Staphylococcal response to oxidative stress

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Staphylococci are a versatile genus of bacteria that are capable of causing acute and chronic infections in diverse host species. The success of staphylococci as pathogens is due in part to their ability to mitigate endogenous and exogenous oxidative and nitrosative stress. Endogenous oxidative stress is a consequence of life in an aerobic environment; whereas, exogenous oxidative and nitrosative stress are often due to the bacteria's interaction with host immune systems. To overcome the deleterious effects of oxidative and nitrosative stress, staphylococci have evolved protection, detoxification, and repair mechanisms that are controlled by a network of regulators. In this review, we summarize the cellular targets of oxidative stress, the mechanisms by which staphylococci sense oxidative stress and damage, oxidative stress protection and repair mechanisms, and regulation of the oxidative stress response. When possible, special attention is given to how the oxidative stress defense mechanisms help staphylococci control oxidative stress in the host.

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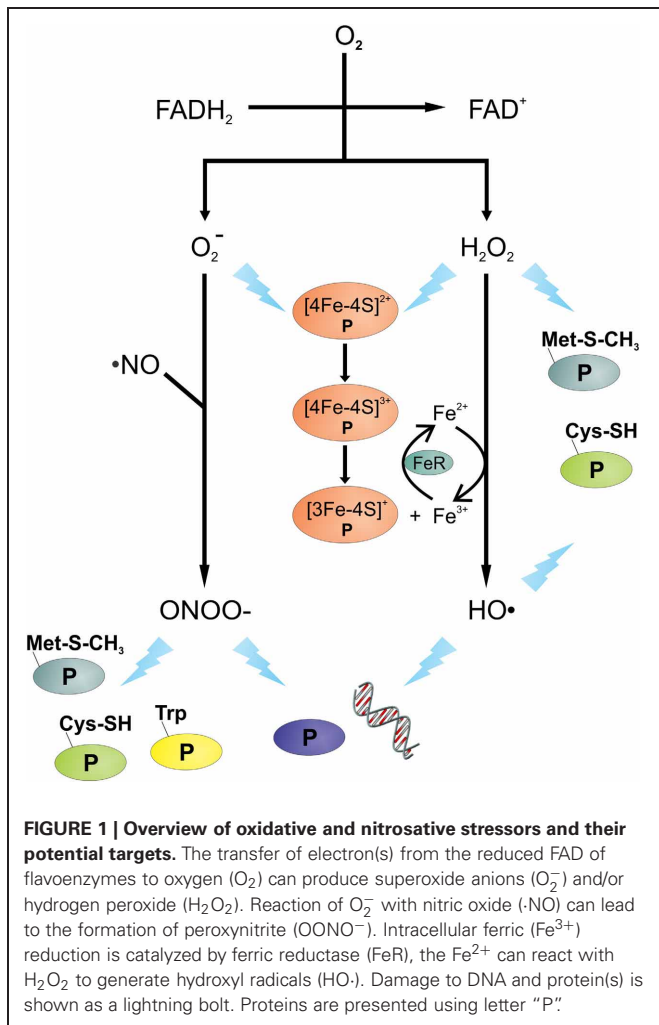
INTRODUCTION

The genus staphylococcus represents a broad family of related species and subspecies that are widely distributed in nature and that colonize skin, skin glands, and mucous membranes of humans and other warm-blooded animals (Götz et al., 2006). Several staphylococcal species have commercial value and are routinely used as starter cultures in fermentation processes (Schleifer and Fischer, 1982; Geisen et al., 1992; Tanasupawat et al., 1992; Hammes et al., 1995; Montel et al., 1996; Probst et al., 1998; Place et al., 2003), whereas others have achieved importance as animal and human pathogens. On this latter point, some staphylococci, such as *Staphylococcus epidermidis*, are pathogens primarily due to their ability to colonize indwelling medical devices and to form biofilms (von Eiff et al., 2002; Vuong and Otto, 2002; McCann et al., 2008; Rogers et al., 2009). In contrast, *Staphylococcus aureus* is capable of causing a variety of diseases ranging from soft tissue infections to life-threatening septicemia. The ability of *S. aureus* to cause this wide array of infections is due to its diverse range of virulence factors and its resistance to numerous antibiotics. In addition, *S. aureus* is a prevalent cause of infections due to the fact that 20–30% of humans carry *S. aureus* in their anterior nares (Kluytmans et al., 1997; von Eiff et al., 2001; Wertheim et al., 2005). Lastly, the prominence of *S. aureus* as a pathogen is also due to its ability to evade or defend itself from the host immune system (Voyich et al., 2005; Palazzolo-Ballance et al., 2008; Foster, 2009). For these reasons, *S. aureus* is the most prominent staphylococcal pathogen of nosocomial and community-acquired infections and a leading cause of human infections worldwide (Lowy, 1998; Diekema et al., 2001; Stevens, 2003; Grundmann et al., 2006; Chambers and DeLeo, 2009; Rosenthal et al., 2010; Johnson, 2011). As oxidative and nitrosative killing mechanisms are important for the host immune response, this review will focus on the ability of staphylococci to resist oxidative stress with an emphasis on *S. aureus*, for which the greatest amount of information is available.

ENDOGENOUS AND EXOGENOUS OXIDATIVE AND NITROSATIVE STRESS

Endogenous oxidative stress can be caused by many things, including aerobic respiration, autooxidation reactions, intracellular redox reactions, and antibiotics (Pomposiello and Demple, 2002; Imlay, 2003; Kohanski et al., 2007; Yeom et al., 2010). During aerobic respiration, oxygen functions as a final electron acceptor in the electron transport chain where its complete reduction results in the formation of H_2O. Occasionally, oxygen undergoes incomplete reduction on interaction with flavoproteins (e.g., oxidases and monooxygenases) and can generate reactive oxygen species (ROS) (Messner and Imlay, 1999). Upon interaction with the reduced FAD cofactor of flavoenzymes, one or two electrons are transferred to molecular oxygen (Müller, 1987), leading to the generation of endogenous superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) (Massey et al., 1969; Korshunov and Imlay, 2010). In addition to flavoenzyme catalyzed reactions generating ROS, other reactions can also produce reactive oxygen intermediates (Imlay et al., 1988); specifically, Fenton chemistry can produce the highly-reactive hydroxyl radicals (HO·). This chemistry occurs when iron reacts with H_2O_2 and generates HO· (**Figures 1** and **2**). Fenton chemistry has been hypothesized to be possible with other metals such as Cu and Cd; however, questions remain about the physiological significance of non-ferrous metals catalyzing this chemistry (Macomber and Imlay, 2009).

In addition to endogenous oxidative stress, exogenous oxidative stress is a common challenge that bacteria must overcome in order to survive. Host innate immune cells such as macrophages, monocytes, and neutrophils have NADPH oxidase (NOX) that is responsible for the generation of the O_2^- during an oxidative

FIGURE 1 | Overview of oxidative and nitrosative stressors and their potential targets. The transfer of electron(s) from the reduced FAD of flavoenzymes to oxygen (O_2) can produce superoxide anions (O_2^-) and/or hydrogen peroxide (H_2O_2). Reaction of O_2^- with nitric oxide (·NO) can lead to the formation of peroxynitrite (OONO$^-$). Intracellular ferric (Fe^{3+}) reduction is catalyzed by ferric reductase (FeR), the Fe^{2+} can react with H_2O_2 to generate hydroxyl radicals (HO·). Damage to DNA and protein(s) is shown as a lightning bolt. Proteins are presented using letter "P".

burst. Superoxide is generated when the catalytic subunit of NOX transfers electrons from NADPH to oxygen; a process requiring FAD and heme (Nauseef, 2004). Once formed, O_2^- can undergo dismutation to H_2O_2. This H_2O_2 that is formed after dismutation of O_2^- can be used by the myeloperoxidase (MPO) complex to produce the bactericidal compound hypochlorite (OCl$^-$).

MPO, a 150 KDa heme-containing protein complex, is released into the phagosome when azurophilic granules fuse with the phagosome. In the phagosome, MPO binds to bacteria and catalyzes the H_2O_2-dependent conversion of Cl$^-$ to OCl$^-$ (Harrison and Schultz, 1976; Klebanoff, 1999). In addition to the generation of OCl$^-$, MPO is involved in the oxidation of L-tyrosine, such as that found in enkephalins, to the potent cytotoxic tyrosyl radical (o,o′-dityrosine) (Heinecke et al., 1993). That being said, the susceptibility of bacteria to MPO-mediated killing varies; hence, MPO is not considered essential for the innate immune response to bacteria (Lehrer et al., 1969; Lanza, 1998; Allen and Stephens, 2011).

