded in a bicistronic operon with an open reading frame of unknown function (70). The amino acid sequences of IsdG and IsdI share 64% identity and the crystal structures of IsdG and IsdI can be superimposed with a root mean square deviation of 1.0 Å, underscoring the significant similarity between these proteins (84). IsdG and IsdI were the first identified members of a family of heme-degrading enzymes that bears their name, the IsdG family of heme oxygenases.

The degradation of heme is a reaction that occurs in both eukaryotes and bacteria. Before the discovery of the IsdG family of heme oxygenases, all characterized heme degradation reactions resulted in the production of biliverdin, carbon monoxide, and iron. In vertebrates, biliverdin is further reduced to the potent antioxidant bilirubin by biliverdin reductase (75). Three major features distinguish the IsdG family of heme oxygenases from the classical heme oxygenase family. First, IsdG and IsdI are structurally distinct from the classical

heme oxygenases (84). Second, the binding of heme to IsdG or to IsdI results in the severe distortion of the planarity of heme to the point where it appears ruffled (44). This represents the highest degree of heme distortion for any known heme-binding protein and plays a significant role in the third distinctive feature of IsdG family members: IsdG and IsdI degrade heme to a novel chromophore. Heme degradation catalyzed by the IsdG family of heme oxygenases results in the production of a mixture of β- and σ-isomers of oxo-bilirubin, molecules collectively referred to as staphylobilin (**Figure 1*b***) (64). The heme degradation reactions occur in vitro in the presence of an electron donor, such as NADPH–cytochrome P450 (70). The electron donor used to catalyze IsdG-family-mediated heme degradation in vivo is unknown. *isdGI* mutants are attenuated in the murine model of systemic infection, implicating that heme degradation and staphylobilin formation are important for pathogenesis (63).

The physiological significance of encoding two seemingly identical enzymes has been the subject of considerable research and has revealed subtle differences in the regulation of IsdG and IsdI. Expression of both *isdG* and *isdI* is transcriptionally regulated by Fur (63). IsdG stability is also dependent on the presence of heme such that IsdG is degraded in the absence of heme, whereas IsdI is not (63). These results suggest that *S. aureus* can tailor the amount of heme degradation in order to fulfill the nutrient iron requirement of this organism. Targeting IsdG family enzymes has significant clinical potential owing to the requirement for these enzymes during colonization and to the absence of this enzyme family within humans.

Although the primary function of heme-uptake systems is to satisfy the nutrient iron need of *S. aureus*, appreciable heme is brought into the cell during iron-replete conditions. In this case, *isdG* and *isdI* are not expressed and heme is instead distributed intact to the bacterial membrane (71). It is likely that this exogenously acquired heme is destined for proteins involved in respiration because heme is an essential cofactor for proteins involved in the transfer of electrons. Consistent with this model, the respiratory pathways of some gram-positive pathogens rely entirely on the ability of the bacteria to scavenge heme. Taken together, these results imply that *S. aureus* differentially utilizes heme depending on its metabolic needs. In conditions of iron starvation, exogenously acquired heme is degraded to release free iron. In contrast, when iron is abundant, heme is acquired and utilized intact to populate cytochromes of the electron transport chain. This reduces the requirement for endogenous heme synthesis and in effect decreases the metabolic burden on the bacterium. How iron levels within the host affect the subcellular fate of heme within *S. aureus* remains to be uncovered.

Heme-regulated transporter (Hrt): an ABC-type transporter composed of a permease (HrtB) and an ATPase (HrtA) that function to alleviate heme toxicity

THE RESPONSE OF *STAPHYLOCOCCUS AUREUS* TO HEME TOXICITY

Heme is used as a cofactor in many biological systems due the redox potential of the encircled iron atom. However, this redox-active molecule can also be toxic at high levels. This creates a paradox for microorganisms that utilize heme as a source of iron. *S. aureus* resolves this paradox by adapting to heme toxicity, as evidenced by its resistance to lethal levels of heme when first exposed to sublethal concentrations (80). A mechanistic explanation for this adaptation was provided by the observation that *S. aureus* exposed to sublethal concentrations of heme upregulates an ABC transport system 45-fold more compared to untreated controls (29). Because of the heme responsiveness of this system, it has been named the *h*eme-*r*egulated *t*ransporter (*hrtAB*) (**Figure 2**). The ATPase of this system (*hrtA*) is encoded in a bicistronic operon with the predicted permease (*hrtB*), and both are required for adaptation to heme-mediated toxicity. The ATPase activity of HrtA is influenced by various physiochemical conditions including the concentration of ATP, temperature, pH, and metal cofactors. Mutants of *hrtA* that are

