Review

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Thiol-based redox switches in the major pathogen *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is a major human pathogen, which encounters reactive oxygen, nitrogen, chlorine, electrophile and sulfur species (ROS, RNS, RCS, RES and RSS) by the host immune system, during cellular metabolism or antibiotics treatments. To defend against redox active species and antibiotics, *S. aureus* is equipped with redox sensing regulators that often use thiol switches to control the expression of specific detoxification pathways. In addition, the maintenance of the redox balance is crucial for survival of *S. aureus* under redox stress during infections, which is accomplished by the low molecular weight (LMW) thiol bacillithiol (BSH) and the associated bacilliredoxin (Brx)/BSH/bacillithiol disulfide reductase (YpdA)/NADPH pathway. Here, we present an overview of thiol-based redox sensors, its associated enzymatic detoxification systems and BSH-related regulatory mechanisms in *S. aureus*, which are important for the defense under redox stress conditions. Application of the novel Brx-roGFP2 biosensor provides new insights on the impact of these systems on the BSH redox potential. These thiol switches of *S. aureus* function in protection against redox active desinfectants and antimicrobials, including HOCl, the AGXX® antimicrobial surface coating, allicin from garlic and the naphthoquinone lapachol. Thus, thiol switches could be novel drug targets for the development of alternative redox-based therapies to combat multi-drug resistant *S. aureus* isolates.

Keywords: bacillithiol; electrophiles; HOCl; ROS; *Staphylococcus aureus*; thiol switches.

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General introduction into redox stress and thiol switches in *Staphylococcus aureus*

Staphylococcus aureus is an opportunistic human pathogen, which asymptomatically colonizes one quarter of the human population (Foster 2004). However, in the hospital setting and in immunocompromised persons the pathogen can cause many serious diseases, ranging from local skin abscesses to life threatening systemic and chronic infections, such as septicemia, endocarditis and pneumonia (Archer 1998; Boucher and Corey 2008; Lowy 1998). During infections, *S. aureus* has to cope with the oxidative burst of activated macrophages and neutrophils, which is a hallmark of the host innate immune defense (Winterbourn and Kettle 2013; Winterbourn et al. 2016; Ulfig and Leichert 2020). The successful infection with *S. aureus* is enabled by an arsenal of secreted and surface-bound virulence factors to damage host tissues and to evade the host immune defense (Tam and Torres 2019). The increasing prevalence of multiple antibiotic resistant isolates, such as methicillin resistant *S. aureus* (MRSA) poses another health burden since treatment options are limited (Chambers and Deleo 2009; Vestergaard et al. 2019).

During host-pathogen interactions, cellular metabolism, aerobic respiration and antibiotics treatment, *S. aureus* is exposed to various redox active species, including reactive oxygen, electrophile, nitrogen, chlorine and sulfur species (ROS, RES, RNS, RCS, RSS) (Loi et al. 2015). These redox active species affect the cellular redox balance and lead to damages of cellular macromolecules, including proteins, lipids and carbohydrates. As defense mechanism against redox active species, *S. aureus* encodes specific detoxification systems, low molecular weight (LMW) thiols, such as bacillithiol (BSH) and coenzyme A (CoASH) and associated thiol-disulfide oxidoreductases, including the Trx/TrxR/NADPH and Brx/BSH/YpdA/NADPH pathways. These defense mechanisms are directed to neutralize the reactive species or to regenerate oxidized proteins and redox homeostasis and often contribute to resistance and virulence properties of the pathogen.

In addition, specific transcriptional regulators are involved in redox sensing of reactive species, which control defense mechanisms to restore the reduced state of the cytoplasm (Antelmann and Helmann 2011; Chen et al. 2011; Hillion and Antelmann 2015). Redox sensing transcription factors often utilize conserved cysteine residues to sense and respond to redox stress conditions via post-translational thiol modifications, including thiol-disulfide switches as the most common theme (Antelmann and Helmann 2011; Chen et al. 2011; Hillion and Antelmann 2015; Vazquez-Torres 2012). Through innovative mass spectrometry-based redox proteomics approaches, many new thiol switches have been discovered over the past years, which are present in metabolic and redox enzymes, transcription and translation factors. Thiol switches are important regulatory devices from bacteria to men, which alter structure and functions of proteins, often leading to their inactivation and reprogramming of cellular metabolism. Thiol oxidation of redox sensing transcription factors controls specific regulons involved in detoxification pathways or regeneration of redox homeostasis (Antelmann and Helmann 2011; Hillion and Antelmann 2015). Here, we provide an overview of thiol-based redox sensing regulators, the associated detoxification pathways and BSH-related thiol-disulfide reducing systems in the human pathogen *S. aureus*. These defense mechanisms provide resistance and enable survival and efficient adaptation to the host environment contributing to the virulence of this major pathogen.

Sources and thiol chemistry of reactive oxygen, electrophile, chlorine, nitrogen and sulfur species (ROS, RES, RCS, RNS and RSS)

In all organisms, the reduced state of protein thiols in the cytoplasm is maintained by LMW thiols and enzymatic thiol-disulfide reducing systems (Van Laer et al. 2013). *S. aureus* encounters various redox active species during infections by the host immune defense, antibiotic treatment or cellular metabolism, which affects the cellular redox balance, including ROS, RES, RNS, RCS and RSS (Hillion and Antelmann 2015). The most frequent source of ROS is the aerobic respiratory chain, when O_2 is incompletely reduced by stepwise one-electron transfer reactions, generating superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) (Figure 1) (Imlay, 2003, 2008). Furthermore, autoxidation of flavin cofactors in redox enzymes contributes to ROS production. In the Fenton reaction, Fe^{2+} and H_2O_2 produce the highly toxic $OH^{\bullet}$, which can oxidize proteins, lipids and DNA (Forman and Torres 2001; Imlay, 2003, 2008).

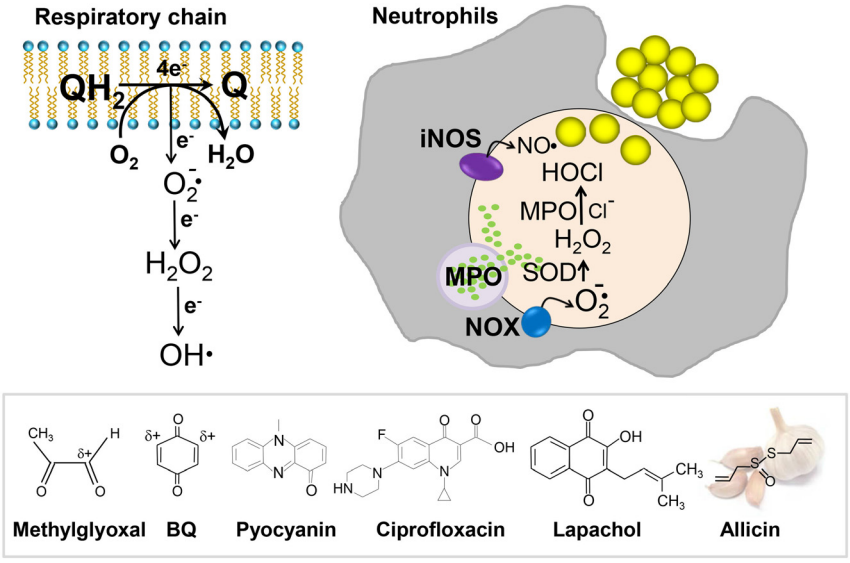

Figure 1: Generation of reactive oxygen, chlorine, nitrogen and electrophile species (ROS, RCS, RNS, RES) during aerobic respiration, infections and antibiotics treatment in *Staphylococcus aureus*. ROS are generated in the respiratory chain by one-electron transfer to O_2, including $O_2^{\bullet -}$ and H_2O_2. The OH• is produced by reaction of H_2O_2 and Fe^{2+} in the Fenton reaction (Imlay 2003). In activated neutrophils, NOX produces $O_2^{\bullet -}$, which is dismutated by SOD to H_2O_2. MPO converts H_2O_2 and Cl^- to the bactericidal HOCl (Winterbourn and Kettle 2013). In neutrophils, iNOS synthesizes NO• from arginine. RES include quinones, such as benzoquinone (BQ), which are electron carriers of the respiratory chain (menaquinone) and structural elements of catechol-type siderophores and antibiotics (e.g. pyocyanin, naphthoquinone and ciprofloxacin). Methylglyoxal is produced as toxic aldehyde in the glycolysis. Allicin is an organosulfur compound that acts as antimicrobial.

In addition, pathogens encounter ROS, RCS and RNS generated during the oxidative burst of activated macrophages and neutrophils as the first line defense of the innate immune system, including $O_2^{\bullet -}$, H_2O_2, nitric oxide (NO), and hypochlorous acid (HOCl) (Winterbourn and Kettle 2013; Winterbourn et al. 2016; Ulfig and Leichert 2020). While $O_2^{\bullet -}$ is produced by the membrane-bound NADPH oxidase (NOX) in neutrophils, superoxide dismutase (SOD) dismutates $O_2^{\bullet -}$ to H_2O_2. The myeloperoxidase (MPO) produces the strong oxidant HOCl from H_2O_2 and chloride anion (Cl^-) in neutrophils after degranulation of azurophilic granules (Figure 1) (Winterbourn and Kettle 2013; Winterbourn et al. 2016; Ulfig and Leichert 2020). While low concentrations of 25 µM $O_2^{\bullet -}$ and 2 µM H_2O_2 are produced in the neutrophil phagosome, MPO generates millimolar levels of HOCl, which are released during infections to kill pathogens (Winterbourn et al. 2006).