Nitric oxide (·NO) is produced by all immune cells and it is important in the control of pathogens; however, like MPO, it is not equally effective against all pathogens (Bogdan et al., 2000). As an example, in mice, the function of ·NO in *S. aureus* control is
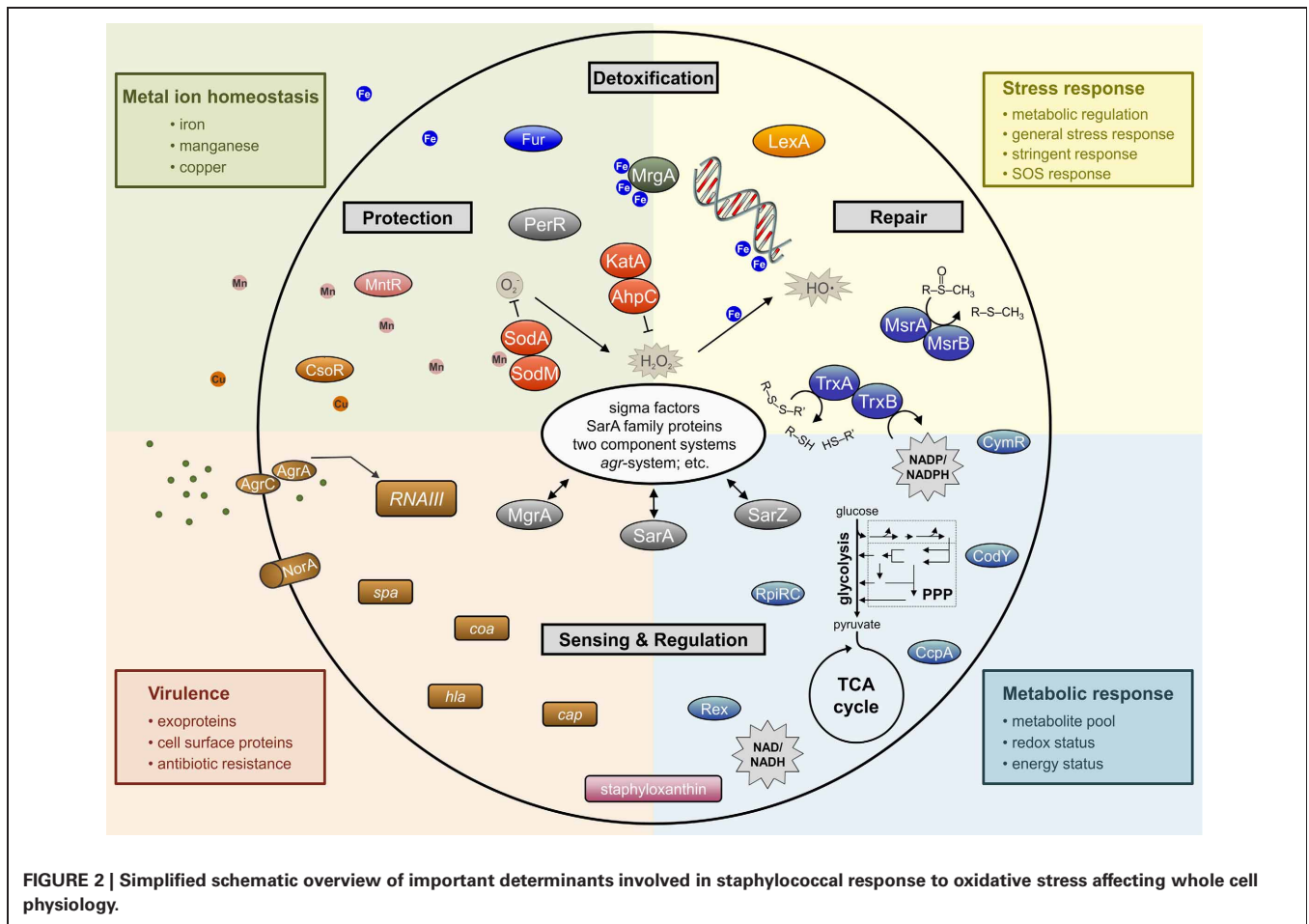
limited, whereas for *Salmonella enterica* serovar Typhimurium it is critical (Nathan and Shiloh, 2000; Vazquez-Torres et al., 2000). Like ROS, NO is a reactive oxidant with potent cytotoxic properties against bacteria. In human macrophage, nitric oxide synthase (iNOS or NOS2) is induced on encountering a pathogen or by activation via cytokines. Once induced, iNOS or NOS2 catalyzes the conversion of L-arginine to L-citrulline and ·NO, a reaction that also reduces oxygen and oxidizes NADPH. While ·NO is toxic to bacteria by itself, ·NO has a synergistic effect with H_2O_2 to facilitate bacterial killing (Brunelli et al., 1995; Woodmansee and Imlay, 2003; Han et al., 2009). In addition, ·NO and O_2^- can form the bactericidal compound peroxynitrite (OONO$^-$) (**Figure 1**), a highly reactive nitrogen intermediate (Huie and Padmaja, 1993).

BACTERIAL TARGETS OF OXIDATIVE DAMAGE

The toxicity of ROS is due to its ability to damage any oxidizable moiety in a biological molecule. In *E. coli*, the importance of ROS damage has been demonstrated in mutants that lack components of the oxidative stress response system (i.e., superoxide dismutase, catalase, and peroxidase) (Carlioz and Touati, 1986; Park et al., 2005b; Jang and Imlay, 2007). In mutants lacking multiple genes of the oxidative stress response, the bacteria were highly sensitive to oxidants such as paraquat and H_2O_2. In this section we will attempt to address some of the consequences of oxidative stress in bacteria.

Superoxide and H_2O_2 can facilitate the release of iron from Fe-S cluster containing proteins, such as aconitase or serine dehydratase. Both O_2^- and H_2O_2 can oxidize the [4Fe-4S]$^{2+}$ to [4Fe-4S]$^{3+}$, which can be further oxidized, leading to the release of iron and the inactivation of the enzyme (Kuo et al., 1987; Flint et al., 1993; Jang and Imlay, 2007). In the presence of H_2O_2, the iron liberated from Fe-S clusters creates an intracellular environment permissive to Fenton chemistry, which generates the highly reactive HO·. Hydroxyl radicals will react with virtually the first molecule that it encounters; hence, to induce damage it must be in close proximity to its cellular target. DNA is a charged molecule that attracts positively charged molecules, like Fe^{2+}; hence, charge-charge interaction brings iron in close proximity to the DNA phosphodiester backbone. The close proximity of Fe^{2+} to DNA means that HO· generated by Fenton chemistry will likely react with DNA, inducing lethal or non-lethal mutations (Keyer and Imlay, 1996).

In addition to DNA being a target of ROS, amino acids, and proteins can be oxidized and/or modified by ROS. H_2O_2 can react with the Fe^{2+} of an iron-containing protein, presumably through Fenton chemistry, to cause irreversible protein carbonylation and the formation of protein aggregates (Dukan et al., 1999; Davies, 2005). Cysteine, methionine, and tryptophan can be oxidized by H_2O_2, HO·, and ONOO$^-$, which can lead to reversible or irreversible enzymatic inactivation. As an example, oxidation of cysteine residues can lead to reversible modifications (i.e., sulfenic acid or S-thiolation) or irreversible modifications (i.e., sulfinic acid, sulfonic acid) (Chouchani et al., 2011). Similarly, oxidation of methionine can lead to the formation of methionine sulfoxides, which are reversible through the action of the methionine sulfoxide reductase (discussed in Section "Methionine sulfoxide

FIGURE 2 | Simplified schematic overview of important determinants involved in staphylococcal response to oxidative stress affecting whole cell physiology.

reductase"). The formation of some important ROS and the potential damage they cause are summarized in **Figure 1**.

OXIDATIVE STRESS RESISTANCE MECHANISMS

PIGMENTATION

As a general rule, most *S. aureus* strains isolated from human infections will form yellowish-orange or golden colonies due to the presence of carotenoid pigments. These pigments become more pronounced after 24 h of growth and when held at room temperature (Willis and Turner, 1962; Jacobs and Willis, 1964). An exception to this rule are the small colony variant (SCV) *S. aureus*, which are non-pigmented and may have auxotrophies for hemin, menadione, thiamine, or thymidine. SCVs are often associated with persistent and recurrent infections and are characterized by numerous phenotypic changes (Proctor et al., 2006). The main pigment of *S. aureus* is the membrane-bound orange-red C_{30} triterpenoid staphyloxanthin, which is synthesized from the enzymes coded within the *crtOPQMN* operon (Marshall and Wilmoth, 1981a,b; Pelz et al., 2005). The synthesis of staphyloxanthin involves the head-to-head condensation of two C_{15} isoprenoid molecules of farnesyl diphosphate to form dehydrosqualene, a reaction catalysed by dehydrosqualene synthase (CrtM). Dehydrosqualene is converted into 4,4′-diaponeurosporene by dehydrosqualene desaturase (CrtN),

which is further oxidized, glycosylated, and esterified to yield staphyloxanthin (Wieland et al., 1994; Pelz et al., 2005). The *crtOPQMN* operon is under positive transcriptional control from the *rsbUVW*-σ[B] system (Kullik et al., 1998; Giachino et al., 2001; Palma and Cheung, 2001; Bischoff et al., 2004) and under negative regulation by the small RNA, SsrA RNA (Liu et al., 2010).