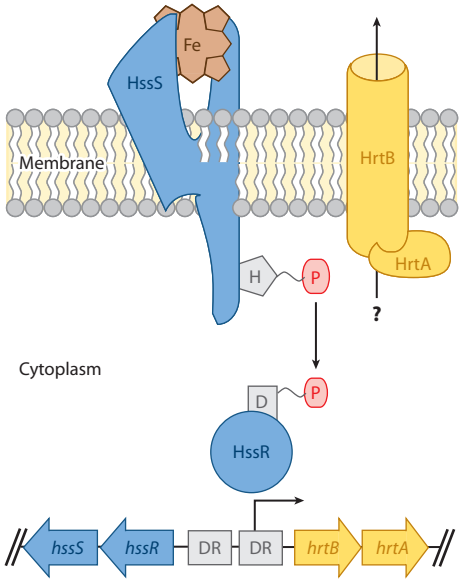


Figure 2

Sensing and alleviation of heme-associated toxicity. HssS senses exposure to heme through an unknown mechanism. This exposure results in the autophosphorylation of HssS, which is followed by a phosphorelay between His279 of HssS and Asp52 of HssR. Phosphorylated HssR binds to a direct repeat (DR) sequence found within the *hrtAB* promoter region, resulting in the expression of those genes. The HrtA ATPase and the HrtB permease form an ABC-type transport system that alleviates heme-mediated toxicity through an unknown mechanism.

unable to hydrolyze ATP are unable to adapt to heme toxicity. These findings mechanistically link the ATPase activity of HrtA with the alleviation of heme toxicity (73).

Whereas the ATPase activity of HrtA has been characterized, the permease activity of HrtB has yet to be confirmed. Moreover, the substrate of HrtAB and the mechanism by which HrtAB detoxifies heme are unknown. The current model predicts that HrtAB acts as an efflux pump that expels a toxic metabolite that accumulates as a result of heme exposure. HrtB expression in the absence of HrtA results in a decrease in the integrity of the membrane and an altered protein secretion profile (4). This phenomenon has a dramatic effect on the virulence of *S. aureus* (discussed below).

The upregulation of HrtA in response to heme is dependent on a two-component system known as the *h*eme *s*ensor *s*ystem (*hssRS*) (**Figure 2**). Bacterial two-component systems are typically composed of a response regulator and a histidine kinase. These systems are critical for the proper physiological response to environmental cues. In the case of HssRS, the environmental cue is heme toxicity and the physiological response is the upregulation of *hrtAB*. Heme exposure activates the histidine kinase, HssS, resulting in autophosphorylation. HssS then transphosphorylates HssR, the response regulator component of the system. Phosphorylated HssR binds to a direct repeat sequence within the *hrtAB* promoter in order to induce transcription of *hrtAB* (74) (**Figure 2**). Expression of *hrtAB* is dependent on HssRS, and inactivation of either *hssS* or *hssR* results in an inability to adapt to heme toxicity. Heme triggers HssRS-dependent *hrtAB* activation; however, the ligand that induces the initial autophosphorylation of HssS is unknown. Identification of the ligand for HssRS will provide much needed information regarding the mechanisms by which heme promotes cellular toxicity, a finding that will have broad biological implications.

THE ROLE OF HEME ACQUISITION DURING INFECTION

Considering the requirement for iron during infection, the Isd system plays a major role during pathogenesis. In addition to binding heme, IsdA binds a wide variety of host proteins, promotes adherence to cells in tissue culture, and acts in concert with IsdB to promote resistance to neutrophil killing (16, 57). Because IsdA binds multiple host proteins, the specific contribution of IsdA-mediated heme binding to infection has been difficult to assess. Inactivation of *isdA* leads to decreased ability to colonize human skin and a decrease in bacterial

Heme sensing system (Hss): a two-component system composed of a histidine kinase (HssS) and a response regulator (HssR) that function to activate Hrt

load in murine kidneys five days postinfection (13, 15). These results suggest that IsdA is important for the colonization and persistence of *S. aureus* in these two host environments. IsdA is expressed in the heart and liver of infected animals, but *isdA* mutants display wild-type levels of colonization in both of these organs. Heme transport via IsdA may be overshadowed in this infection model because in vitro data have demonstrated the NEAT domains of IsdH and IsdB can bind heme and transfer it directly to IsdC or IsdE (52). Similar to the *isdA* mutant, inactivation of *isdC* leads to only a modest decrease in bacterial loads in infected kidneys five days postinfection (13). In light of the in vitro evidence demonstrating that heme can be passed directly from IsdH and IsdB to the heme lipoprotein receptor IsdE, it remains possible that heme transport across the cell wall could occur via this route in vivo (**Figure 1*b***).