ROS react with Cys thiols to form unstable sulfenic acids (R-SOH), which are further oxidized to intramolecular or intermolecular protein disulfides or *S*-thiolations of proteins with LMW thiols, such as *S*-bacillithiolations or *S*-CoAlations in *S. aureus* (Figure 2) (Antelmann and Helmann 2011; Imber et al. 2019; Tsuchiya et al. 2018). In the absence of proximal thiols, ROS can irreversibly oxidize Cys thiols to sulfinic ($R\text{-}SO_2H$) or sulfonic acids ($R\text{-}SO_3H$) (Antelmann and Helmann 2011). While H_2O_2 reacts slowly with biological thiols according to second-order rate constants in the range of k = 18–26 $M^{-1}s^{-1}$ (Winterbourn and Metodiewa 1999), the reaction of HOCl with thiols is several orders of magnitude faster and occurs with rate constants of $k > 10^8$ $M^{-1}s^{-1}$ (Storkey et al. 2014). HOCl chlorinates Cys thiols to generate unstable sulfenylchloride (R-SCl), which is further oxidized to form reversible or irreversible thiol oxidation products (Davies 2011; Hawkins et al. 2003). Apart from Cys thiols, HOCl reacts with methionine residues to methionine sulfoxides (Davies 2011; Rosen et al. 2009). The reaction of HOCl with primary amines leads to chloramine formation (Green et al. 2017). Chloramines formed with free amino acids can further decompose to electrophiles, such as glyoxal, acrolein and *p*-hydroxyphenylacetaldehyde, which react with thiols via *S*-alkylation (Beavers and Skaar 2016; Hazen et al. 1998). HOCl can modify proteins by *N*-chlorination as another reversible modification, which activates chaperone functions of proteins (Davies 2011; Hawkins et al. 2003; Müller et al. 2014; Ulfig and Leichert 2020). In *S. aureus* and *Bacillus* species, we have shown that HOCl causes widespread *S*-bacillithiolations of redox sensing regulators and metabolic enzymes, which function in redox regulation of protein activities and protection against overoxidation to Cys sulfonic acids (Chi et al., 2011,2013; Imber et al. 2019).

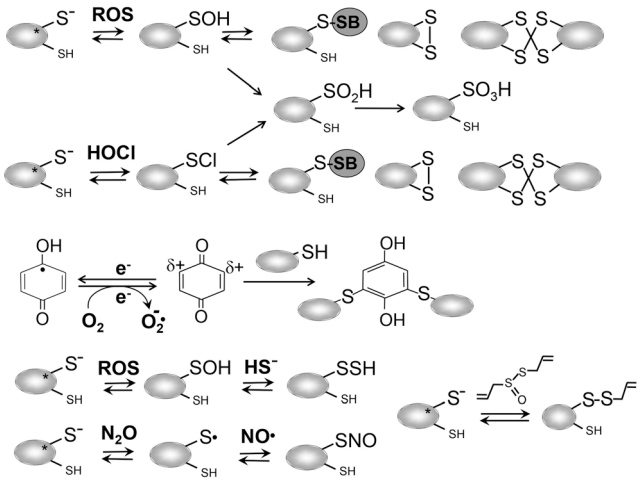

Figure 2: Post-translational thiol modifications of proteins by ROS, RCS, RES, RNS, RSS in *S. aureus*. H_2O_2 causes thiol oxidation of Cys to the Cys-SOH intermediate, which reacts further to protein disulfides or *S*-thiolations, such as *S*-bacillithiolations and *S*-CoAlations. Overoxidation of Cys-SOH leads to $Cys\text{-}SO_2H$ or $Cys\text{-}SO_3H$. HOCl causes Cys chlorination to the unstable Cys-SCl, resulting in thiol oxidation to protein disulfides, such as *S*-thiolations (Hawkins et al. 2003). Additionally, Cys-SCl can be overoxidized to $Cys\text{-}SO_2H$ or $Cys\text{-}SO_3H$. Quinones modify Cys residues by thiol-*S*-alkylation as electrophiles or by thiol oxidation as oxidants. As oxidants, quinones are reduced to semiquinone anions, leading to ROS generation. HS^- reacts with Cys-SOH or protein disulfides (not shown here) leading to persulfidations (Cys-SSH) (Walsh and Giedroc 2020). Allicin leads to *S*-thioallylation of thiols. NO• reacts with oxygen to generate dinitrogen oxide and trioxide (N_2O and N_2O_3). N_2O activates Cys thiols to thiyl radicals, which react with NO• leading to *S*-nitrosylation (Cys-SNO). N_2O_3 can directly react with Cys thiolates to Cys-SNO (not shown here) (Fernando et al. 2019; Foster et al. 2003; Stomberski et al. 2019). The star (*) indicates the active site Cys of the protein.

Moreover, neutrophils use the inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO•) from arginine as bactericidal RNS. In bacteria, endogenous NO• is generated by the nitrate reductase from nitrate, which is used as anaerobic electron acceptor (Foster et al. 2003; Stomberski et al. 2019). NO• is a weak oxidant and can further react with $O_2^{\bullet -}$ to generate peroxynitrite ($ONOO^-$) as highly reactive intermediate between ROS and RNS (Radi 2018). NO• was shown to interact with H_2S to form nitroxyl (HNO) (Peng et al. 2017a; Walsh and Giedroc 2020). Thus, this crosstalk between ROS, RNS and RSS yields new reactive species that could partly explain the broad specificity of certain redox sensors. RNS can lead to post-translational thiol modifications of proteins and LMW thiols resulting in *S*-nitrosylation (Cys-SNO), which occurs via different chemical routes (Fernando et al. 2019; Foster et al. 2003; Stomberski et al. 2019). NO• autoxidation by oxygen yields dinitrogen oxide and trioxide (N_2O and

N_2O_3). N_2O activates Cys thiols to thiyl radicals (Cys-S•), which react with NO• to Cys-SNO (Figure 2). N_2O_3 directly reacts with Cys thiolates to form Cys-SNO (Fernando et al. 2019; Foster et al. 2003; Stomberski et al. 2019). In addition, the NO group can be transferred from metal-NO complexes or LMW-SNOs to protein Cys thiolates via *trans-S*-nitrosylation (Stomberski et al. 2019). Thus, *S. aureus* must be equipped with effective protection systems against ROS, RCS and RNS encountered endogenously and during interaction with host immune cells.

Additionally, *S. aureus* is exposed to antibiotics, xenosiderophores and metabolites, which have electron-deficient centers and are termed as reactive electrophilic species (RES). In *S. aureus*, endogenous RES are present as menaquinones in the electron chain, catechol-type siderophores and glycolytic aldehydes, such as methylglyoxal. Host-derived electrophiles are generated during macrophage and neutrophil infections by RNS and ROS as secondary reactive metabolites from oxidation of amino acids, unsaturated fatty acids and carbohydrates (Marnett et al. 2003). Electrophilic fatty acids accumulate especially during inflammation and can exert anti-inflammatory signaling effects (Delmastro-Greenwood et al. 2014). Electrophilic quinones have two modes of actions, an oxidative and an alkylating mode (Monks et al. 1992; O'Brien 1991). In the oxidative mode, quinones are reduced by one-electron transfer reactions to the highly reactive semiquinone anion radicals, leading to $O_2^{\bullet -}$ generation (Figure 2). In the alkylation mode, quinones react irreversibly with thiol groups via thiol-*S*-alkylation, resulting in protein aggregation and thiol depletion in the proteome and thiol metabolome (O'Brien, 1991,Monks et al., 1992,Liebeke et al., 2008). In general, the ability of quinones to alkylate protein thiols decreases with the number of substitutions of the quinone ring adjacent to the keto groups (O'Brien 1991; Smith 1985). Unsubstituted quinones, such as benzoquinones have a high ability to alkylate and aggregate protein thiols (Brunmark and Cadenas 1989; Liebeke et al. 2008). However, the fully substituted naphthoquinone lapachol was recently shown to act only via the oxidative mode in *S. aureus* (Linzner et al. 2020). In addition, the electrophile methylglyoxal is produced as toxic byproduct during glycolysis from dihydroxyacetone phosphate in many organisms (Booth et al. 2003; Ferguson et al. 1998; Kalapos 2008). Methylglyoxal conjugates nucleophilic thiols and amino groups of amino acids, DNA and RNA bases, leading to glycation end products (Kalapos 2008).

In addition, host and bacterial cells produce reactive sulfur species (RSS), such as H_2S, HS^- and S^{2-}, which have important signaling functions and act as gasotransmitters (Walsh and Giedroc 2020). RSS can lead to persulfidations of protein thiols and LMW thiols (RSSH). Persulfides have nucleophilic and electrophilic properties (Benchoam et al. 2020). While host phagocytes generate ROS and HOCl as microbicidal killing agents, RSS have beneficial properties as antioxidants to provide protection of bacterial pathogens against oxidative stress and antibiotics during infections (Shatalin et al. 2011; Walsh and Giedroc 2020). H_2S has been shown to confer tolerance to antibiotics by counteracting ROS production in many bacteria (Mironov et al. 2017; Shatalin et al. 2011). Mechanistically, H_2S has been proposed to sequester free iron to protect bacteria against oxidative stress elicited by antibiotics by inhibition of the Fenton reaction (Mironov et al. 2017). In *S. aureus*, endogenous H_2S is generated by the enzymes cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) in the transsulfuration pathway from cysteine and homocysteine (Walsh and Giedroc 2020). The *S. aureus cbs/cbe* mutant, deficient in H_2S biogenesis, was impaired in survival under macrophage and neutrophil infections, indicating that RSS are important antioxidants and defense mechanisms during host-pathogen interactions (Toliver-Kinsky et al. 2019; Walsh and Giedroc 2020). HS^- reacts with Cys-SOH or protein disulfides leading to widespread persulfidations of proteins in the proteome of *S. aureus* (Figure 2). Persulfidation of the major virulence regulator MgrA inhibits its DNA binding activity resulting in lower secretion of cytotoxins in the secretome (Peng et al. 2017bPeng et al. 2017). Furthermore, *S. aureus* encounters RSS in the form of the antimicrobial organosulfur compound and common foodstuff allicin from garlic, which is composed of the diallylthiosulfinate (Borlinghaus et al. 2014). In *S. aureus*, allicin reacts with redox sensitive protein thiols and LMW thiols via *S*-thioallylation, leading to inactivation of protein functions (Loi et al. 2019).