Carotenoid pigments protect *S. aureus* against desiccation and photosensitization, and are known to quench toxic singlet oxygen. On this latter point, carotenoids are potent antioxidants due to their numerous conjugated double bonds, which make them an important survival factor for detoxifying ROS (Grinsted and Lacey, 1973; Mathews-Roth et al., 1974; Dahl et al., 1989; Krinsky, 1993; El-Agamey et al., 2004). The importance of staphyloxanthin in protecting *S. aureus* against ROS is seen in non-pigmented *crt* mutants, that grow normally, but have increased sensitivity toward ROS, OONO⁻, and HOCl (Liu et al., 2005; Clauditz et al., 2006). One consequence of this increased sensitivity to oxidants is that *S. aureus* strains deficient in carotenoid biosynthesis are more readily cleared by the innate immune response (Salamah, 1992; Liu et al., 2005, 2008; Clauditz et al., 2006; Olivier et al., 2009). In a mouse subcutaneous abscess model as well as a systemic *S. aureus* infection model, non-pigmented *S. aureus* have reduced virulence and survival relative to the pigmented wild-type strain (Liu et al., 2005, 2008). The resistance to phagocytic

killing that is conferred by pigments is primarily attributed to the antioxidant properties of carotenoids, since the effect is not seen in NOX deficient mice or when the oxidative burst is inhibited (Liu et al., 2005). In addition to the antioxidant properties of pigments, staphyloxanthin can also alter membrane rigidity, which is important in protecting against non-oxidative host defences mediated by cationic peptides (Mishra et al., 2011). Because of the contribution of carotenoid pigments to staphylococcal fitness, inhibition of carotenoid biosynthesis is viewed as a potential therapeutic target in treating *S. aureus* infections (Daum, 2008; Haebich and von Nussbaum, 2008; Liu et al., 2008; Walsh and Fischbach, 2008; Song et al., 2009a,b; Oldfield, 2010).

DETOXIFYING ENZYMES

In addition to pigments, most staphylococci possess several enzymes that are used in the detoxification of reactive oxygen and nitrogen intermediates; particularly, superoxide dismutases, catalases, glutathione peroxidases, globins, and peroxiredoxins (**Figure 2**).

Superoxide dismutase (SOD)

Superoxide dismutases are metalloenzymes that catalyse the dismutation of O_2^- to oxygen and H_2O_2, which can be further reduced to water and oxygen by catalase or alkyl hydroperoxide reductase. By preventing the accumulation of O_2^-, SODs not only protect the bacterium from damage caused by O_2^-, but also against products that are derived from reactions requiring O_2^-, such as $OONO^-$ (**Figure 1**). SODs are classified into one of four types based on the metal ion cofactor; specifically, these are the copper-zinc type (Cu/Zn-SOD), the manganese type (Mn-SOD), the iron type (Fe-SOD), and the nickel type (Ni-SOD) (Fridovich, 1995; Kim et al., 1996, 1998). *S. aureus* possess two monocistronic superoxide dismutase genes, *sodA* and *sodM* (Poyart et al., 1995; Clements et al., 1999; Valderas and Hart, 2001); whereas, coagulase-negative staphylococci lack the *sodM* gene (Barrière et al., 2001a,b; Valderas et al., 2002).

The *S. aureus sodA* gene was identified during a screen for amino acid starvation survival mutants (Watson et al., 1998) and during characterization of that mutant, it was observed in a zymogram analysis that *S. aureus* had three zones of SOD activity (Clements et al., 1999). The three zones of activity were attributed to the presence of two homodimers and a heterodimer, which confirmed the presence of a previously identified second SOD (SodM) (Poyart et al., 1995; Valderas and Hart, 2001). During *in vitro* aerobic growth of *S. aureus*, the transcription and activity of both SODs increase in the post-exponential growth phase and remain high during the stationary phase, with SodA being responsible for the majority of SOD activity (Clements et al., 1999; Valderas and Hart, 2001; Karavolos et al., 2003). The transcription and activity of both SODs can also be increased by the addition of oxidants; specifically, *sodA* is induced by internal stressors and *sodM* by exogenous O_2^- stress. That being said, both SODs function in maintaining cell viability during exogenous O_2^- stress (Clements et al., 1999; Valderas and Hart, 2001; Karavolos et al., 2003). *In vivo*, the importance of superoxide dismutase in *S. aureus* infections is unclear, as some reports suggest that SOD is important (Kanafani and Martin, 1985; Karavolos et al., 2003;

Das et al., 2008; Das and Bishayi, 2009), while others suggest that SOD has only a minimal effect on virulence (Mandell, 1975; Clements et al., 1999; Schneider et al., 2002). The difficulty in determining the function of SOD in virulence may be due to the observation that calprotectin sequesters Mn and Zn, decreasing SodA and SodM activity, and rendering the bacteria more susceptible to neutrophil-dependent killing (Kehl-Fie et al., 2011).

Both SODs in *S. aureus* are transcibed from σ^A-type promoters. The transcription of *sodA* initiates from one of two σ^A-type promoters; however, the first promoter is negatively influenced by σ^B. While the effect of σ^B on *sodA* transcription and activity remains unclear, transcription of *sodM* and activity are elevated in σ^B-deficient strains (Karavolos et al., 2003; Bischoff et al., 2004). Additionally, the staphylococcal accessory regulator (SarA) functions as a repressor of *sodM* transcription independent of σ^B. Other proteins of the SarA family have only slight effects (i.e., SarR) or no effect on *sodM* transcription. A less pronounced regulatory effect of SarA on *sodA* transcription has also been observed (Ballal and Manna, 2009).

Catalase and peroxiredoxin AhpC

As mentioned above, the detoxification of H_2O_2 is accomplished by catalases that catalyze the degradation of H_2O_2 to water and oxygen. The catalase family of proteins is divided into monofunctional or typical catalases, bifunctional catalase-peroxidases, and manganese-containing catalases (Chelikani et al., 2004). In the *Staphylococcus* genus, all species are catalase-positive with the exception of *S. saccharolyticus* and *S. aureus* subspecies *anaerobius* (Götz et al., 2006). *S. aureus* has a single monofunctional heme-containing tetrameric catalase encoded by the monocistronic *katA* gene (Sanz et al., 2000; Horsburgh et al., 2001a). In contrast, *S. xylosus*, *S. equorum*, and *S. saprophyticus* each have two catalase genes (Blaiotta et al., 2010). In addition to catalase, staphylococci have several peroxiredoxins that are induced upon treatment of *S. aureus* with H_2O_2 (i.e., Tpx, Ohr-like protein, and AhpC) (Wolf et al., 2008; Chen et al., 2009). Peroxiredoxins detoxify alkyl hydroperoxides by converting them to their corresponding alcohols using NADH or NADPH as the reducing equivalents. This reducing activity is dependent on redox-active cysteines in the active site. The alkyl hydroperoxide reductase (*ahpC*) gene forms an operon with *ahpF*, which codes for a homodimeric flavoenzyme that acts as a dedicated disulfide reductase to facilitate the reducing equivalent-dependent reduction and regeneration of AhpC (Poole, 2005). In *E. coli*, AhpC detoxifies low levels of H_2O_2, whereas KatA is the primary scavenger of H_2O_2 at high levels (Seaver and Imlay, 2001). Similar to *E. coli*, the *S. aureus* catalase is the major determinant in resistance toward H_2O_2 (Martin and Chaven, 1987; Horsburgh et al., 2001a), while AhpC confers resistance to a broader spectrum of ROS (Cosgrove et al., 2007). In *S. aureus* mutants lacking both catalase and AhpC, their ability to scavenge exogenous and endogenous H_2O_2 is inhibited, leading to the accumulation of H_2O_2 (Cosgrove et al., 2007).