IsdB appears to be the lynchpin of heme acquisition in vivo. *S. aureus* inactivated for *isdB* displays a log decrease in the number of bacteria recovered from the spleen and kidneys following systemic infection of mice (60). IsdB expression is prominent in the heart of infected animals, and colonization of the heart by the *isdB* mutant is reduced by two orders of magnitude (60). This latter result suggests that hemoglobin recognition is particularly important to cardiac colonization. Inactivation of *isdH* alone does not affect bacterial burden in this model, implicating IsdB as the critical hemoprotein receptor during systemic infection. The importance of IsdB to *S. aureus* infection combined with the surface-exposed localization of the protein establishes IsdB as a potential vaccine candidate. Mice immunized with purified IsdB exhibit greater survival after subsequent challenge with *S. aureus* than mock-immunized mice (43). Full protection is observed when the IsdB vaccine includes the cell wall–anchored proteins IsdA, SdrD, and SdrE (76). Moreover, antibodies against IsdA and IsdB rescue animals from lethal staphylococcal challenge and inhibit abscess formation (39). Future studies will focus on determining the efficacy of this vaccination strategy in human subjects.

Use of animal models of infection are limited because virulence factors expressed by human pathogens have evolved to bind human-specific molecules. Advances in the genetic manipulation of mice have made it possible to engineer "humanized" mice that express human versions of proteins that are targeted by bacterial virulence factors. This technology has been used to investigate the interaction between *S. aureus* and human hemoglobin. There is a considerable amount of divergence between the surface-exposed amino acids of human and murine hemoglobin. These amino acids likely represent the binding platform for the *S. aureus* hemoglobin receptor IsdB. This is supported by the observation that the affinity of IsdB for human hemoglobin is significantly higher than the affinity for mouse hemoglobin. Moreover, human hemoglobin is more readily utilized as an iron source by *S. aureus* in culture. In keeping with this, the burden of *S. aureus* is increased by an order of magnitude in the heart and liver of mice expressing a human hemoglobin transgene following systemic infection (61). This increase in virulence is dependent on IsdB, as *S. aureus* mutants inactivated for *isdB* proliferate to similar levels in both wild-type mice and mice expressing human hemoglobin. These results establish the human-hemoglobin-expressing mice as a humanized mouse model that more accurately represents the nutrient environment encountered by *S. aureus* during infection. This infection model will be valuable for determining the contribution of hemoglobin-iron acquisition to colonization of a variety of infection sites, and in the search for inhibitors specific for the human hemoglobin-IsdB interaction.

The last step to obtaining iron from heme is mediated by the heme oxygenases IsdG and IsdI. These two enzymes are differentially regulated at the posttranslational level through proteolytic degradation of IsdG in the absence of heme (63). A clue to the physiological importance of this differential regulation comes from the organ-specific colonization profile

of *isdG* and *isdI* mutants. Inactivation of *isdG* alone or in combination with *isdI* results in a decrease in bacterial burden in both the heart and kidneys of infected animals. Inactivation of *isdI* leads to a decrease in bacterial burden only in the heart (63). The finding that both IsdI and IsdG are independently required for full virulence suggests that these enzymes have distinct roles during vertebrate colonization. In addition, the requirement for each of these enzymes during systemic infection establishes the heme degradation machinery as a target for therapeutic intervention.