Defense mechanisms of *S. aureus* against ROS, RES, RCS, RNS and RSS

Enzymatic detoxification systems

Superoxide anion and H_2O_2 detoxification

S. aureus encodes several antioxidant enzymes, including superoxide dismutases (SODs), catalases and peroxiredoxins, which are involved in detoxification of $O_2^{\bullet -}$ and H_2O_2 and provide protection under infection conditions (Imlay 2008; Mishra and Imlay 2012). Two superoxide dismutases (SodA and SodM) are present in *S. aureus* that

catalyze the dismutation of $O_2^{\bullet -}$ to H_2O_2 (Clements et al. 1999; Valderas and Hart 2001; Valderas et al. 2002). While other bacteria possess Fe^{2+}- and Mn^{2+}-containing SODs, SodA and SodM of *S. aureus* are both Mn-dependent enzymes. Both SODs are induced by the redox cycling agents paraquat and methyl viologen as well as during the post-exponential growth phase in *S. aureus* (Clements et al. 1999; Karavolos et al. 2003; Valderas and Hart 2001; Valderas et al. 2002). We have recently shown that transcription of *sodA* and *sodM* is strongly enhanced under lapachol stress, which exerts its toxicity via redox cycling in *S. aureus*, leading to $O_2^{\bullet -}$ and H_2O_2 production (Linzner et al. 2020). In addition, SodM expression was upregulated in *S. aureus* in airway environments of cystic fibrosis patients (Treffon et al. 2018).

Both SodA and SodM are important for virulence in a murine abscess model and required for nutritional immunity in *S. aureus*, when Mn^{2+} is restricted in the host due to sequestering by calprotectin during infections (Garcia et al. 2017; Gaupp et al. 2012; Karavolos et al. 2003; Kehl-Fie et al. 2011). SodA is required for oxidative stress resistance and virulence in a murine infection model in the presence of Mn^{2+}, while SodM is more important under Mn^{2+}-starvation and provides resistance to nutritional immunity (Garcia et al. 2017). The cambialistic nature of SodM has been demonstrated *in vitro*, showing similar activity with Mn^{2+} and Fe^{2+} as metal cofactor. Thus, SodA is strictly Mn-dependent and SodM can switch from a Mn^{2+}- to a Fe^{2+}-dependent enzyme under conditions of Mn^{2+} depletion during infections (Garcia et al. 2017). Interestingly, expression of *sodA* is regulated by the MntR-dependent small non-coding RNA RsaC, which is induced under Mn^{2+}-starvation and basepairs with the *sodA* mRNA, thereby inhibiting translation of SodA under conditions of nutritional immunity, when expression of the cambialistic SodM is favored to combat oxidative stress (Lalaouna et al. 2019). The altered metal specificity of SodM is caused by two mutations, which evolved in close proximity to the active site, but without direct contacts of the sidechains to the metal or metal-coordinating ligands (Barwinska-Sendra et al. 2020).

Catalases (Kat) and peroxiredoxins (Prx) are the primary antioxidant enzymes for detoxification of H_2O_2 in most aerobic bacteria (Imlay, 2003, 2008; Mishra and Imlay 2012). While catalases operate at high H_2O_2 levels under oxidative stress, peroxiredoxins scavenge physiological levels of H_2O_2 produced during aerobic respiration (Imlay, 2003, 2008; Mishra and Imlay 2012). H_2O_2 detoxification by the major vegetative catalase involves heme iron for disproportionation of H_2O_2 to H_2O and O_2. In contrast, peroxiredoxins use a peroxidative Cys (C_P) for reduction of H_2O_2, leading to Prx-SOH and subsequent disulfide formation between C_P and the resolving Cys (C_R) (Poole et al. 2011). Recycling of oxidized Prx requires electron donors, such as the Trx/TrxR/NADPH pathway (Mishra and Imlay 2012).

S. aureus exhibits a remarkable H_2O_2 resistance and can survive 100 mM H_2O_2 (Weber et al. 2004), which depends on the major catalase KatA. KatA is peroxide-inducible, mediates resistance to oxidative stress and is controlled by the metalloregulatory Fur-family PerR repressor (Horsburgh et al. 2001a,b). While PerR was required for full virulence in a murine skin abscess model of infection, KatA was not essential for pathogenicity (Horsburgh et al. 2001a). However, KatA is important for survival under glucose starvation and desiccation conditions and promotes nasal colonization in *S. aureus* (Cosgrove et al. 2007; Horsburgh et al. 2001a). In addition, transcription of *katA* was strongly enhanced under oxidative and electrophile stress in *S. aureus*, including HOCl, AGXX®, MHQ, allicin and lapachol (Fritsch et al. 2019; Loi et al., 2018a,b, 2019; Linzner et al. 2020). Moreover, the *katA* mutant was sensitive to lapachol-induced ROS generation, indicating that KatA provides protection under quinone stress (Linzner et al. 2020).

The peroxiredoxins of *S. aureus* include the alkyl hydroperoxide reductase (AhpCF), the thiol peroxidase (Tpx), the bacterioferritin comigratory protein (Bcp) and two thiol-dependent peroxidases (GpxA1 and GpxA2). As a typical 2-Cys peroxiredoxin, bacterial AhpC reacts with peroxides to the C_P-SOH, resulting in intersubunit disulfide formation between C_P and C_R in the AhpC dimer and oligomerization to an inactive AhpC decamer complex (Parsonage et al., 2008,2015). Oxidized AhpC is regenerated by the flavin disulfide oxidoreductase AhpF, which uses NADPH or NADH as electron donors (Mishra and Imlay 2012; Poole and Ellis 1996). In *S. aureus*, the *ahpCF* and *bcp* peroxiredoxins are both PerR-controlled and H_2O_2-inducible (Horsburgh et al. 2001a). KatA and AhpCF have compensatory roles in H_2O_2 resistance, persistence and nasal colonization (Cosgrove et al. 2007). In the *katA* mutant, the *ahpCF* operon showed increased expression, contributing to H_2O_2 detoxification. *Vice versa*, expression of *katA* was elevated in the *ahpCF* mutant, providing H_2O_2 resistance (Cosgrove et al. 2007). The *katA ahpCF* double mutant exhibits growth defects during aerobic cultivations due to elevated endogenous ROS levels leading to DNA damage and cell death (Cosgrove et al. 2007).

In addition, *S. aureus* encodes two homologs of the thiol-dependent peroxidase Ohr, which are involved in detoxification of organic hydroperoxides, originating from lipid peroxidation (Dubbs and Mongkolsuk 2007; Mongkolsuk and Helmann 2002). The Ohr paralogs are redox-controlled by the thiol-based MarR/OhrR-family repressors MgrA and SarZ as well as by MsaB in *S. aureus* (Chen et al.

2011; Pandey et al. 2019). However, many other peroxiredoxins respond to oxidative stress, such as Tpx, Bcp or GpxA1 and GpxA2, which remain to be, characterized in future studies.

HOCl detoxification

While the functions of KatA and AhpCF in peroxide detoxification are widely studied, the enzymatic pathways for removal of HOCl are only beginning to emerge. We have recently identified the NADPH-dependent flavin disulfide oxidoreductase MerA, which responds most strongly to HOCl and is controlled by the Rrf2-family HypR repressor in *S. aureus* (Loi et al. 2018b). MerA conferred protection under oxidative stress provoked by HOCl, AGXX® and allicin in *S. aureus*. In addition, the *merA* mutant was impaired in survival under macrophage infections, indicating important roles of MerA in the virulence of *S. aureus* (Loi et al., 2018a,b, 2019). Thus, we hypothesize that the physiological function of MerA could be the reduction of strong oxidants, such as HOCl or OH•, which are generated under infection, aerobic growth or by ROS-producing antimicrobials.

The MerA flavin disulfide reductase harbors a $C_{43}XXXXC_{48}$ active site, which forms a disulfide during catalysis to transfer electrons from NADPH via a FAD cofactor to the substrate, such as allicin and possibly HOCl (Argyrou and Blanchard 2004). Accordingly, Cys43 was required for survival under HOCl stress and infections in *S. aureus* (Loi et al. 2018b). Moreover, MerA is a homolog of the RclA flavin disulfide reductase of *Escherichia coli* and *Salmonella* Typhimurium, which is controlled by the HOCl specific RclR regulator and was shown to promote survival of *S.* Typhimurium inside the phagosome (Baek et al. 2020; Parker et al. 2013). The crystal structure of RclA of *S.* Typhimurium revealed the typical flavin disulfide reductase-fold with two Cys in the active site and a FAD cofactor (Baek et al. 2020). Interestingly, Cu^{2+} enhanced the RclA-mediated oxidation of NADH, resulting in decreased oxygen levels, which might inhibit the oxidative burst of macrophages (Baek et al. 2020). In *Pseudomonas aeruginosa*, an alkyl hydroperoxidase D-like protein PA0565 (RcsA) was shown to reduce HOCl via its catalytic $C_{60}XXC_{63}$ motif and provides resistance under HOCl and infections (Nontaleerak et al. 2020). Thus, AhpD-like peroxiredoxins, MerA and RclA flavoenzymes could be responsible for HOCl removal in different bacterial pathogens.

Quinone and aldehyde detoxification

Furthermore, *S. aureus* encodes enzymes for detoxification of quinones, including quinone reductases (Frp, AzoR1), a nitroreductase (YodC), an FMN-linked monooxygenase, thiol-dependent dioxygenases (CatE, CatE2, MhqE) and a phospholipase/carboxylesterase (MhqD) (Fritsch et al. 2019; Ji et al. 2013). The quinone and nitroreductases catalyze reduction of quinones to hydroquinones. The thiol-dependent dioxygenases function in ring cleavage of quinones for their detoxification (Leelakriangsak et al. 2008). These quinone detoxification enzymes confer resistance to quinones and quinone-like antimicrobials and are controlled by the quinone-sensing MarR-type repressors QsrR and MhqR in *S. aureus* (Fritsch et al. 2019; Ji et al. 2013).