In *S. aureus*, the *katA* gene and the *ahpCF* operon are negatively regulated by PerR (discussed in Section "PerR"), and putative PerR boxes are found in the promoter regions of both genes (Horsburgh et al., 2001a). In addition, the transcription of *katA* is positively affected by the ferric uptake regulator [Fur,

discussed in Section "Ferric uptake regulator (Fur)"] (Horsburgh et al., 2001b). As Fur normally functions as a repressor, it is likely that the positive regulation of *katA* is due to the repression of a positive regulator of *katA*, such as a small regulatory RNA. Together, PerR and Fur regulate transcription of the *katA* gene in response to peroxide and the availability of manganese and iron. As expected, maximal transcription of *katA* and catalase activity occur under aerobic conditions in the post-exponential growth phase, when tricarboxylic acid (TCA) cycle activity and electron transport are also maximal (Martin and Chaven, 1987; Horsburgh et al., 2001b; Cosgrove et al., 2007). The function of catalase in *S. aureus* virulence is not completely understood (Mandell, 1975; Kanafani and Martin, 1985; Horsburgh et al., 2001a; Messina et al., 2002; Cosgrove et al., 2007; Das et al., 2008; Das and Bishayi, 2009; Martínez-Pulgarín et al., 2009; Sen et al., 2009); however, both KatA and AhpC are important for nasal colonization (Cosgrove et al., 2007).

Flavohemoglobin (Hmp)

The discovery of a hemoglobin-like protein in *E. coli* (Vasudevan et al., 1991), and subsequent genome sequencing projects, led to realization that globins are widely distributed in nature. In *S. aureus*, the hemoglobin-like protein is a flavohemoglobin (Hmp), which has a N-terminal heme-containing globin domain and C-terminal NAD- and FAD-binding domains that together form a ferredoxin-NADP$^+$ oxidoreductase-like domain (Ermler et al., 1995). Hmp family members commonly demonstrate three enzymatic activities: NO-reductase, NO-dioxygenase, and alkyl-hydroperoxide reductase (Bonamore and Boffi, 2008). During aerobic growth the Hmp from *E. coli* utilizes NAD(P)H and O_2 to convert ·NO to nitrate; however, under anaerobic conditions it converts ·NO to N_2O, albeit less efficiently (Gardner et al., 1998; Kim et al., 1999). *In vitro* experiments using *E. coli* Hmp have demonstrated an NADH-dependent alkyhydroperoxide reductase activity; however, the *in vivo* significance of this activity remains to be elucidated (Bonamore et al., 2003). Like *E. coli*, *S. aureus* Hmp activity is greatest during microaerobic/anaerobic growth or during nitrosative stress conditions (Gonçalves et al., 2006; Richardson et al., 2006). Regulation of Hmp activity in response to reduced oxygen tension is mediated in part by the SrrAB two-component system; presumably, at the transcriptional level. That being said, the regulators of *hmp* transcription remain unknown in *S. aureus*, although, it has been postulated to involve the NO_2^--sensing transcription repressor, NsrR (Richardson et al., 2006).

METAL HOMEOSTASIS

Transition metal ions (i.e., Fe, Cu, Mn, and Zn) give structure to proteins, act as cofactors to enzymes, and are essential for electron transfer; hence, they are required by all forms of life (Lippard and Berg, 1994). The ability of transition metals to transfer electrons is beneficial under some circumstances; however, it is this same ability that facilitates the generation of ROS through Fenton chemistry (Gutteridge et al., 1982; Imlay et al., 1988). For this reason, the transport of metal ions is very tightly regulated to maintain an appropriate intracellular concentration and to avoid the accumulation of metals to toxic levels (**Figure 2**). To maintain

metal ion homeostasis, bacteria have evolved active transporters, efflux systems, and metallochaperones (Finney and O'Halloran, 2003; Maier et al., 2007; Bagai et al., 2008).

Iron

Iron is an important cofactor for numerous enzymes; hence, it is essential for bacterial viability (Griffiths, 1999). Despite iron being one of the most abundant elements in nature, it primarily exists in the insoluble Fe^{3+} form, which is difficult for bacteria to acquire (Ratledge and Dover, 2000). In the host, the low solubility of iron and the presence of heme, ferritin, and lactoferrin, create an environment in which free iron is essentially non-existent (Brown and Holden, 2002). This iron-limited environment coupled with the bacterial need for iron, creates a major challenge for bacteria in the host (Weinberg, 1978). To counter this challenge, *S. aureus* have adapted to extract iron from heme, which is carried out in part using the proteins encoded by the iron-regulated surface determinant (Isd) genes, *isdA*, *isdB*, *isdCDEF*, *isdG*, *isdH*, and *isdI* (Skaar et al., 2004; Skaar and Schneewind, 2004; Torres et al., 2006). In addition, *S. aureus* can extract iron from transferrin using the siderophores staphyloferrin A and B (encoded by *sfaABCD* and *sbnABCDEFGHI*, respectively) (Dale et al., 2004; Park et al., 2005a; Cheung et al., 2009; Cotton et al., 2009). When complexed with iron, these siderophores are transported into the bacterial cytoplasm through the HtsABC and SirABC transport systems using the energy from FhuC catalyzed ATP hydrolysis to drive the importation (Speziali et al., 2006; Beasley et al., 2011). In addition, *S. aureus* can acquire iron from hydroxymate siderophores produced by other bacteria and from catecholamine (Morrissey et al., 2000; Sebulsky et al., 2003, 2004; Beasley et al., 2011). In *S. aureus*, these iron acquisition and transport systems are regulated in part by the Fur [(Xiong et al., 2000); discussed in Section "Ferric uptake regulator (Fur)"].

After iron has been acquired by *S. aureus*, it is utilized or it is bound by ferritin, bacterioferritin comigratory protein (Bcp), or the Dps homolog MrgA (Metallo regulated gene A), which function as iron chelator/storage proteins. Ferritin, a polypeptide with a ferroxidase center, is encoded by *ftnA* in *S. aureus* and *sefA* in *S. epidermidis* (Horsburgh et al., 2001a; Morrissey et al., 2004) and both function primarily as iron-storage proteins (Andrews, 1998). In contrast, MrgA has an iron-chelating function and protects DNA from oxidative damage (discussed in Section "MrgA"). Bcp is homologous to peroxiredoxin and as such is likely involved in the thiol-dependent reduction of peroxides. In *S. aureus*, transcription of *bcp*, *ftnA*, and *mrgA* is regulated by PerR, highlighting the importance of sequestering iron during periods of oxidative stress (Horsburgh et al., 2001a; Chang et al., 2006; Wolf et al., 2008).

Manganese

Like iron, manganese is an essential cofactor in bacteria that is involved in diverse cellular functions such as, sugar metabolism, signal transduction, the stringent response, and oxidative stress resistance (Kehres and Maguire, 2003; Papp-Wallace and Maguire, 2006). In contrast to iron, Mn^{2+} is soluble in a physiological pH range and it has a higher reduction potential than Fe^{2+}, meaning that it is less likely to facilitate deleterious redox reactions.

For these reasons, Mn^{2+} is important for the detoxification of ROS in bacteria. As examples, the activity of SodA (discussed in Section "Superoxide dismutase") requires Mn^{2+} and SodM is also believed to require Mn^{2+} (Clements et al., 1999; Valderas and Hart, 2001). To supply the *S. aureus* Mn^{2+} requirement, there are two transporters for manganese, encoded by *mntABC* (MntABC-type) and *mntH* (Nramp-type) and in *S. epidermidis*, *sitABC* codes for the MntABC-type transporter (Cockayne et al., 1998; Horsburgh et al., 2002).

The importance of Mn^{2+} to bacteria can be inferred from the observation that host phagocytic cells transport Mn^{2+} out of the phagosome upon engulfing a bacterium. To do this, phagocytic cells recruit the efflux protein Nramp1 to the phagosome; thus, reducing the availability of Mn^{2+} to the bacterium (Jabado et al., 2000). In addition, the heterodimeric host protein calprotectin decreases Mn^{2+} availability by chelating it (Corbin et al., 2008). Because bacteria need Mn^{2+} and host cells attempt to deny the bacteria Mn^{2+}, it is not surprising that *S. aureus* virulence is attenuated in mutant strains lacking both *mntA* and *mntH* relative to the isogenic wild-type bacteria. Similarly, mutation of *mntA*, *mntH*, or *mntR* reduces *S. aureus* survival in human endothelial cells (Horsburgh et al., 2001a, 2002; Ando et al., 2003).

Regulation of Mn^{2+} transport is critical for maintaining metal ion homeostasis. In *S. epidermidis*, the DtxR homolog SirR binds to a Sir box consensus sequence in the promoter region of *sitABC*, in a Mn/Fe-dependent manner, to repress transcription (Cockayne et al., 1998; Hill et al., 1998). Similarly, in *S. aureus*, the DtxR homolog MntR represses *mntABC*, but not *mntH*, in a Mn^{2+}-dependent manner by binding to a MntR box in the promoter region. In addition to MntR, PerR also regulates *mntABC* transcription and a putative PerR box is located in the promoter region of this operon. This communal regulation of *mntABC* by MntR and PerR is also seen with other members of the PerR regulon (Horsburgh et al., 2002).