HEME TOXICITY AND INFECTION

S. aureus strains inactivated for individual components of the Isd system are unable to satisfy their nutrient iron need and are therefore attenuated for growth within vertebrates. Conversely, an inability to cope with heme stress through inactivation of *hssR* and *hrtA* results in a hypervirulent phenotype. In a murine abscess model of infection, *hssR* or *hrtA* mutants exhibit more overt symptoms of infection and the number of abscesses in the liver is elevated compared with mice infected with wild-type *S. aureus* (80). The hypervirulence of Δ*hrtA* is specific to the liver and is due to increased expression of immunomodulatory proteins in response to these mutant strains (80). This results in an impairment in neutrophil migration and activation that promotes bacterial survival. The increased secretion of immunomodulatory proteins that leads to the hypervirulent phenotype is due to a loss of membrane integrity in the *hrtA* mutant (4). The mechanism leading to the hypervirulence of the *hssR* mutant has yet to be elucidated. The Δ*hrtA* phenotype highlights the delicate balance of the host-pathogen interaction and raises questions regarding the mechanism by which *S. aureus* senses and responds to membrane damage induced by the host immune response. The observation that orthologs of *hssRS* and *hrtAB* are conserved in many gram-positive pathogens that come in contact with vertebrate blood provides further support that the ability to sense heme-dependent toxicity is important for pathogenesis.

CONCLUDING REMARKS

Nutritional immunity protects the host from invading microorganisms by sequestering iron and other essential nutrients. To combat this immune strategy, *S. aureus* utilizes a multifaceted approach that targets the most abundant iron reservoirs within the host. The efficiency with which *S. aureus* is able to overcome nutritional immunity is clinically relevant given the significant public health threat posed by this pathogen. The successful treatment of staphylococcal infections with antibiotics is proving to be difficult due to the prevalence of antibiotic-resistant strains. Therefore, it is necessary to explore alternative strategies to combat this pathogen.

The importance of iron acquisition systems to *S. aureus* virulence suggests these systems are viable targets for therapeutic intervention. *S. aureus* siderophores steal iron from host proteins, and the Isd system consumes host heme and releases iron. Both pathways are expressed within the iron-limited host environment. The functional redundancy built into staphylococcal iron acquisition systems guarantees the pathogen obtains enough iron to successfully colonize a variety of diverse niches within the host. Colonization is decreased but not abolished when either pathway is inactivated, suggesting that both siderophore-iron and heme-iron are critical to pathogenesis. The finding that *S. aureus* grown in iron-replete conditions transports exogenous heme to the membrane intact implies that *S. aureus* has alternative uses for heme. Clearly, there is much to be learned regarding the nutrient-uptake and processing pathways that allow this pathogen to proliferate during infection.

SUMMARY POINTS

1. The host sequesters iron through a process known as nutritional immunity. *S. aureus* overcomes nutritional immunity by acquiring iron through the production of siderophores and heme acquisition systems.
2. *S. aureus* responds to the iron-restricted environment of the host by activating the Fur regulon. The genes involved in both siderophore-mediated iron scavenging and heme-iron acquisition systems are under the control of this transcriptional repressor.
3. Staphylococcal siderophores staphyloferrin A and staphyloferrin B compete for iron with host iron-binding proteins in the serum. Staphyloferrin A transport into the bacterial cell is mediated via the HtsABC system, while the SirABC system imports staphyloferrin B.
4. The Isd system mediates the binding of hemoglobin and the transport of heme across the cell wall and plasma membrane.
5. The cell wall–anchored hemoglobin receptor IsdB and heme receptor IsdA are colocalized on the cell surface and each is required for full *S. aureus* virulence. IsdB binds human hemoglobin with a greater affinity than hemoglobin from other species.
6. The heme oxygenases IsdG and IsdI degrade heme to release free iron. This reaction also results in the production of a chromophore unique to the IsdG family of heme oxygenases called staphylobilin.
7. Heme is a redox-active molecule that can be toxic. *S. aureus* responds to heme toxicity through the activation of the HssRS two-component system.
8. Activation of HssRS leads to expression of HrtAB, an ABC transporter that alleviates heme toxicity. Disruption of *hrtA* leads to hepatic hypervirulence because of the increased secretion of immunomodulatory toxins.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. 1984. Classics in infectious diseases. “On abscesses”. Alexander Ogston (1844–1929). *Rev. Infect. Dis.* 6:122–28
2. Andrade MA, Ciccarelli FD, Perez-Iratxeta C, Bork P. 2002. NEAT: a domain duplicated in genes near the components of a putative Fe3+ siderophore transporter from Gram-positive pathogenic bacteria. *Genome Biol.* 3:RESEARCH0047