In addition, several uncharacterized thiol-dependent glyoxalases and aldehyde dehydrogenases are present in *S. aureus*, which might be involved in the detoxification of methylglyoxal and other aldehyde substrates. We have recently characterized the aldehyde dehydrogenase AldA, which showed broad-spectrum activity for oxidation of various aldehyde substrates, including methylglyoxal (Imber et al. 2018b). However, AldA was shown to confer resistance to HOCl stress and could be involved in methylglyoxal detoxification, which is elevated under HOCl stress (Imber et al. 2018b). In *E. coli*, methylglyoxal detoxification involved the GSH-dependent glyoxalase pathway, converting methylglyoxal to lactate (Booth et al. 2003; Ferguson et al. 1998). While there are structural and biochemical studies on putative glyoxalase enzymes in *S. aureus* (Chirgadze et al. 2018; Kim et al. 2017), a functional glyoxalase pathway has not been characterized. Of particular interest for future studies should be the DJ-1/ThiJ/PfpI superfamily protein HchA, which is induced under oxidative and electrophile stress conditions in *S. aureus*, such as AGXX®, HOCl, MHQ, allicin and lapachol (Fritsch et al. 2019; Loi et al., 2018a,b, 2019; Linzner et al. 2020). Purified HchA showed glyoxalase III and chaperone holdase activity in biochemical assays *in vitro* (Kim et al. 2017). However, the role of HchA in *S. aureus* cells under various stress conditions remains to be investigated.

H_2S and NO• detoxification

S. aureus cells possess detoxification enzymes for removal of toxic H_2S, which are encoded by the CstR-controlled *cstAB-sqr* operon and provide protection against RSS (Luebke et al. 2014). The sulfide:quinone oxidoreductase SQR catalyzes the two-electron oxidation of HS^-, leading to formation of LMW persulfides. CstA functions as sulfurtransferase reacting with inorganic polysulfides and persulfides of the cysteine desulfurase SufS. The persulfide dioxygenase CstB oxidizes LMW persulfides, such as

bacillithiol persulfides (BSSH) and coenzyme A persulfides (CoASSH) to produce thiosulfate and LMW thiols (Higgins et al. 2015; Shen et al., 2015,2016; Walsh and Giedroc 2020).

S. aureus adapts to NO• stress by expression of the lactate dehydrogenases *ddh*, *ldh1* and *lqo* and two terminal oxidases *qoxABCD* and *cydAB* for maintenance of the redox balance (Fuller et al. 2011). The flavohemoglobin Hmp catalyzes the oxidation of NO• to NO_3^-, representing the main enzymatic mechanism of RNS detoxification in *S. aureus* (Goncalves et al. 2006). NO• leads to activation of the SrrAB two-component system, which controls expression of *hmp*, *qoxABCD* and *cydAB* (Grosser et al. 2016; Kinkel et al. 2013). Apart from Hmp, the flavodiiron NO• reductase Nor contributes to NO• detoxification, which is induced under anaerobic conditions and important for the virulence in *S. aureus* (Favazzo et al. 2019; Lewis et al. 2015).

Functions of bacillithiol in detoxification and antibiotics resistance

LMW thiols are non-proteinogenous thiol compounds that are present in millimolar concentrations in the bacterial cytoplasm and function in maintenance of redox homeostasis (Chandrangsu et al. 2018; Loi et al. 2015; Van Laer et al. 2013). While the tripeptide glutathione (GSH) is utilized as LMW thiol by most Gram-negative bacteria, *Staphylococcus* species and other firmicutes produce bacillithiol (BSH) as alternative LMW thiol, which functions as GSH surrogate to maintain redox homeostasis (Chandrangsu et al. 2018). BSH derivatives are present in *Thermus thermophiles* and in phototrophic *Chlorobiaceae* (Hiras et al. 2018; Newton and Rawat 2019; Norambuena et al. 2018) and widely distributed in archaea (Rawat and Maupin-Furlow 2020). In addition, *S. aureus* uses coenzyme A (CoASH) as alternative LMW thiol, which is maintained in its reduced state by the CoASH disulfide reductase Cdr (delCardayre and Davies 1998; delCardayré et al. 1998).

Bacillithiol (BSH, Cys-GlcN-malate) is an α-anomeric glycoside of L-cysteinyl-D-glucosamine with L-malic acid of 398 Da (Figure 3), which is synthesized by the glycosyltransferase BshA, the deacetylase BshB and the Cys ligase BshC in *S. aureus* (Chandrangsu et al. 2018; Gaballa et al. 2010; Newton et al. 2009). Using biophysical methods, the BSH standard redox potential was determined as $E^{0'}$(BSSB/BSH) of –221mV (Sharma et al. 2013). However, Brx-roGFP2 measurements inside *S. aureus* revealed a more negative BSH redox potential (E_{BSH}) ranging from –282 to –295 mV during the growth (Loi et al. 2017). Under oxidative stress provoked by H_2O_2 and HOCl stress, BSH is oxidized to BSH disulfide (BSSB) as shown *in vitro* and *in vivo* in *S. aureus* (Dickerhof et al. 2020). At higher levels, HOCl leads to BSH sulfonamide formation and overoxidation to BSH sulfonic acids (Dickerhof et al. 2020). The reaction of HOCl with BSH is very fast and occurs with second order rate constants of 6 x 10^7 $M^{-1}s^{-1}$ (Dickerhof et al. 2020).

The reduced state of BSH is maintained by the NADPH-dependent flavin disulfide reductase YpdA in *S. aureus*, which functions as a BSSB reductase (Figure 3) (Linzner et al. 2019; Mikheyeva et al. 2019). YpdA acts in the Brx/BSH/YpdA/NADPH redox pathway to regenerate *S*-bacillithiolated proteins under oxidative stress in *S. aureus* as outlined in the next sections (Linzner et al. 2019).

Importantly, BSH was identified as virulence factor in clinical MRSA isolates, such as COL and the highly virulent USA300 strain. BSH promotes survival inside murine macrophages and neutrophils in human whole-blood infection assays (Posada et al. 2014; Pöther et al. 2013; Rajkarnikar et al. 2013). Moreover, *S. aureus* isolates of the NCTC8325-4 lineage evolved mutations in the *bshC* gene, leading to the lack of BSH synthesis (Newton et al. 2012;

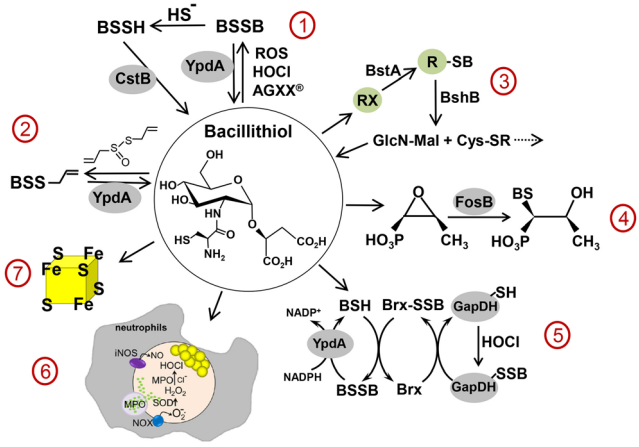

Figure 3: Bacillithiol functions in detoxification, antibiotics resistance and virulence in *S. aureus*. BSH is involved in detoxification of ROS, RES, HOCl, RSS and confers resistance to the redox active antibiotics fosfomycin, allicin, lapachol and AGXX® in *S. aureus*. (1) ROS, HOCl and AGXX® oxidize BSH to bacillithiol disulfide (BSSB), which is recycled by the BSSB reductase YpdA. BSSB could further react with HS^- generating BSH persulfides (BSSH), which are regenerated by the dioxygenase CstB. (2) Allicin reacts with BSH to *S*-allylmercaptobacillithiol (BSSA) as another substrate of YpdA. (3) The *S*-transferase BstA conjugates RES to BSH, forming BS-electrophiles (BS-R), which are cleaved by the amidase BshB to GlcN-Mal and mercapturic acids (Cys-SR), followed by their export. (4) The *S*-transferase FosB conjugates BSH to fosfomycin for its inactivation. (5) HOCl leads to *S*-bacillithiolations of proteins, which are reversed by the Brx/BSH/YpdA/NADPH pathway. (6) The Brx/BSH/YpdA/NADPH pathway is important under infections in *S. aureus*. (7) BSH functions in FeS cluster assembly in *S. aureus*.

Pöther et al. 2013). Thus, the *S. aureus* strain SH1000 of the NCTC8325-4 lineage was impaired in survival in infection assays compared to the SH1000 *bshC* repaired strain (Pöther et al. 2013). Interestingly, the *S. aureus bshA, ypdA* and *brxAB* mutants showed similar defects in survival inside J774.1 macrophages and neutrophils, indicating that the Brx/BSH/YpdA/NADPH system is essential under infections to regenerate BSH and protein thiols (Linzner et al. 2019; Mikheyeva et al. 2019; Pöther et al. 2013; Posada et al. 2014). Future studies should investigate the physiological role of BSH and the Brx/BSH/YpdA/NADPH redox pathway in *S. aureus* in murine infection models with defects in the NADPH oxidase and myeloperoxidase to elucidate if BSH homeostasis provides protection against the oxidative burst of neutrophils and macrophages.

Phenotype analyses of BSH-deficient mutants revealed important functions of BSH in the defense against ROS, HOCl, electrophiles, metals, xenobiotics, fosfomycin, rifampicin, AGXX®, allicin and lapachol in *S. aureus* (Figure 3)(Chandrangsu et al. 2018; Linzner et al. 2020; Loi et al., 2018a, 2019).