Zinc

Zinc is an essential nutrient that is required as a cofactor for a few enzymes; however, it's more important function is in protein stability (Lippard and Berg, 1994). While Zn may be essential for bacterial viability, an over abundance of Zn is toxic due to the fact that it competes with other metals for binding to the active centers of enzymes (Beard et al., 1997; Xiong and Jayaswal, 1998). The essential nature of Zn and its potential to be toxic make obvious the importance of Zn ion homeostasis, and demonstrate why bacteria have evolved zinc transporters (Blencowe and Morby, 2003). In *S. aureus*, Zn homeostasis is maintained by the plasmid encoded CadCA (*cadCA*) transporter and/or the chromosomally encoded ZntRA (*zntRA* or *czrAB*) transporter (Endo and Silver, 1995; Xiong and Jayaswal, 1998; Kuroda et al., 1999). Both transport systems code for ArsR/SmtB family transcriptional regulators (CadC and ZntR) (Busenlehner et al., 2003) and metal-exporting membrane proteins CadA and ZntA (Guffanti et al., 2002). In the presence of excess zinc, CadC and ZntR bind Zn, decreasing their affinity for their cognate promoters and this de-represses transcription of *cadA* and *zntA* (Nucifora et al., 1989; Singh et al., 1999; Ye et al., 2005).

In *B. subtilis*, Zn transport is primarily mediated by the proteins encoded within the *znuABC* operon, and *zosA*. Transcription of *znuABC* is regulated by the Zn-responsive Fur homolog known as Zur, while the transcription of *zosA* is regulated by PerR [discussed in Section "PerR" (Lee and Helmann, 2007)]. In complex with Zn, the Zur homodimer functions as a transcriptional repressor by binding to a Zur-box (AAATCGTAATNATTACGATTT) present in the promoter/operator region of *znuABC* (Gaballa et al., 2002; Ma et al., 2011). When the availability of Zn is low, Zur releases from the DNA, de-repressing transcription of the ATP binding cassette Zn transporter coded by *znuABC*. The P-type metal-transporting ATPase coded by *zosA* is induced in response to H_2O_2, consistent with its proposed function in oxidative stress resistance (Gaballa and Helmann, 2002; Gaballa et al., 2002). Although *zosA* has not been identified in *S. aureus*, it does have genes homologous to *znuA* and *znuB*; designated as *mreA* and *mreB*, respectively. As stated, Zn has important physiological functions; however, the role of Zn transport in *S. aureus* pathogenesis remains to be elucidated (Lindsay and Foster, 2001).

Copper

Although limited in number, copper-requiring enzymes have critical roles in bacterial respiration, biosynthesis, and oxidative stress resistance; hence, Cu is considered an essential trace element (Halliwell and Gutteridge, 1984; Puig and Thiele, 2002). The enzymatic utility of Cu is due in part to its ability to act as an electron donor or acceptor by cycling between the Cu^{2+} to Cu^{1+} oxidation states. Like iron, the properties of Cu that make it a useful redox cofactor, also allow it to facilitate the generation of ROS (Baker et al., 2010). For this reason, bacteria have evolved mechanisms to protect themselves from the toxic effects of Cu, while maintaining the intracellular concentration of Cu at the minimum necessary for growth (Solioz and Stoyanov, 2003; Liu et al., 2007; Wolschendorf et al., 2011). In *S. aureus*, copper homeostasis is primarily maintained by the P_1-type ATPase CopA and the copper chaperone CopZ; CopZ sequesters intracellular Cu^{1+} and delivers it to the Cu exporter CopA (Sitthisak et al., 2007). While CopA and CopZ are highly conserved in *S. aureus*, some strains also have a second P_1-type ATPase copper transporter, CopB, and a Cu oxidizing enzyme, multicopper oxidase (*mco*) (Sitthisak et al., 2005). In contrast to the chromosomally encoded genes *copA* and *copZ*, both *copB* and *mco* are carried on a plasmid or on a plasmid integrated into the chromosome (Holden et al., 2004; Baker et al., 2011). Importantly, the plasmid carrying *copB* and *mco* can be transferred between *S. aureus* strains (Baker et al., 2011). Regulation of *copA*, *copZ*, *copB*, and *mco* is dependent upon the Cu^{1+} responsive copper-sensitive operon repressor (CsoR) (Baker et al., 2011; Grossoehme et al., 2011). CsoR binds to DNA in the absence of Cu^{1+} and represses transcription; however, as the intracellular concentration of copper increases, CsoR complexes with Cu^{1+}, releases from the DNA, and de-represses transcription of copper resistance genes. The *in vivo* importance of Cu resistance for *S. aureus* is likely due to the fact that macrophage increase the Cu concentration in phagosomes via the ATP7A Cu transporter, which enhances the bactericidal activity of the phagosome (Wagner et al., 2005; White et al., 2009).

DNA PROTECTION AND REPAIR

MrgA

The genomic DNA of bacteria is organized in a nucleoid that requires DNA supercoiling, molecular crowding, and several architectural proteins (e.g., Hu, H-NS, Fis, Dps) (Dame, 2005; Luijsterburg et al., 2006). Dps (DNA-binding protein from starved cells) is a member of the ferritin super family of proteins and a non-specific DNA binding protein that functions as a major determinant for protecting DNA by nucleoid condensation (Martinez and Kolter, 1997; Wolf et al., 1999; Nair and Finkel, 2004). During the stationary phase of growth in *E. coli*, the conformation of DNA changes from a relaxed state to a compacted state, a process requiring Dps (Kim et al., 2004; Ohniwa et al., 2006). This complex DNA condensation process is regulated during the exponential growth phase by Fis and H-NS binding to the *dps* promoter and repressing transcription. (Ohniwa et al., 2006; Grainger et al., 2008). Unlike *E. coli*, *S. aureus* appears to lack both Fis and H-NS homologs (Ohniwa et al., 2011); hence, transcription of the Dps homolog MrgA is likely linked to growth via other means. One possible linkage between growth and regulation of *mrgA* is PerR (discussed in Section "PerR").

Transcription of *mrgA* is induced by H_2O_2 and iron (Horsburgh et al., 2001a; Morrissey et al., 2004; Chang et al., 2006; Morikawa et al., 2006; Wolf et al., 2008), suggesting that PerR and Fur regulate *mrgA* transcription. This suggestion is partially true as PerR is a repressor of *mrgA* transcription; whereas, *mrgA* transcription is independent of Fur. Consistent with PerR regulation of *mrgA*, inactivation of *perR* results in a compacted nucleoid in the absence of oxidative stress, which is similar to that found in a strain overexpressing *mrgA* (Morikawa et al., 2006, 2007). The activating effect of iron on *mrgA* transcription, while not completely understood, is related to the fact that the MrgA/Dps protein is a ferritin-like Fe^{2+} binding and storage protein (Grant et al., 1998; Zhao et al., 2002; Su et al., 2005). The compact nature of the nucleoid and the susceptibility of DNA to oxidative damage (discussed in Section "Bacterial targets of oxidative damage") likely led to the evolution of a bi-functional protein involved in DNA condensation and protection from Fe^{2+} generated HO· (**Figure 2**).

Excision repair

Oxidative damage to DNA can occur at the bases or sugars, producing lesions such as strand breakage or base alterations (Demple and Harrison, 1994; Lu et al., 2001). Due to the obvious importance of DNA in species propagation, DNA repair mechanisms have evolved to maintain genetic integrity. These DNA repair mechanisms can be divided into two broad categories; excision repair [i.e., base excision repair (BER), mismatch repair (MMR), and nucleotide excision repair (NER)] and recombinational repair. As with many aspects of bacterial physiology, much of our knowledge has been derived from the study of model bacteria; therefore, we will use this knowledge to draw inferences into staphylococcal DNA repair mechanisms. In time, these inferences will likely be proven correct as *S. aureus* has homologs of many of the DNA repair enzymes discussed below; specifically, for BER/MMR *S. aureus* has Nfo, MutM, MutY, MutT, MutS, MutL, RecJ, and Nth (O'Neill and Chopra, 2002; Prunier and Leclercq, 2005; Ambur et al., 2009).