3. Athanasopoulos AN, Economopoulou M, Orlova VV, Sobke A, Schneider D, et al. 2006. The extracellular adherence protein (Eap) of *Staphylococcus aureus* inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* 107:2720–27
4. Attia AS, Benson MA, Stauff DL, Torres VJ, Skaar EP. 2010. Membrane damage elicits an immunomodulatory program in *Staphylococcus aureus*. *PLoS Pathog.* 6:e1000802
5. Baichoo N, Wang T, Ye R, Helmann JD. 2002. Global analysis of the *Bacillus subtilis* Fur regulon and the iron starvation stimulon. *Mol. Microbiol.* 45:1613–29
6. **Beasley FC, Vines ED, Grigg JC, Zheng Q, Liu S, et al. 2009. Characterization of staphyloferrin A biosynthetic and transport mutants in *Staphylococcus aureus*. *Mol. Microbiol.* 72:947–63**
7. Benito N, Miro JM, de Lazzari E, Cabell CH, del Rio A, et al. 2009. Health care-associated native valve endocarditis: importance of non-nosocomial acquisition. *Ann. Intern. Med.* 150:586–94
8. Brock JH, Ng J. 1983. The effect of desferrioxamine on the growth of *Staphylococcus aureus*, *Yersinia enterocolitica* and *Streptococcus faecalis* in human serum—uptake of desferrioxamine-bound iron. *FEMS Microbiol. Lett.* 20:439–42
9. Bullen JJ. 1981. The significance of iron in infection. *Rev. Infect. Dis.* 3:1127–38
10. Carlsson F, Stalhammar-Carlemalm M, Flardh K, Sandin C, Carlemalm E, Lindahl G. 2006. Signal sequence directs localized secretion of bacterial surface proteins. *Nature* 442:943–46
11. Centers for Disease Control and Prevention. 2007. HIV/AIDS surveillance report. Vol. 17. Revised Edition
12. Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, et al. 2002. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat. Med.* 8:687–93
13. **Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. 2009. Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. *FASEB J.* 23:3393–404**
14. Cheung J, Beasley FC, Liu S, Lajoie GA, Heinrichs DE. 2009. Molecular characterization of staphyloferrin B biosynthesis in *Staphylococcus aureus*. *Mol. Microbiol.* 74:594–608
15. Clarke SR, Mohamed R, Bian L, Routh AF, Kokai-Kun JF, et al. 2007. The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. *Cell Host Microbe* 1:199–212
16. Clarke SR, Wiltshire MD, Foster SJ. 2004. IsdA of *Staphylococcus aureus* is a broad spectrum, iron-regulated adhesin. *Mol. Microbiol.* 51:1509–19
17. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, et al. 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science* 319:962–65
18. Cotton JL, Tao J, Balibar CJ. 2009. Identification and characterization of the *Staphylococcus aureus* gene cluster coding for staphyloferrin A. *Biochemistry* 48:1025–35
19. Courcol RJ, Trivier D, Bissinger MC, Martin GR, Brown MR. 1997. Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. *Infect. Immun.* 65:1944–48
20. Dale SE, Doherty-Kirby A, Lajoie G, Heinrichs DE. 2004. Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*: identification and characterization of genes involved in production of a siderophore. *Infect. Immun.* 72:29–37
21. Dale SE, Sebulsky MT, Heinrichs DE. 2004. Involvement of SirABC in iron-siderophore import in *Staphylococcus aureus*. *J. Bacteriol.* 186:8356–62
22. DeDent A, Bae T, Missiakas DM, Schneewind O. 2008. Signal peptides direct surface proteins to two distinct envelope locations of *Staphylococcus aureus*. *EMBO J.* 27:2656–68
23. Dmitriev BA, Toukach FV, Holst O, Rietschel ET, Ehlers S. 2004. Tertiary structure of *Staphylococcus aureus* cell wall murein. *J. Bacteriol.* 186:7141–48
24. Drabkin D. 1951. Metabolism of the hemin chromoproteins. *Physiol. Rev.* 31:345–431
25. Dryla A, Gelbmann D, Von Gabain A, Nagy E. 2003. Identification of a novel iron regulated staphylococcal surface protein with haptoglobin-haemoglobin binding activity. *Mol. Microbiol.* 49:37–53
26. Dryla A, Hoffmann B, Gelbmann D, Giefing C, Hanner M, et al. 2007. High-affinity binding of the staphylococcal HarA protein to haptoglobin and hemoglobin involves a domain with an antiparallel eight-stranded beta-barrel fold. *J. Bacteriol.* 189:254–64

6. Identifies the genes involved in staphyloferrin A biosynthesis and transport and describes the FhuC-dependent import of siderophores.