BSH is an important cofactor for the thiol-*S*-transferase FosB, which confers fosfomycin resistance. FosB catalyzes the ring cleavage of fosfomycin leading to formation of the BS-fosfomycin-conjugate (Figure 3) (Chandrangsu et al. 2018; Roberts et al. 2013). Both *fosB* and *bshA* mutants were similarly susceptible under fosfomycin stress in *S. aureus*, supporting the function of FosB as a BSH-dependent thiol-*S*-transferase (Posada et al. 2014; Rajkarnikar et al. 2013). In addition, the BSH-dependent *S*-transferase BstA was shown to conjugate BSH to electrophiles, such as 1-chloro-2,4-dinitrobenzene, monobromobimane and the antibiotic cerulenin in *S. aureus* (Newton et al., 2011,2012; Perera et al. 2014). BstA belongs to the widely conserved DinB/YfiT superfamily of *S*-transferases in BSH-, MSH- and GSH-producing bacteria (Newton et al. 2011; Perera et al. 2014). The resulting BS-electrophile conjugates are cleaved by the BSH *S*-conjugate amidase BshB to GlcN-Mal and mercapturic acids (CysSR), which are subsequently exported (Figure 3) (Newton et al. 2011). Thus, the thiol-*S*-transferases FosB and BstA are important determinants of antibiotics resistance in *S. aureus*.

Additionally, BSH and the Brx/BSH/YpdA/NADPH pathway were shown to confer protection against the naphthoquinone lapachol in *S. aureus* (Linzner et al. 2020). Lapachol acts via the oxidative mode, resulting in ROS formation, which leads to increased thiol oxidations and an oxidative shift in the E_{BSH} in *S. aureus*. Thus, the *bshA, brxAB* and *ypdA* mutants were more sensitive in growth and survival assays under lapachol treatment, indicating that the Brx/BSH/YpdA/NADPH pathway is essential to restore thiol homeostasis under lapachol stress (Linzner et al. 2020).

Similarly, BSH was shown to promote resistance to the antimicrobial surface coating AGXX® (Largentech GmbH, Berlin), which is composed of Ag^+ and Ru^+ ions and can be electroplated on medical devices and implants (Clauss-Lendzian et al. 2018; Gupta et al. 1999; Guridi et al. 2015; Heiss et al. 2017; Loi et al. 2018a; Vaishampayan et al. 2018). The Ag^+ and Ru^+ ions form a micro-galvanic cell, resulting in H_2O_2 and $OH^\bullet$ generation due to the electric field. In support of ROS production, AGXX® caused strong oxidative, quinone and metal stress responses in the *S. aureus* transcriptome (Loi et al. 2018a). AGXX® induced an oxidative shift in the E_{BSH} and protein *S*-bacillithiolation of GapDH. The *bshA* mutant was strongly impaired in growth and survival under AGXX® stress, indicating an important role of BSH in protection against ROS generated by AGXX® (Loi et al. 2018a). Thus, BSH is a beneficial antioxidant in *S. aureus*, to ensure survival during host-pathogen interactions and under ROS-producing antimicrobials.

Furthermore, BSH functions in detoxification of RSS and RNS in *S. aureus*, such as H_2S and nitroxyl (HNO), the latter being an intermediate of $NO^\bullet$ and H_2S (Peng et al. 2017a,b). H_2S caused widespread persulfidations in the proteome and thiol metabolome of *S. aureus* as revealed by elevated amounts of BSH persulfide (BSSH), CoASH persulfide (CoASSH) and cysteine persulfide (CysSSH) after exposure to Na_2S and HNO (Figure 3) (Peng et al. 2017a,b). Among the targets for persulfidations are the glycolytic GapDH and the major virulence regulator MgrA, which both were redox-controlled and inactivated by persulfidations of the active site Cys residues. The reversal of persulfidations was controlled by the novel thioredoxins TrxP and TrxQ (Peng et al. 2017bPeng et al. 2017).

In addition, BSH is crucial for resistance towards allicin and polysulfanes from garlic in *S. aureus* and *Bacillus subtilis* (Borlinghaus et al. 2014; Chi et al. 2019; Loi et al. 2019). The diallylthiosulfinate allicin decomposes upon heating to diallyl polysulfanes with up to seven sulfur chains (Münchberg et al. 2007; Tocmo et al. 2017). Allicin and diallyl tetrasulfane (DAS4) caused a strong oxidative and sulfur stress response in the transcriptome and widespread *S*-thioallylations of redox sensitive enzymes and transcriptional regulators in the proteome (Figure 2) (Chi et al. 2019; Loi et al. 2019). BSH-deficient mutants of *S. aureus* and *B. subtilis* are strongly impaired in growth and survival under allicin and DAS4 treatment (Arbach et al. 2019; Chi et al. 2019; Loi et al. 2019). In *S. aureus*, allicin caused a strong oxidative shift in the E_{BSH}, which is caused by BSH depletion and formation of *S*-allylmercaptobacillithiol (BSSA) (Figure 3). BSSA was identified as

substrate of the BSSB reductase YpdA to regenerate BSH by production of allyl thiol (Loi et al. 2019). In addition, the disulfide reductase MerA was shown to function in reduction of allicin to allyl thiols and the Brx/BSH/YpdA/NADPH pathway was important for regeneration of *S*-thioallylated proteins in *S. aureus*. Consistent with their biochemical functions in allicin detoxification, the *bshA*, *brxAB* and *ypdA* mutants were susceptible to allicin stress in *S. aureus* (Loi et al. 2019). In *B. subtilis*, the toxicity of polysulfanes was increased with longer sulfur chains (Arbach et al. 2019). Exposure to DAS3 and DAS4 caused strong depletion of BSH and cysteine, which was accompanied by increased formation of allyl thiols (Arbach et al. 2019), suggesting detoxification of polysulfanes in *B. subtilis* via related pathways as revealed in *S. aureus* (Loi et al. 2019).

During host-pathogen interactions, *S. aureus* has to cope with nutritional immunity caused by restriction of iron and other metal ions by host proteins (Maresso and Schneewind 2006; Marchetti et al. 2020). Thus, *S. aureus* must develop strategies to maintain Fe^{2+} homeostasis to ensure the biosynthesis of FeS clusters and Fe^{2+}-containing enzymes, such as catalase and superoxide dismutase (Maresso and Schneewind 2006; Marchetti et al. 2020). BSH plays an important role in FeS-cluster biogenesis in *S. aureus* (Figure 3). Specifically, BSH-deficient mutants were impaired in growth under iron starvation (Rosario-Cruz and Boyd 2016; Rosario-Cruz et al. 2015). BSH mutants showed growth defects in the absence of branched chain amino acids due to decreased synthesis of FeS cluster-containing enzymes, involved in leucine or isoleucine biosynthesis (Rosario-Cruz and Boyd 2016; Rosario-Cruz et al. 2015). It was suggested that BSH functions in FeS cluster assembly and transport to target proteins independently of the SufA and Nfu carrier proteins (Rosario-Cruz and Boyd 2016; Rosario-Cruz et al. 2015). However, the role of BSH in FeS cluster assembly is still unknown. It might be possible that BSH coordinates FeS cluster biogenesis together with the bacilliredoxins BrxAB as shown for GSH and glutaredoxins in eukaryotes (Lill 2020).

Redox regulation of proteins by protein *S*-bacillithiolation

During infections, *S. aureus* encounters the highly bactericidal HOCl as part of the oxidative burst by activated neutrophils (Winterbourn and Kettle 2013; Winterbourn et al. 2016). HOCl stress leads to formation of BSH mixed protein disulfides, termed as protein *S*-bacillithiolations, which are widespread across *Bacillus* and *Staphylococcus* species (Chi et al., 2011, 2013; Lee et al. 2007). These *S*-bacillithiolated proteins include metabolic and antioxidant enzymes, translation factors and redox sensing regulators, which respond to oxidative stress (Figure 4) (Chi et al., 2011,2013; Imber et al., 2018a, 2019). *S*-bacillithiolation was proposed to function in redox regulation of HOCl-specific transcription factors and to prevent irreversible overoxidation of vulnerable Cys thiols to sulfonic acids (Imber et al. 2019). In *B. subtilis*, the OhrR repressor was shown to sense HOCl and organic hydroperoxides (OHP) by *S*-bacillithiolation at its lone Cys15 residue, resulting in expression of the OhrA peroxidredoxin, which confers OHP and HOCl resistance (Chi et al. 2011; Lee et al. 2007).

In *S. aureus*, two OhrR homologs SarZ and MgrA are important virulence and antibiotics resistance regulators and share the conserved N-terminal Cys residue of the OhrR family, which senses oxidative stress via thiol oxidation (Figure 4) (Chen et al. 2011; Hillion and Antelmann 2015). The crystal structure of SarZ was resolved with Cys13 in the reduced, Cys-SOH and *S*-thiolated form with benzene thiol (Poor et al. 2009). Only the *S*-thiolated SarZ resulted in conformational changes in the DNA-binding helix-turn-helix motif, resulting in dissociation of SarZ from the target gene promoter (Poor et al. 2009). Increased thiol oxidation of MgrA and SarZ was found under HOCl stress in the redox proteome of *S. aureus*, supporting the hypothesis that both might be redox-controlled by *S*-bacillithiolation *in vivo* (Imber et al. 2018a). Furthermore, MgrA was regulated by persulfidation under sulfide stress, which impacts virulence factor secretion in the secretome of *S. aureus* (Peng et al. 2017bPeng et al. 2017).

Additionally, conserved *S*-bacillithiolated proteins were identified as metabolic enzymes with redox active catalytic centers, such as the methionine synthase MetE, the inosine monophosphate (IMP) dehydrogenase GuaB, the glyceraldehyde 3-phosphate dehydrogenase GapDH and the aldehyde dehydrogenase AldA (Figure 4) (Chi et al. 2011; Imber et al., 2018a, 2019). MetE represents the most abundant *S*-bacillithiolated protein in *B. subtilis* cells, which forms the BSH mixed disulfide at Cys730 in the active site Zn^{2+} center, resulting in inactivation of the enzyme and methionine auxotropy under HOCl stress. Moreover, the enzymes SerA, PpaC, MetI and YxjG were *S*-bacillithiolated under HOCl stress, which act in the same pathway as MetE (Chi et al. 2011; Imber et al., 2018a, 2019).