Multistep BER pathways rely on damage-specific DNA glycosylases that scan DNA, recognize base lesions, and initiate removal by cleaving the base–deoxyribose glycosyl bond, forming apurinic/apyrimidimic sites (AP site). The next step in the repair process is restoration of the correct DNA sequence via short-patch (1-nucleotide patch size) or long-patch (multiple nucleotide patch size) pathways. Depending on the enzymatic properties of the initiating DNA glycoslyase, the activities of AP endonucleases and/or DNA deoxyribosephosphodiesterase (drPase) are required to break the DNA during this process. In *E. coli*, AP endonuclease activity is primarily due to exonuclease III (ExoIII or Xth) and endonuclease IV (EndoIV or Nfo), while drPase activity is due to RecJ and exonuclease I (ExoI) (Ljungquist, 1977; Lindahl, 1979; Rogers and Weiss, 1980; Franklin and Lindahl, 1988; Sandigursky and Franklin, 1992; Mol et al., 2000).

During periods of oxidative stress, a common lesion found in DNA is the oxidized base 7,8-dihydro-8-oxoguanine (8-oxoG or GO lesion), which can mispair with adenine (Shibutani et al., 1991). In *E. coli*, the formamidopyrimidine DNA glycosylase (Fpg or MutM) acts on oxidized and ring-opened purines, while the adenine DNA glycosylase MutY prevents mutagenic transversions by removing already misincorporated adenine (Michaels et al., 1992a,b; Michaels and Miller, 1992). In addition to repairing DNA, oxidized guanine is removed from the nucleotide pool by the pyrophosphohydrolase activity of MutT (Maki and Sekiguchi, 1992).

The 5,6-double bond of pyrimidines is susceptible to reaction with HO· creating a number of oxidatively damaged products, such as thymine glycol. In *E. coli*, pyrimidine lesions are recognized by endonuclease III (EndoIII or Nth) and endonuclease VIII (EndoVIII or Nei) (Radman, 1976; Demple and Linn, 1980; Katcher and Wallace, 1983; Breimer and Lindahl, 1984; Wallace, 1988). The removal of misincorporated bases occurs by the MMR system, which requires MutSL for recognition and initiation of excision repair (Modrich, 1991; Marti et al., 2002). Genetic defects in MMR and/or GO systems are associated with mutator phenotypes and as such, these damage repair systems are important in adaptive mutagenesis and the generation of genetic diversity, which was also demonstrated for *S. aureus* (O'Neill and Chopra, 2002; Chopra et al., 2003; Prunier and Leclercq, 2005; Vidales et al., 2009).

In contrast to BER, NER lesions are repaired in 12–13 nucleotide-long segments, followed by synthesis of a repair patch using the intact strand as a template and ligation of the repaired ends. Briefly, damaged DNA is detected by a complex of UvrA and UvrB. After the DNA damage is detected, UvrB binds the damaged DNA, displacing UvrA, and allowing UvrC to complex with UvrB. It is this UvrBC complex that cleaves the phosphodiester backbone to create a 12 base excision. UvrD facilitates dissociation of base pairing, causing the release of the nucleotide segment. At this point, the deletion can be filled by DNA polymerase I and then the newly synthesized DNA is ligated to the existing DNA. In addition to UvrAB, the transcription-repair coupling factor Mfd can recruit the DNA excision-repair machinery to damaged DNA (Sancar, 1996). This process is likely similar in *S. aureus* as it has the *uvrABC* genes and *mfd* (Ambur et al., 2009).

Recombinational repair

The predominant consequence of oxidative damage to the sugar in DNA is strand breakage, which can be repaired by mechanisms used in recombination. In *E. coli*, repair of DNA strand breaks initiates when RecBCD binds to the blunt end of a double stranded DNA break and the helicase activity of RecB and RecD unwinds the DNA. Due to severe consequences of strand breakage (i.e., death), there is redundancy in the initiation of recombinational repair systems (i.e. RecF and SbcCD pathways). While the repair of most double strand DNA breaks in *E. coli* are initiated by the RecBCD exonuclease/helicase complex, low-GC content Gram-positive bacteria, including staphylococci, lack this pathway. Gram-positive bacteria initiate double strand break repair using the AddAB nuclease/helicase complex, functional homologs of RecBCD, or homologs of the RecF and SbcCD pathways (Alonso et al., 1993; Eisen and Hanawalt, 1999; Ambur et al., 2009; Yeeles and Dillingham, 2010). After initiating recombinational repair and as the DNA is unwound, RecA binds to the single stranded DNA and pairs with the homologous DNA sequence and initiates strand invasion. Following strand invasion, RuvAB drive branch migration and then in concert with RuvC, cuts the DNA to resolve the Holliday junction. The process of branch migration and resolution can be catalyzed by RecG as well. These additional components of double strand break repair are conserved in *S. aureus* (i.e., RecA, RuvAB, and RecG) (Niga et al., 1997; Ambur et al., 2009).

PROTEIN DAMAGE REPAIR

Thioredoxin

In bacteria, the cytoplasm is in a reduced state; hence, protein thiols can be maintained in their reduced form (Pollitt and Zalkin, 1983; Derman and Beckwith, 1991). This reduced state of the bacterial cytoplasm depends heavily on the thioredoxin and glutaredoxin systems and the low-molecular-weight thiol reductants coenzyme A (CoASH) and bacillithiol (BSH) (Brown, 1959; Derman et al., 1993; Prinz et al., 1997; Di Simplicio et al., 2003). While many bacteria have both the thioredoxin and glutaredoxin systems, most Gram-positive bacteria, including *S. aureus*, lack the glutaredoxin system (Vido et al., 2005; Diep et al., 2006); thus, these bacteria rely heavily on the thioredoxin system to maintain a reduced cytoplasm (Scharf et al., 1998; Uziel et al., 2004). The importance of thioredoxin cannot be overstated as it is essential for a large variety of cellular processes, including acting as a hydrogen donor to ribonucleotide reductase and methionine sulfoxide reductases (Russel and Model, 1986; Aberg et al., 1989). As important, thioredoxins are major contributors to oxidative stress resistance by facilitating the reduction of H_2O_2, scavenging HO·, and donating reducing equivalents to peroxiredoxins and peroxidase (Arnér and Holmgren, 2000) (**Figure 2**).

The thioredoxin system is comprised of thioredoxin (*trxA*) and the thioredoxin reductase (*trxB*). Thioredoxins are small disulfide reductase proteins, while thioredoxin reductase uses the electrons from NADPH to maintain thioredoxin in a reduced state (Holmgren, 1985). In *B. subtilis*, *trxA* and *trxB* transcription is maintained at a basal level during growth but it is increased in response to diamide, H_2O_2, heat, salt, or ethanol stress (Scharf et al., 1998; Leichert et al., 2003; Mostertz et al., 2004). These

increases in *trxA* and *trxB* transcription are primarily mediated by σ^A, σ^B, and Spx (Scharf et al., 1998), an RNA polymerase-dependent transcriptional activator that responds to diamide stress (Nakano et al., 2003). Similar to *B. subtilis*, transcription of *trxA* and *trxB* in *S. aureus* is maintained at a basal level under aerobic and anaerobic growth conditions, with Spx being required for transcription under all growth conditions (Horsburgh et al., 2001a; Pamp et al., 2006; Ballal and Manna, 2010). Also similar to *B. subtilis*, stressors such as copper, diamide, menadione, and tert-butyl hydroperoxide induce transcription of *trxA* and *trxB* (Uziel et al., 2004; Wolf et al., 2008; Baker et al., 2010).

As stated above, bacteria rely on the cysteine-containing small proteins thioredoxin and glutaredoxin to carry out the thiol-disulfide redox cycling reactions and maintain a reduced cytoplasm (Holmgren, 1989); therefore, cysteine biosynthesis is critical for sustaining the reducing environment of the cytoplasm. This can be seen by the fact that under diamide or H_2O_2-induced oxidative stress, *S. aureus* increases cysteine biosynthesis and uptake (Chang et al., 2006; Wolf et al., 2008). In *B. subtilis* and *S. aureus*, cysteine biosynthesis and transport are negatively regulated by CymR in complex with CysK [o-acetyl serine (OAS) thiol-lyase] (Even et al., 2006; Soutourina et al., 2009). Although cysteine is needed to maintain the reducing environment of the cytoplasm via thioredoxin, the intracellular concentration of cysteine is kept low due the ability of free cysteine to reduce Fe^{3+} to Fe^{2+}, which can facilitate Fenton chemistry (Park and Imlay, 2003). Thus, the intracellular concentration of cysteine must be finely balanced, or the reducing environment of the cytoplasm will be compromised. This can be seen in *cymR* mutants where cysteine accumulates in the cytoplasm and susceptibility to H_2O_2 also increases (Soutourina et al., 2009, 2010).