13. Demonstrates that the heme-scavenging factors IsdA and IsdB, as well as SdrD and protein A, are necessary for abscess formation and persistence in the murine model of infection.

27. Etz H, Minh DB, Henics T, Dryla A, Winkler B, et al. 2002. Identification of in vivo expressed vaccine candidate antigens from *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA* 99:6573–78
28. Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, et al. 2005. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* 293:3012–21
29. Friedman DB, Stauff DL, Pishchany G, Whitwell CW, Torres VJ, Skaar EP. 2006. *Staphylococcus aureus* redirects central metabolism to increase iron availability. *PLoS Pathog.* 2(8):e87
30. Ghuysen JM, Strominger JL. 1963. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. I. Preparation of fragments by enzymatic hydrolysis. *Biochemistry* 2:1110–19
31. Gladstone GP, Walton E. 1971. The effect of iron and haematin on the killing of staphylococci by rabbit polymorphs. *Br. J. Exp. Pathol.* 52:452–64
32. Grigg JC, Cheung J, Heinrichs DE, Murphy ME. 2010. Specificity of staphyloferrin B recognition by the SirA receptor from *Staphylococcus aureus*. *J. Biol. Chem.* 285:34579–88
33. Grigg JC, Cooper JD, Cheung J, Heinrichs DE, Murphy ME. 2010. The *Staphylococcus aureus* siderophore receptor HtsA undergoes localized conformational changes to enclose staphyloferrin A in an arginine-rich binding pocket. *J. Biol. Chem.* 285:11162–71
34. Grigg JC, Vermeiren C, Heinrichs D, Murphy M. 2007. Haem recognition by a *Staphylococcus aureus* NEAT domain. *Mol. Microbiol.* 63:139–49
35. Ho KM, Robinson JO. 2009. Risk factors and outcomes of methicillin-resistant *Staphylococcus aureus* bacteraemia in critically ill patients: a case control study. *Anaesth. Intensive Care* 37:457–63
36. Hussain M, Becker K, von Eiff C, Schrenzel J, Peters G, Herrmann M. 2001. Identification and characterization of a novel 38.5-kDa cell surface protein of *Staphylococcus aureus* with extended-spectrum binding activity for extracellular matrix and plasma proteins. *J. Bacteriol.* 183:6778–86
37. Hwang PK, Greer J. 1980. Interaction between hemoglobin subunits in the hemoglobin · haptoglobin complex. *J. Biol. Chem.* 255:3038–41
38. Johnson M, Cockayne A, Morrissey JA. 2008. Iron-regulated biofilm formation in *Staphylococcus aureus* Newman requires *ica* and the secreted protein Emp. *Infect. Immun.* 76:1756–65
39. Kim HK, DeDent A, Cheng AG, McAdow M, Bagnoli F, et al. 2010. IsdA and IsdB antibodies protect mice against *Staphylococcus aureus* abscess formation and lethal challenge. *Vaccine* 28:6382–92
40. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, et al. 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 298:1763–71
41. Konetschny-Rapp S, Jung G, Meiwes J, Zahner H. 1990. Staphyloferrin A: a structurally new siderophore from staphylococci. *Eur. J. Biochem.* 191:65–74
42. Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, et al. 2006. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. *J. Infect. Dis.* 193:172–79
43. Kuklin NA, Clark DJ, Secore S, Cook J, Cope LD, et al. 2006. A novel *Staphylococcus aureus* vaccine: Iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine *S. aureus* sepsis model. *Infect. Immun.* 74:2215–23
44. Lee WC, Reniere ML, Skaar EP, Murphy ME. 2008. Ruffling of metalloporphyrins bound to IsdG and IsdI, two heme-degrading enzymes in *Staphylococcus aureus*. *J. Biol. Chem.* 283:30957–63
45. Lindsay JA, Riley TV. 1994. Staphylococcal iron requirements, siderophore production, and iron-regulated protein expression. *Infect. Immun.* 62:2309–14
46. Liu M, Tanaka WN, Zhu H, Xie G, Dooley DM, Lei B. 2008. Direct hemin transfer from IsdA to IsdC in the iron-regulated surface determinant (Isd) heme acquisition system of *Staphylococcus aureus*. *J. Biol. Chem.* 283:6668–76
47. Mason WJ, Skaar EP. 2009. Assessing the contribution of heme-iron acquisition to *Staphylococcus aureus* pneumonia using computed tomography. *PLoS One* 4:e6668
48. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. 2000. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc. Natl. Acad. Sci. USA* 97:5510–15
49. Mazmanian SK, Liu G, Ton-That H, Schneewind O. 1999. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science* 285:760–63
50. Mazmanian SK, Skaar E, Gaspar A, Humayun M, Gornicki P, et al. 2003. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* 299:906–9