The glycolytic GapDH displayed 29% increased thiol oxidation under HOCl stress and was the most abundant *S*-bacillithiolated protein in *S. aureus* cells, representing 4% of the total Cys proteome (Imber et al., 2018a). GapDH *S*-bacillithiolation occurs under oxidative stress at the active site Cys151, resulting in enzyme inactivation and metabolic switching from glycolysis to the pentose phosphate

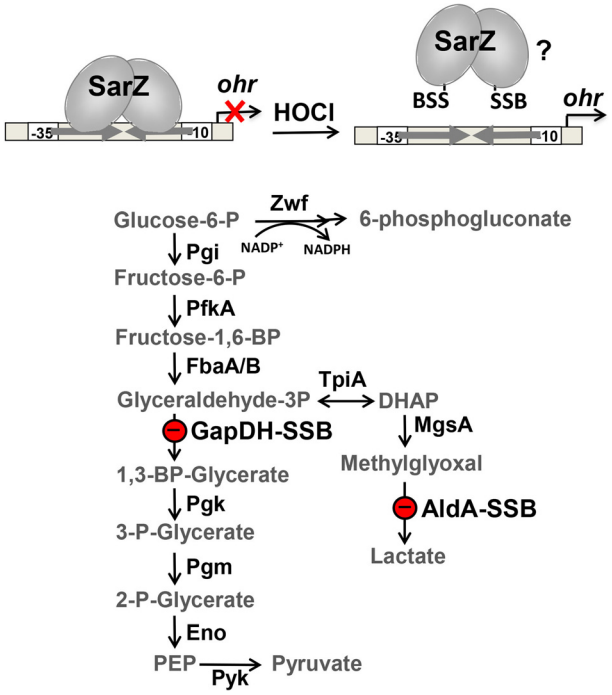

Figure 4: Physiological roles of *S*-bacillithiolations of GapDH, AldA and SarZ under HOCl stress in *S. aureus*. The OhrR-type repressors MgrA and SarZ were identified as HOCl-sensitive using OxICAT and *S*-thioallylated at Cys13 by allicin in *S. aureus*, indicating their possible redox regulation by *S*-bacillithiolation under HOCl stress (Imber et al. 2018a; Loi et al. 2019). SarZ controls the *ohr* peroxiredoxin, efflux pumps for antibiotics (*norB, tet38*), virulence factors and metabolic genes. SarZ confers resistance to OHP and antibiotics and contributes to virulence (Chen et al. 2011). HOCl stress causes *S*-bacillithiolation of the glycolytic GapDH and the aldehyde dehydrogenase AldA in *S. aureus,* resulting in the switch from glycolysis to the pentose phosphate pathway for NADPH regeneration (Deng et al. 2013; Imber et al. 2018a). AldA might be involved in methylglyoxal detoxification under HOCl stress. Abbreviations are: Pgi, glucose 6 phosphate isomerase; Pfk, phosphofructokinase; FbaA/B, fructose-1,6-bisphosphate aldolase; GapDH, glyceraldehyde 3-phosphate dehydrogenase; Pgk, phosphoglycerate kinase; Pgm, phosphoglycerate mutase; Eno, enolase; Pyk, pyruvate kinase; Zwf, glucose 6 phosphate dehydrogenase; TpiA, triose phosphate isomerase; MgsA, methylglyoxal synthase.

pathway (Deng et al. 2013; Imber et al. 2018a). Interestingly, GapDH inactivation by *S*-bacillithiolation proceeds faster compared to its inhibition by overoxidation in the absence of BSH *in vitro* (Imber et al. 2018a). Thus, *S*-bacillithiolation functions in redox regulation of GapDH activity and can prevent overoxidation of the active site (Imber et al. 2018a). Using molecular docking, BSH was modeled into the Cys151 active site of the apo- and holoenzymes. Interestingly, BSH occupies two different positions in the GapDH active site depending on the NAD^+ cofactor. While the BSH moiety is located in the active site with Cys151 in the attacking state position in the holoenzyme, *S*-bacillithiolation of the apoenzyme occurs with Cys151 in the resting state in the absence of the NAD^+ cofactor (Imber et al., 2018a,2019). However, *S*-bacillithiolation of GapDH did not cause major structural changes.

Similarly, the NAD^+-dependent aldehyde dehydrogenase AldA showed 29% increased thiol oxidation at its active site Cys279 and was inactivated by *S*-bacillithiolation under HOCl stress in *S. aureus* (Imber et al. 2018a,b). Computational chemistry revealed similar attacking and resting state positions of Cys279 upon *S*-bacillithiolation of the AldA holo- and apoenzymes, respectively, as shown for GapDH. In addition, *S*-bacillithiolation of AldA elicits no major structural and conformational changes (Imber et al., 2018b,2019). AldA was shown to catalyze oxidation of formaldehyde, methylglyoxal, acetaldehyde and glycolaldehyde *in vitro*, but its physiological substrate *in vivo* is unknown. Transcription of *aldA* is elevated under oxidative and electrophile stress, such as formaldehyde, methylglyoxal, HOCl, allicin and AGXX®, and depends of an unknown redox regulator. Phenotype analyses revealed that AldA is required for survival under HOCl stress, but dispensable under aldehyde stress. Thus, the physiological role of AldA under HOCl stress and its regulation remain to be elucidated in future studies (Imber et al. 2018b).

Apart from GapDH and AldA, GuaB is another NAD^+-containing enzyme with a highly conserved Cys308 active site that forms an adduct with the IMP substrate. GuaB is the most conserved target for *S*-glutathionylation, *S*-bacillithiolation and *S*-mycothiolation across prokaryotes and eukaryotes (Imber et al. 2019; Loi et al. 2015). Based on the different conformations of Cys308 in the substrate bound enzyme or in the apoenzyme structures, the BSH moiety may adopt a similar position in the GuaB active site with Cys308 in the attacking or resting state, respectively, as shown for GapDH and AldA (Imber et al., 2018a,b,2019). Altogether, *S*-bacillithiolations were shown to occur at accessible active site Cys residues of redox sensitive metabolic enzymes or transcriptional regulators. While *S*-bacillithiolation of GapDH and AldA does not lead to conformational changes, major structural rearrangements in the DNA binding HTH motif are required to accommodate redox regulation of the 1-Cys type OhrR-family repressors.

Reversal of protein *S*-bacillithiolation by the Brx/BSH/YpdA/NADPH pathway

The bacilliredoxins BrxA, BrxB and BrxC were previously identified as *S*-bacillithiolated in *B. subtilis* and *Staphylococcus carnosus* under HOCl stress, which suggested their role in the reversal of protein *S*-bacillithiolations (Chi et al. 2013).

BrxA and BrxB are paralogs of the DUF1094 family with a Trx fold and a CGC active site motif, which co-occur together with the BSH synthesis enzymes in BSH-producing bacteria (Gaballa et al. 2014). However, the redox potential of BrxA is relatively positive with −130 mV, and rather in the range of disulfide isomerases (Derewenda et al. 2009). BrxC has a TCPIS active site motif suggesting its possible function as monothiol Brx.

The functions of BrxA and BrxB as bacilliredoxins in reduction of *S*-bacillithiolated proteins have been first demonstrated for the *S*-bacillithiolated OhrR and MetE proteins in *B. subtilis* (Gaballa et al. 2014). DNA binding assays revealed the reactivation of OhrR after de-bacillithiolation of OhrR-SSB with the BrxB resolving Cys mutant protein, but not with the BrxB active site mutant. The removal of BSH from *S*-bacillithiolated MetE in *B. subtilis* cell extracts was shown with both BrxA and BrxB using BSH specific Western blots and mass spectrometry (Gaballa et al. 2014). In *S. aureus*, Brx activity assays were conducted with purified GapDH as most abundant *S*-bacillithiolated protein *in vivo* (Imber et al. 2018a). The *S. aureus* BrxA homolog catalyzed the de-bacillithiolation of GapDH, as shown by reactivation of the glycolytic activity of GapDH to oxidize the glyceraldehyde 3-phosphate substrate to 1,3-bis-phoshoglycerate with generation of NADH. While the BrxA resolving Cys mutant could restore GapDH activity, the BrxA active site mutant did not. The de-bacillithiolation reaction could be monitored using non-reducing BSH specific Western blots (Imber et al. 2018a). These results established the function of BrxA and BrxB in reversal of *S*-bacillithiolations *in vitro*.

Furthermore, the flavin disulfide reductase YpdA was phylogenetically associated with the BSH biosynthesis enzymes and bacilliredoxins (Gaballa et al. 2010). Transcriptome analyses revealed increased transcription of *ypdA, brxA, brxB, bshA*, *bshB* and *bshC* under oxidative and electrophile stress, such as H_2O_2, HOCl, diamide, AGXX®, lapachol, allicin and azurophilic granule proteins in *S. aureus* (Linzner et al. 2020; Loi et al., 2018a,b,2019; Palazzolo-Ballance et al. 2008). These data provide evidence for a functional Brx/BSH/YpdA/NADPH redox cycle in *S. aureus* (Figure 3), which is co-regulated under redox stress conditions. YpdA was characterized as a NADPH-dependent BSSB reductase in *S. aureus* to regenerate BSH homeostasis during recovery from oxidative stress and under infections (Linzner et al. 2019; Mikheyeva et al. 2019). Moreover, the *S. aureus brxAB*, *bshA* and *ypdA* mutants were more sensitive in growth and survival under HOCl stress and in macrophage infection assays *in vivo* (Linzner et al. 2019; Mikheyeva et al. 2019; Pöther et al. 2013; Posada et al. 2014). In addition, the *ypdA* mutant showed a 2-fold increased basal level of BSSB compared to the wild type (WT). Overproduction of YpdA, in turn, resulted in higher BSH levels (Mikheyeva et al. 2019). While the basal BSH levels of the *S. aureus* COL WT and the *ypdA* mutant were determined in a similar range of 1.5–1.9 µmol/g raw dry weight (rdw), the basal BSSB levels were measured as ~0.05 µmol/g and ~0.09 µg rdw in WT and *ypdA* mutant cells, respectively (Linzner et al. 2019). Thus, the BSH/BSSB ratio was determined as 35:1 for the WT and decreased to 17:1 in the *ypdA* mutant under non-stress conditions. Based on the high BSH levels in both WT and *ypdA* mutant strains, the basal E_{BSH} was not affected under non-stress conditions along the growth curve as measured using the Brx-roGFP2 biosensor, which is described in more detail in the following section (Linzner et al. 2019). However, due to the increased BSSB levels, the *ypdA* mutant was impaired to regenerate the reduced E_{BSH} upon recovery from oxidative stress, supporting the function of YpdA as BSSB reductase especially under oxidative stress in *S. aureus* (Linzner et al. 2019; Mikheyeva et al. 2019).