CoA reductase

Coenzyme A functions as a substrate for biosynthesis and the oxidation of pyruvate and fatty acids. These functions rely on the ability of CoA to form high-energy thioester bonds, such as that found in acetyl-CoA (Magnuson et al., 1993; del Cardayre et al., 1998). In addition to the metabolic importance of CoA, staphylococci use CoA and, possibly, bacillithiol as low molecular weight free thiols to help maintain the reducing environment of the cytoplasm (Newton et al., 1993; del Cardayre et al., 1998; Fahey, 2001; Pöther et al., 2009). To fill the need for reduced CoA (CoASH), coenzyme A disulfide reductase catalyzes the NADPH-dependent reduction of CoASSCoA to CoASH and protein-SH (Coulter et al., 1998). The importance of coenzyme A disulfide reductase is reflected in the observation that inactivation of the *S. aureus* coenzyme A disulfide reductase results in the attenuation of virulence for mice relative to the isogenic strain (Coulter et al., 1998; Schneider et al., 2002). Because of the metabolic demand for CoA and the use of CoA as a free thiol, it is difficult to predict which function is more responsible for this attenuation.

Methionine sulfoxide reductase

Methionine is highly susceptible to oxidation (Dean et al., 1997; Grimaud et al., 2001), which can lead to structural changes that alter or inhibit enzymatic functions. When oxidized, methionine forms diastereomeric S and R forms of methionine sulfoxide

(Brot et al., 1981; Moskovitz et al., 1996; Sharov et al., 1999). To repair this change, most life forms rely on methionine sulfoxide reductases (Moskovitz et al., 1996). The oxidized S form of methionine is reduced by the enzyme MsrA, while the R form is reduced by MsrB. The reduction of methionine sulfoxide to methionine is dependent on the thioredoxin thiol-disulfide redox system or other reducing agents that can donate electrons (Russel and Model, 1986). The importance of methionine sulfoxide reductase can be seen in *E. coli* where GroEL, a chaperone involved in the folding of polypeptides, is highly susceptible to inactivation by host immune cell generated $ONOO^-$ and $HOCl$ (Khor et al., 2004; Sasindran et al., 2007).

In most bacteria genes encoding for the enzymes, MsrA and MsrB, are commonly present as a single copy for each one (Ezraty et al., 2005). In *S. aureus*, there are three paralogs of *msrA* (i.e., *msrA1*, *msrA2*, *msrA3*), and a single *msrB* gene. *msrA1* and *msrB* are present in an operon with a PTS permease and a fourth gene of unknown function; whereas, *msrA2* and *msrA3* are unlinked with *msrA1* and *msrB* (Singh et al., 2001; Singh and Moskovitz, 2003). As expected, *msrA1* deletion makes *S. aureus* more sensitive to H_2O_2; however, the other *msrA* paralogs do not complement for the *msrA1* deletion. In the case of *msrA2*, this may be due poor transcription rather than inability to function as a methionine sulfoxide reductase. Interestingly, *msrA1* transcription can be induced by oxacillin and other cell wall antibiotics, but not by peroxide (Singh et al., 2001; Singh and Moskovitz, 2003). While some work has been performed on methionine sulfoxide reductase in *S. aureus*, the fitness benefit of having three *msrA* paralogs remains to be determined.

Fe-S cluster repair

Iron-sulfur clusters are ubiquitous prosthetic groups that are involved in diverse cellular processes such as electron transfer, enzyme activity, environmental sensing, and gene regulation. The biogenesis of these Fe-S clusters requires the presence of assembly systems (e.g., *isc*, *suf*, and *csd* operons/systems), containing cysteine desulfurases, Fe-S scaffold proteins, and other accessory proteins (Johnson et al., 2005; Fontecave and Ollagnier-de-Choudens, 2008). As mentioned in Section "Bacterial targets of oxidative damage", Fe-S clusters are very susceptible to oxidative inactivation; hence, to survive in an aerobic environment, bacteria have evolved Fe-S cluster repair mechanisms. In some bacteria, the *suf* system appears to maintain Fe-S cluster assembly under oxidative stress conditions, while IscS is thought to repair Fe-S clusters (Nachin et al., 2003; Djaman et al., 2004). In addition to IscS, the recovery of aconitase and fumarase activity after oxidative damage is facilitated by the Fe-S cluster repair proteins YtfE in *E. coli* and ScdA in *S. aureus* (Justino et al., 2006, 2007; Overton et al., 2008; Vine et al., 2010). ScdA is a di-iron protein that is widely distributed among bacteria and is believed to be a new protein family that repairs Fe-S clusters (Overton et al., 2008). In *S. aureus*, the monocistronic *scdA* gene was first identified as being involved in autolysis and cell division (Brunskill et al., 1997). Transcription of *scdA* is de-repressed in the post-exponential growth phase when TCA cycle activity and electron transport are maximal and the bacterial demand for iron is at its greatest (Brunskill et al., 1997; Somerville et al., 2003), a

process mediated by MgrA [(Ingavale et al., 2003); discussed in Section "MgrA"]. Consistent with the MgrA-dependent repression of *scdA*, transcription of *scdA* can be induced by exposure to H_2O_2 (Chang et al., 2006) and *scdA* inactivation leads to increased sensitivity to H_2O_2 (Overton et al., 2008). The importance of *scdA* to staphylococcal pathogenesis remains to be determined.

SENSING AND REGULATION

As oxidative stress affects all aspect of bacterial physiology, it is understandable that regulation of cellular processes that respond to oxidative stress is complex (**Figure 2**). In fact, the complexity is significantly increased when one considers that the staphylococcal response to oxidative stress depends on the chemical nature of the oxidant. This was demonstrated when *S. aureus* was treated with three different oxidative stress inducing compounds (i.e., paraquat, H_2O_2, diamide) and the protein profiles for each stress had only limited overlap (Wolf et al., 2008). Because many of the regulators that affect the oxidative stress response are discussed in other areas of this Frontiers Research Topic, we have limited our discussion to only a few of the sensing and regulatory systems in staphylococci.

ENVIRONMENTAL SENSING—THE METABOLIC RESPONSE

Despite the large percentage of the staphylococcal genomes dedicated to metabolism and physiology, staphylococci only require 13 biosynthetic intermediates to synthesize all macromolecules in the cell. These 13 biosynthetic intermediates are derived from the three metabolic pathways of central metabolism: glycolysis, the pentose phosphate pathway (PPP), and the TCA cycle. Because of the importance of these 13 intermediates, staphylococci have evolved metabolite responsive regulators (e.g., CcpA, CodY, RpiR) to "sense" the availability of these intermediates or compounds derived from them (Somerville and Proctor, 2009). As stated above, oxidative stress leads to the rapid inactivation of Fe-S cluster containing enzymes and the reversible and irreversible oxidation of some cysteine and methionine-containing proteins. Hence, oxidative stress alters enzymatic activity, resulting in changes in metabolite concentrations as well as the redox poise. These changes in the bacterial metabolic status create signals that alter the activity of redox-responsive and metabolite-responsive regulators (e.g., Rex, CcpA, CodY, RpiR) (Egeter and Brückner, 1996; Seidl et al., 2008a,b; Pagels et al., 2010). It is for this reason that metabolite-responsive regulators, such as CodY (Majerczyk et al., 2010) and RpiRC (Zhu et al., 2011) are involved in regulating components of the oxidative stress response (**Figure 2**).

FERRIC UPTAKE REGULATOR (Fur)

The ferric uptake regulator (Fur) is a transcriptional regulator that is partially responsible for maintenance of iron homeostasis in many bacteria, including *S. aureus* and *S. epidermidis* (Ernst et al., 1978; Hantke, 1981; Heidrich et al., 1996; Xiong et al., 2000). Fur is a homodimeric metalloprotein with an N terminal DNA binding domain and C terminal dimerization domain that may be occupied by structural zinc (Jacquamet et al., 1998; Gonzalez de Peredo et al., 1999; Sheikh and Taylor, 2009). Fur when complexed with iron can regulate the transcription of

genes by binding to a 19 bp inverted repeat sequence known as the Fur box (GATAATTGATAATCATTATC) in the promoter region (Ochsner et al., 1995; Escolar et al., 1999; Xiong et al., 2000; Baichoo and Helmann, 2002). Fur primarily functions as a repressor; therefore, changes in gene expression during growth in iron-limited medium are similar to those changes observed in a *fur* mutant (Johnson et al., 2011). Interestingly, *in vitro* studies of *fur* mutants have demonstrated that the number of genes regulated by iron far outweighs the number of genes identified as being directly regulated by Fur. It is hypothesized that Fur-independent transcription of genes that are regulated by the availability of iron may be regulated by *agr*, *rot*, and *sae*, as the transcription of these regulators is influenced by Fur during iron-limited growth. As stated, Fur is primarily a transcriptional repressor; however, Fur is a positive effector of *katA* transcription and consistent with this positive effect, *fur* mutants have reduced catalase activity and increased sensitivity to peroxide stress (Horsburgh et al., 2001b). The more likely explanation for the positive effect of Fur on *katA* transcription is that Fur represses transcription of a positive regulator of *katA* transcription; specifically, small RNAs have been implicated in regulating *S. aureus* transcription and translation (Allard et al., 2006; Felden et al., 2011). In *S. aureus*, Fur is speculated to regulate the accumulation of immunomodulatory proteins, cytolytic proteins, and to protect against neutrophil-mediated killing (Torres et al., 2010). Despite this speculation, *fur* inactivation has a slight effect on *S. aureus* virulence in one type of experimental model (Horsburgh et al., 2001b).