50. Identifies the Isd system as the primary heme acquisition system of *S. aureus*.

51. Meiwes J, Fiedler HP, Haag H, Zahner H, Konetschny-Rapp S, Jung G. 1990. Isolation and characterization of staphyloferrin A, a compound with siderophore activity from *Staphylococcus hyicus* DSM 20459. *FEMS Microbiol. Lett.* 55:201–5

52. Demonstrates the in vitro transfer of heme between the NEAT domain-containing proteins of the Isd system.

52. Muryoi N, Tiedemann MT, Pluym M, Cheung J, Heinrichs DE, Stillman MJ. 2008. Demonstration of the iron-regulated surface determinant (Isd) heme transfer pathway in *Staphylococcus aureus*. *J. Biol. Chem.* 283:28125–36

53. Navarre WW, Schneewind O. 1999. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol. Mol. Biol. Rev.* 63:174–229

54. Nizet V. 2007. Understanding how leading bacterial pathogens subvert innate immunity to reveal novel therapeutic targets. *J. Allergy Clin. Immunol.* 120:13–22

55. Noble WC, Valkenburg HA, Wolters CH. 1967. Carriage of *Staphylococcus aureus* in random samples of a normal population. *J. Hyg.* 65:567–73

56. Oliviero S, Morrone G, Cortese R. 1987. The human haptoglobin gene: transcriptional regulation during development and acute phase induction. *EMBO J.* 6:1905–12

57. Palazzolo-Ballance AM, Reniere ML, Braughton KR, Sturdevant DE, Otto M, et al. 2008. Neutrophil microbicides induce a pathogen survival response in community-associated methicillin-resistant *Staphylococcus aureus*. *J. Immunol.* 180:500–9

58. Park RY, Sun HY, Choi MH, Bai YH, Shin SH. 2005. *Staphylococcus aureus* siderophore-mediated iron-acquisition system plays a dominant and essential role in the utilization of transferrin-bound iron. *J. Microbiol.* 43:183–90

59. Pilpa RM, Robson SA, Villareal VA, Wong ML, Phillips M, Clubb RT. 2009. Functionally distinct NEAT (NEAr Transporter) domains within the *Staphylococcus aureus* IsdH/HarA protein extract heme from methemoglobin. *J. Biol. Chem.* 284:1166–76

60. Pishchany G, Dickey SE, Skaar EP. 2009. Subcellular localization of the *Staphylococcus aureus* heme iron transport components IsdA and IsdB. *Infect. Immun.* 77:2624–34

61. Uses a transgenic mouse expressing human hemoglobin to demonstrate that *S. aureus* preferentially recognizes human hemoglobin over murine hemoglobin.

61. Pishchany G, McCoy AL, Torres VJ, Krause JC, Crowe JE Jr, et al. 2010. Specificity for human hemoglobin enhances *Staphylococcus aureus* infection. *Cell Host Microbe* 8:544–50

62. Rahav G, Volach V, Shapiro M, Rund D, Rachmilewitz EA, Goldfarb A. 2006. Severe infections in thalassaemic patients: prevalence and predisposing factors. *Br. J. Haematol.* 133:667–74

63. Reniere ML, Skaar EP. 2008. *Staphylococcus aureus* haem oxygenases are differentially regulated by iron and haem. *Mol. Microbiol.* 69:1304–15

64. Elucidates the structure and chemical features of staphylobilin.

64. Reniere ML, Ukpabi GN, Harry SR, Stec DF, Krull R, et al. 2010. The IsdG family of haem oxygenases degrades haem to a novel chromophore. *Mol. Microbiol.* 75:1529–38

65. Sebulsky MT, Hohnstein D, Hunter MD, Heinrichs DE. 2000. Identification and characterization of a membrane permease involved in iron-hydroxamate transport in *Staphylococcus aureus*. *J. Bacteriol.* 182:4394–400

66. Sebulsky MT, Shilton BH, Speziali CD, Heinrichs DE. 2003. The role of FhuD2 in iron(III)-hydroxamate transport in *Staphylococcus aureus*. Demonstration that FhuD2 binds iron(III)-hydroxamates but with minimal conformational change and implication of mutations on transport. *J. Biol. Chem.* 278:49890–900