These results confirm previous findings, which revealed unchanged cytosolic GSH levels, but increased GSSG amounts in a yeast mutant deficient for the glutathione disulfide reductase Glr (Morgan et al., 2011,2013). Grx-roGFP2 biosensor measurements showed that the basal GSH redox potential (E_{GSH}) was only slightly increased by ~20 mV in the yeast *glr* mutant, whereas the H_2O_2 response was much stronger in the *glr* mutant with an impaired recovery of the reduced state (Morgan et al. 2011). It was further shown that Grx2 and Trx2 can compensate for GSSG reduction in the absence of Glr (Morgan et al. 2013). However, in *S. aureus* BrxA did not show BSSB reductase activity *in vitro* and the BSSB levels were not affected in the *brxAB* mutant, indicating that BrxAB cannot replace YpdA in regeneration of BSH (Linzner et al. 2019; Mikheyeva et al. 2019).

YpdA is a flavin disulfide reductase, which contains a conserved Cys14 residue in the glycine-rich Rossmann-fold NADPH binding domain ($GGGPC_{14}G$) (Bragg et al. 1997; Mikheyeva et al. 2019). Using NADPH coupled electron transfer assays, we demonstrated that YpdA consumes NADPH in the presence of BSSB, but not with other LMW thiol disulfides, such as GSSG, cystine or $CoAS_2$, confirming its function as BSSB reductase in *S. aureus in vitro* (Linzner et al. 2019). The BSSB reductase activity of YpdA was dependent on the conserved Cys14, which is unique in YpdA homologs and represents a novel active site. In support of its active site function, Cys14 of YpdA was identified as HOCl-sensitive with >10% increased oxidation using the redox proteomics approach OxICAT in *S. aureus* (Imber et al. 2018a). Thus, Cys14 might be *S*-bacillithiolated by BSSB, and regenerated by electron transfer from NADPH

via the FAD co-factor. Moreover, YpdA works in concert with BrxA and BSH to establish a functional Brx/BSH/YpdA/NADPH redox pathway for reduction of *S*-bacillithiolated proteins in *S. aureus*. In biochemical assays with the complete Brx/BSH/YpdA/NADPH pathway, BrxA was sufficient for the complete de-bacillithiolation of GapDH. In contrast, YpdA cannot regenerate *S*-bacillithiolated proteins, but functions instead in BSSB reduction to complete the Brx/BSH/YpdA/NADPH cycle (Linzner et al. 2019). In this redox cycle, the BrxA active site Cys attacks the *S*-bacilithiolated protein, resulting in Brx-SSB formation (Gaballa et al. 2014). Brx-SSB is resolved with BSH, leading to BSSB formation, which is recycled by YpdA to BSH (Linzner et al. 2019). However, structural analyses are required to resolve the detailed catalytic mechanism of YpdA in BSSB reduction. Future studies should be directed to identify the substrates of the Brx/BSH/YpdA/NADPH pathway under infection conditions inside host cells, which promote virulence, survival and persistence of *S. aureus* inside the host.

Monitoring the changes in the E_{BSH} using the Brx-roGFP2 biosensor

To monitor E_{BSH} changes inside *S. aureus*, BrxA were fused to the redox sensitive green fluorescent protein (roGFP2), generating a genetically encoded Brx-roGFP2 biosensor (Loi et al. 2017; Loi and Antelmann 2020). Upon oxidation, the two Cys residues in roGFP2 form a disulfide, influencing the spectral properties of roGFP2 (Meyer and Dick 2010; Schwarzländer et al. 2016). Specifically, the 488 nm excitation maximum is decreased and the 405 nm maximum increased, resulting in ratiometric changes in the 405/488 excitation ratio, which is quantified as oxidation degree (OxD). The fusion of Brx to roGFP2 facilitates the equilibration of roGFP2 with the BSH/BSSB redox pair enabling specific measurements of the E_{BSH} in *S. aureus* (Loi and Antelmann 2020; Loi et al. 2017; Meyer and Dick 2010; Schwarzländer et al. 2016). ROS exposure causes oxidation of cellular BSH to BSSB, which reacts with the Brx active site Cys of the Brx-roGFP2 probe, leading to Brx-SSB formation. The BSH moiety of Brx-SSB is transfered to the coupled roGFP2, which rearranges to the roGFP2 disulfide resulting in ratiometric changes of the excitation spectrum (Figure 5). Assuming that roGFP2 and the BSH/BSSB pair are in equilibrium, E_{BSH} is equal to the calculated E_{roGFP2}. Consequently, E_{BSH} can be calculated based on the OxD values of the Brx-roGFP2 biosensor and the previously determined $E^{o'}_{roGFP2}$ of −280 mV (Dooley et al. 2004), according to the Nernst equation as described (Loi et al. 2017; Morgan et al. 2011).

We have used the Brx-roGFP2 biosensor to monitor the changes in the E_{BSH} during the growth, under oxidative stress and antbiotics treatments in *S. aureus* isolates and in different mutant backgrounds (Loi and Antelmann 2020; Loi et al. 2017). First, purified Brx-roGFP2 was shown to respond fast and specific to low levels of 10–100 µM BSSB, but not to other LMW disulfides *in vitro*. The changes in E_{BSH} were determined in *S. aureus* COL *bshA*, *brxAB* and *ypdA* mutants along the growth and under oxidative stress to investigate the impact of the Brx/BSH/YpdA/NADPH pathway on the basal E_{BSH} and in response to oxidative stress (Linzner et al. 2019; Loi et al. 2017). Brx-roGFP2 measurements along the growth revealed a highly reducing basal level E_{BSH} in the range from −282 to −295 mV in the *S. aureus* COL WT, which was similar in the *ypdA* and *brxAB* mutants (Linzner et al. 2019; Loi et al. 2017). However, in the *bshA* mutant the biosensor was fully oxidized (Figure 5) (Loi et al. 2017). This indicates an impaired redox balance in the BSH-deficient strain, which might be caused by ROS increase. Interestingly, the *ypdA* mutant showed a strong Brx-roGFP2 oxidation after exposure to HOCl and H_2O_2, but was unable to regenerate the reduced state of E_{BSH} during recovery from oxidative stress due to its higher BSSB levels (Figure 5) (Linzner et al. 2019). In addition, the *ypdA* mutant was impaired in H_2O_2 detoxification as measured using the Tpx-roGFP2 biosensor (Linzner et al. 2019). These results confirmed the function of YpdA to regenerate reduced BSH after oxidative stress.

In addition, Brx-roGFP2 oxidation was analyzed after treatment of *S. aureus* COL WT with oxidants, different antibiotics and ROS-producing antimicrobials to investigate E_{BSH} changes (Loi et al., 2017,2018a, 2019). Brx-roGFP2 responds very fast to low levels of 50–100 µM HOCl, resulting in complete oxidation and slow recovery of the reduced E_{BSH} in *S. aureus* COL. Due to the high level of H_2O_2 resistance, Brx-roGFP2 was less reactive to 100 mM H_2O_2 and the *S. aureus* cells were able to recover faster their reduced E_{BSH} (Loi et al. 2017). Determination of the E_{BSH} changes of *S. aureus* COL after infection inside THP-1 macrophages revealed about 87% oxidation after 1 h, supporting that *S. aureus* experiences the oxidative burst (Loi et al. 2017). However, Brx-roGFP2 did not respond to sub-lethal concentrations of different antibiotics classes, such as rifampicin, fosfomycin, ampicillin, oxacillin, vancomycin, aminoglycosides and fluoroquinolones in *S. aureus* log phase cells (Loi et al. 2017).