PerR

PerR is a second member of the Fur family of regulators in staphylococci, and it has been identified as a peroxide sensing protein (Horsburgh et al., 2001a; Lee and Helmann, 2006a, 2007). The PerR regulon includes many genes involved in the oxidative stress response and iron storage, including *katA*, *ahpCF*, *mrgA*, *bcp*, and *trxA* genes. The fact that PerR regulates part of the oxidative stress response would suggest it is important for surviving the host immune response during an infection; however, in a mouse model of infection, *perR* mutants are only slightly attenuated in virulence relative to the parental strain (Horsburgh et al., 2001a). As a member of the Fur family of regulators, the activity of PerR is dependent upon metal ions. In *B. subtilis*, PerR carries structural zinc and its DNA binding activity is enhanced when complexed with either Fe or Mn (Lee and Helmann, 2006a). PerR containing Fe or Mn will function as a transcriptional repressor by binding to a consensus DNA sequence, termed the PerR box (AAGTATTATTTATTATTATTA) (Chen et al., 1995; Horsburgh et al., 2001a). In the presence of H_2O_2, the iron in PerR leads to formation of HO·, which oxidizes the iron-coordinating histidines, causing the loss of iron and DNA binding activity (Lee and Helmann, 2006b). When PerR is complexed with Mn, it is less likely to be inactivated by H_2O_2 because Mn is a poor mediator of Fenton chemistry; thus, in the absence of HO· there is little oxidation of the metal coordinating histidines and PerR retains its DNA binding properties. Based on this mechanism of activity regulation, it is understandable that in the presence of high Mn^{2+} and low Fe^{2+} PerR regulon members remain repressed in

the presence of H_2O_2 (Horsburgh et al., 2001a, 2002; Fuangthong et al., 2002).

MgrA

MgrA (multiple gene regulator) is a member of MarR family of regulators that positively affects capsule biosynthesis and nuclease accumulation, represses α-toxin, coagulase, and protein A synthesis, and represses autolysis (Ingavale et al., 2003; Luong et al., 2003, 2006). In addition, MgrA regulates transcription of several multidrug efflux pumps (i.e., NorA, NorB, NorC, and Tet38) (**Figure 2**); thus, MgrA functions in staphylococcal resistance to different antibiotics including fluoroquinolones, tetracycline, vancomycin or penicillin (Truong-Bolduc et al., 2003, 2006; Cui et al., 2005; Kaatz et al., 2005; Truong-Bolduc et al., 2005; Chen et al., 2006; Truong-Bolduc et al., 2006). In total, transcriptional profiling has revealed that MgrA affects the transcription of as many as 350 genes (Luong et al., 2006). This global reach of MgrA is achieved by binding to target genes as well as by indirect regulation through its affects on other regulators (i.e., SarS, SarV, SigB, LytRS, and ArlRS) (Ingavale et al., 2003, 2005; Truong-Bolduc et al., 2003; Manna et al., 2004; Luong et al., 2006). The global nature of MgrA is also reflected in the fact that it is required for the establishment and progression of *S. aureus* infections in murine abscess, septic arthritis, and sepsis models (Chen et al., 2006; Jonsson et al., 2008; Sun et al., 2011). *S. aureus* MgrA is structurally similar to the MarR of *E. coli* in that it contains a DNA binding, helix-turn-helix domain and a dimerization domain (Chen et al., 2006). In addition, MgrA contains a single cysteine (Cys12) in the dimerization domain that is accessible to oxidizing agents. This dimer interface domain is similar to that of the *B. subtilis* peroxide-sensing regulator OhrR (Fuangthong et al., 2001; Fuangthong and Helmann, 2002; Lee et al., 2007; Soonsanga et al., 2007), which when the cysteines of the two monomers are oxidized leads to dissociation of MgrA from the DNA (Chen et al., 2006). In addition, to regulating activity via Cys oxidation, the activity of MgrA is modulated by the eukaryotic-like serine/threonine kinase (Stk1 or PknB) (Truong-Bolduc et al., 2008).

SarZ

In addition to MgrA, a second MarR family regulator, SarZ, is involved in sensing oxidative stress. Like MgrA, SarZ has a DNA-binding helix–turn–helix motif, a single cysteine (Cys13), and a dimerization domain. The oxidation of Cys13 to sulfenic acid by peroxides is insufficient to disrupt SarZ DNA-binding properties; however, generation of a mixed disulfide or further oxidation to sulfinic acid or sulfonic acid leads to a de-repression of transcription. Thus, in *S. aureus*, SarZ and MgrA function as thiol switches, similar to the *B. subtilis* OhrR (Chen et al., 2006, 2011; Poor et al., 2009). Among the SarZ affected genes, many code for proteins involved in intermediary, amino acid, fatty acid, nucleotide, and sugar metabolism, including regulators of pyrimidine synthesis (*pyrR*) and gluconate catabolism (*gntR*, GntR-like protein). In addition, SarZ regulates transcription of the H_2O_2-inducible Ohr-like peroxiredoxin (Chen et al., 2009). Interestingly, there is little regulatory overlap between SarZ and MgrA affected genes (Luong et al., 2006; Chen et al., 2009). Since the Cys oxidation

mechanisms for regulating the activity of MgrA and SarZ are similar, this absence of overlap may only partially explain why different oxidants create different proteome profiles (Wolf et al., 2008).

SarA

The SarA (Cheung et al., 1992) is a promiscuous DNA binding protein containing a single cysteine (Cys9) at the dimerization interface that may be involved in oxidative stress sensing; however, SarA is more sensitive to alkylation than it is to oxidation (Chen et al., 2011). The DNA binding activity of SarA may be influenced by the redox poise or the oxidative status of the cytoplasm (Chan and Foster, 1998; Lindsay and Foster, 1999; Fujimoto et al., 2009). This may explain why SarA is a negative effector of superoxide dismutase and thioredoxin reductase transcription (Ballal and Manna, 2009, 2010). In addition to regulating some aspects of the oxidative stress response, SarA affects transcription of genes involved in many cellular processes, including virulence related genes and amino acid, nucleotide, and cell wall metabolism genes (Dunman et al., 2001).

SOS RESPONSE

If the ROS burden is high and the general stress response systems (e.g., σ^B-system) are overwhelmed, then the SOS response can become activated. As mentioned above, oxidative stress frequently induces DNA damage. For this reason, it is not surprising that exposure of S. aureus to H_2O_2 can induce the LexA regulated SOS response (Chang et al., 2006; Wolf et al., 2008). The SOS response is a highly conserved global DNA damage repair system that can be triggered by numerous DNA damaging agents, including fluoroquinolone or β-lactam antibiotics (Anderson et al., 2006; Cirz et al., 2007; Erill et al., 2007). During an SOS response, the sensor protein RecA becomes activated by non-specific binding to single-stranded DNA that is derived from recombinational repair or stalled replication. Activated RecA stimulates the autocatalytic cleavage of the SOS transcriptional repressor LexA in the C-terminal dimerization domain and in the N-terminal DNA binding domain, leading to the de-repression of SOS genes. When RecA no longer encounters ssDNA, the concentration of non-cleaved LexA increases and the SOS repair system is deactivated. While the SOS system is important for staphylococcal survival, it has also been linked to virulence, antibiotic resistance, and the dissemination of mobile genetic elements (Úbeda et al., 2005, 2007; Goerke et al., 2006; Kelley, 2006; Maiques et al., 2006). As an example, the gene encoding fibronectin binding protein B (fnbpB) is part of the LexA regulon in S. aureus (Bisognano et al., 2004).

CONCLUSION

Staphylococci face the near constant challenge of surviving in the presence of exogenous and endogenous oxidants. To meet this challenge, staphylococci have evolved a multitude of oxidative defense strategies that require a coordinated regulatory response (**Figure 2**). This regulatory response relies on molecular sentinels to detect oxidative stress or the damage caused by oxidative stress and to transduce these signals to regulators that enhance or repress transcription of the defence genes in proportion to the challenge. Once activated, the defence machinery must repair or degrade and replace damaged DNA and proteins. Disruptions in the ability of staphylococci to sense, respond, or repair oxidative stress, and the damage caused by oxidative stress, results in a fitness cost that makes the bacterium more sensitive to oxidative damage.

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