67. Sebulsky MT, Speziali CD, Shilton BH, Edgell DR, Heinrichs DE. 2004. FhuD1, a ferric hydroxamate-binding lipoprotein in *Staphylococcus aureus*: a case of gene duplication and lateral transfer. *J. Biol. Chem.* 279:53152–59

68. Sharp KH, Schneider S, Cockayne A, Paoli M. 2007. Crystal structure of the heme-IsdC complex, the central conduit of the isd iron/heme uptake system in *Staphylococcus aureus*. *J. Biol. Chem.* 282:10625–31

69. Singh N, Sun HY. 2008. Iron overload and unique susceptibility of liver transplant recipients to disseminated disease due to opportunistic pathogens. *Liver Transpl.* 14:1249–55

70. Assigns IsdG and IsdI as a novel class of heme-degrading enzymes present in gram-positive bacteria.

70. Skaar EP, Gaspar AH, Schneewind O. 2004. IsdG and IsdI, heme-degrading enzymes in the cytoplasm of *Staphylococcus aureus*. *J. Biol. Chem.* 279:436–43

71. Demonstrates that *S. aureus* preferentially imports heme-iron to transferrin-iron.

71. Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O. 2004. Iron-source preference of *Staphylococcus aureus* infections. *Science* 305:1626–28

72. Speziali CD, Dale SE, Henderson JA, Vines ED, Heinrichs DE. 2006. Requirement of *Staphylococcus aureus* ATP-binding cassette-ATPase FhuC for iron-restricted growth and evidence that it functions with more than one iron transporter. *J. Bacteriol.* 188:2048–55

73. Stauff DL, Bagaley D, Torres VJ, Joyce R, Anderson KL, et al. 2008. *Staphylococcus aureus* HrtA is an ATPase required for protection against heme toxicity and prevention of a transcriptional heme stress response. *J. Bacteriol.* 190:3588–96
74. Stauff DL, Torres VJ, Skaar EP. 2007. Signaling and DNA-binding activities of the *Staphylococcus aureus* HssR-HssS two-component system required for heme sensing. *J. Biol. Chem.* 282:26111–21
75. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. 1987. Bilirubin is an antioxidant of possible physiological importance. *Science* 235:1043–46
76. Stranger-Jones YK, Bae T, Schneewind O. 2006. Vaccine assembly from surface proteins of *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA* 103:16942–47
77. Tolosano E, Altruda F. 2002. Hemopexin: structure, function, and regulation. *DNA Cell Biol.* 21:297–306
78. Torres VJ, Attia AS, Mason WJ, Hood MI, Corbin BD, et al. 2010. *Staphylococcus aureus fur* regulates the expression of virulence factors that contribute to the pathogenesis of pneumonia. *Infect. Immun.* 78:1618–28
79. Torres VJ, Pishchany G, Humayun M, Schneewind O, Skaar EP. 2006. *Staphylococcus aureus* IsdB is a hemoglobin receptor required for heme iron utilization. *J. Bacteriol.* 188:8421–29
80. Torres VJ, Stauff DL, Pishchany G, Bezbradica JS, Gordy LE, et al. 2007. A *Staphylococcus aureus* regulatory system that responds to host heme and modulates virulence. *Cell Host Microbe* 1:109–19
81. Trivier D, Courcol RJ. 1996. Iron depletion and virulence in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 141:117–27
82. von Eiff C, Becker K, Machka K, Stammer H, Peters G. 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study group. *N. Engl. J. Med.* 344:11–16
83. Weinberg ED. 1978. Iron and infection. *Microbiol. Rev.* 42:45–66
84. Wu R, Skaar EP, Zhang R, Joachimiak G, Gornicki P, et al. 2005. *Staphylococcus aureus* IsdG and IsdI, heme-degrading enzymes with structural similarity to monooxygenases. *J. Biol. Chem.* 280:2840–46
85. Zhu H, Xie G, Liu M, Olson JS, Fabian M, et al. 2008. Pathway for heme uptake from human methemoglobin by the iron-regulated surface determinants system of *Staphylococcus aureus*. *J. Biol. Chem.* 283:18450–60

80. This work identified the HssRS two-component system as being responsible for the heme-dependent expression of HrtAB.

84. Unequivocally establishes the IsdG family of heme oxygenases as the second family of heme-degrading enzyme identified in nature.

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