In contrast, the Brx-roGFP2 biosensor was highly oxidized by the redox active antimicrobials AGXX®, allicin and lapachol resulting in an oxidative shift of E_{BSH} in *S. aureus* (Loi et al., 2018a, 2019, Linzner et al. 2020). Exposure of *S. aureus* COL to sub-lethal concentrations of

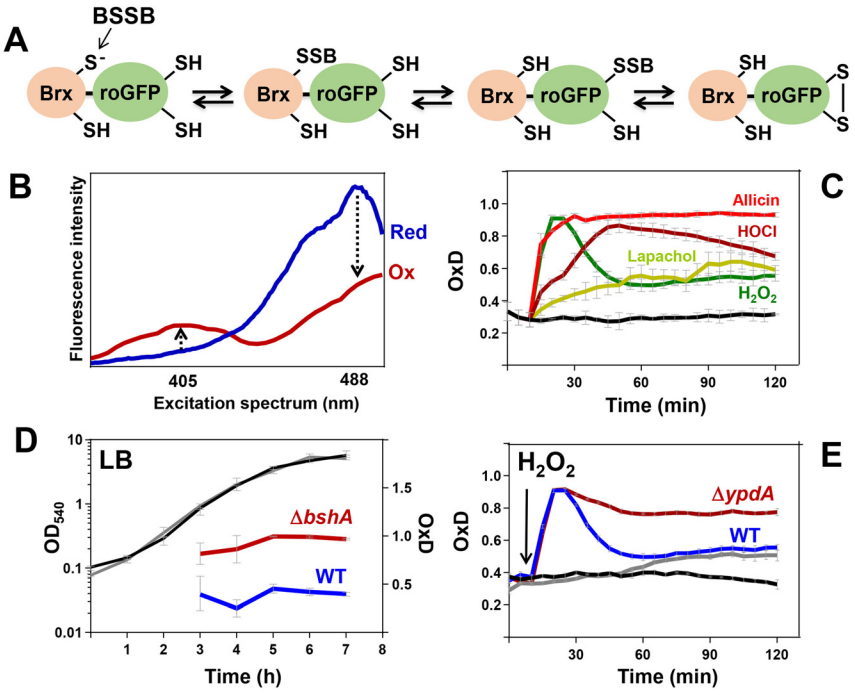

Figure 5: Principle, ratiometric changes and results of the Brx-roGFP2 biosensor in *S. aureus*. (A) The Brx active site thiolate of the Brx-roGFP2 biosensor reacts with BSSB, resulting in Brx-SSB formation, transfer of BSH to roGFP2, and re-arrangement to the roGFP2 disulfide (Loi et al. 2017). (B) Brx-roGFP2 oxidation results in ratiometric changes at the 405 and 488 nm maxima of the excitation spectrum, shown reduced (blue) and oxidized (red). The 405/488 excitation ratio is used for calculation of the biosensor oxidation degree (OxD). (C) *S. aureus* cells respond differentially to 100 mM H_2O_2, 100 µM HOCl, 50 µM allicin and 100 µM lapachol (Linzner et al. 2020; Loi et al. 2017; Loi et al. 2019). (D) The *bshA* mutant showed a fully oxidized basal redox state and (E) the *ypdA* mutant was impaired in recovery of the reduced E_{BSH} under oxidative stress (Linzner et al. 2019; Loi et al. 2017).

AGXX® leads to strong biosensor oxidation, which is caused by OH• and other ROS generated by AGXX® (Clauss-Lendzian et al. 2018; Loi et al. 2018a). Allicin causes *S*-thioallylations of BSH, resulting in BSSA formation, which oxidizes Brx-roGFP2 similar as BSSB (Loi et al. 2019). Thus, 50–100 µM allicin lead to fast and complete biosensor oxidation within 10 min in *S. aureus*, which was reversible with DTT, supporting the strong thiol reactivity of allicin (Figure 5) (Loi et al. 2019). The response of the Brx-roGFP2 biosensor was different for the naphthoquinone lapachol, which acts via the redox cycling mode in *S. aureus* (Linzner et al. 2020). Upon exposure to 100 µM lapachol, Brx-roGFP2 oxidation was slowly increased within 2 h with no recovery of reduced E_{BSH} (Linzner et al. 2020). This indicates constant generation of H_2O_2 by lapachol as measured with the Tpx-roGFP2 biosensor. In conclusion, Brx-roGFP2 is a valuable tool to screen for ROS-production and intracellular redox potential changes by redox active antibiotics in *S. aureus*.

Redox regulation of proteins by protein *S*-CoAlation

S. aureus produces millimolar CoASH and encodes a CoASH disulfide reductase Cdr, which reduces CoASH disulfide ($CoAS_2$) back to CoASH *in vitro* (delCardayre and Davies 1998,1998). Thus, before the discovery of BSH, CoASH was proposed to function as LMW thiol in *S. aureus* (delCardayre and Davies 1998; Tsuchiya et al. 2018). CoASH is an important thiol cofactor, which activates sugar metabolites of the central carbon catabolism by formation of energy-rich CoA thioesters, such as acetyl-CoA and succinyl-CoA to ensure ATP generation (Jackowski and Rock 1986). Recently, evidence was provided that CoASH can function in redox modification of proteins via *S*-CoAlations, which may substitute for *S*-bacillithiolation in the absence of BSH in *S. aureus* (Gout 2019; Tsuchiya et al. 2018). *S*-CoAlated proteins were enriched using pulldown with monoclonal anti-CoASH antibodies and identified by mass spectrometry (Gout 2019; Tsuchiya et al. 2018). In *S. aureus*, about 356 *S*-CoAlated proteins were identified under diamide stress, including conserved redox sensitive metabolic and antioxidant enzymes (GapDH, AldA, GuaB, Trx, AhpC, Tpx) and transcriptional regulators (SarR, CtsR, AgrA, PerR, SarS), which harbor active site Cys residues and overlap with *S*-bacillithiolated proteins under HOCl stress (Gout 2019; Tsuchiya et al. 2018).

Furthermore, protein *S*-CoAlation of the active site Cys151 of GapDH resulted in enzyme inactivation, which was reversible by DTT reduction (Tsuchiya et al. 2018). Computational chemistry proposed that the ADP moiety of CoASH occupies the NAD^+ binding pocket in the apoenzyme, facilitating *S*-CoAlation of Cys151 of GapDH (Tsuchiya et al. 2018). However, the physiological role of *S*-CoAlation in relation to sugar catabolism remains to be elucidated in *S. aureus*. Based on its metabolic role, enzymes involved in the activation of pyruvate and succinate by CoASH, such as pyruvate dehydrogenase (Pdh) and

succinyl-CoA-synthetase (SucCD) could be redox-controlled by *S*-CoAlation, which may impact the metabolic flux through the TCA cycle. Similarly, the pathways for reversal of *S*-CoAlations and the physiological role of Cdr under oxidative stress and infections are important subjects of future studies in *S. aureus*.

Thiol-based and other redox switches in *S. aureus*

PerR as Fur-family metal-based peroxide sensor

The peroxide specific PerR repressor belongs to the Fur family of metalloregulators and was characterized in *B. subtilis* and *S. aureus* (Faulkner and Helmann 2011; Pinochet-Barros and Helmann 2018). PerR negatively controls the adaptive response to H_2O_2 and confers H_2O_2 resistance in both bacteria (Faulkner and Helmann 2011; Horsburgh et al. 2001a; Ji et al. 2015; Mongkolsuk and Helmann 2002). The members of the PerR regulon function in ROS detoxification and iron storage, including genes for catalase, peroxiredoxins and thioredoxin reductase (*katA*, *ahpCF*, *bcp*, *trxB*), the iron storage ferritin and miniferritin (*ftnA*, *dps*), the ferric uptake (*fur*) repressor and the FeS cluster machinery (*sufCDSUB*) in *S. aureus* (Figure 6) (Horsburgh et al. 2001a). PerR binds to a conserved inverted repeat sequence ATTATAATTATTATAAT in the promoter region of the PerR regulon genes (Horsburgh et al. 2001a). In *S. aureus*, PerR is required for virulence in murine skin abscess and *Caenorhabditis elegans* infection models. In contrast, the catalase KatA is dispensable for pathogenicity (Ji et al. 2015; Horsburgh et al. 2001a).

The structures of *B. subtilis* and *S. aureus* PerR proteins contain two metal binding sites each, a structural Zn^{2+} site coordinated by four Cys residues and the regulatory Fe^{2+} or Mn^{2+} site, which includes His and Asp residues (Faulkner and Helmann 2011; Pinochet-Barros and Helmann 2018). The PerR repressors sense H_2O_2 by metal-catalyzed histidine oxidation in the regulatory Fe^{2+} binding sites, leading to their inactivation and derepression of transcription of the PerR regulons in *B. subtilis* and *S. aureus* (Ji et al. 2015; Lee and Helmann 2006; Pinochet-Barros and Helmann 2018). While the His and Asp residues in the regulatory site can bind both Fe^{2+} or Mn^{2+} as corepressors, only the Fe^{2+}-bound PerR can sense H_2O_2 by iron-catalyzed His oxidation. Specifically, Fe^{2+} in the regulatory site is oxidized by H_2O_2 in a Fenton reaction to generate $OH^{\bullet}$, leading to oxidation of His37 or His91 to form 2-oxo-histidine in the

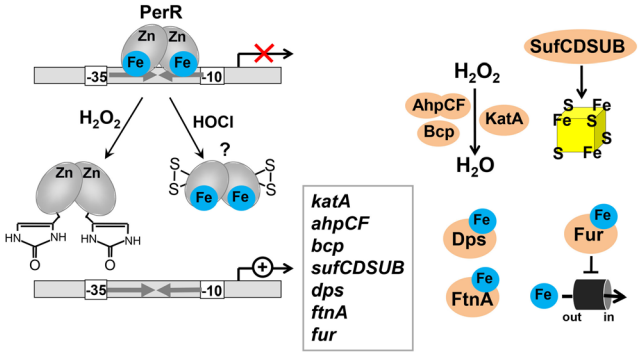

Figure 6: Redox regulation of the PerR repressor by iron-catalyzed His oxidation in response to H_2O_2 and by a putative thiol switch under HOCl stress in *S. aureus*. PerR senses H_2O_2 by Fe^{2+} catalyzed oxidation in the regulatory Asp/His site, leading to HO• formation and His37/His91 oxidation to 2-oxo-histidines, resulting in derepression of the PerR regulon (Ji et al. 2015; Lee and Helmann 2006). HOCl treatment leads most likely to PerR inactivation by a thiol switch in the structural Zn^{2+} site as identified in *Bacillus subtilis* cells using mass spectrometry *in vivo* (Chi et al. 2011). PerR controls genes encoding catalase, peroxiredoxins and thioredoxin reductase (*katA, ahpCF, bcp, trxB*), Fe-storage ferritin and miniferritin (*ftnA, dps*), the FeS cluster machinery (*sufCDSUB*) and the ferric uptake (*fur*) repressor in *S. aureus*. This figure is adapted from (Hillion and Antelmann 2015).

B. subtilis and *S. aureus* PerR proteins (Duarte and Latour 2010; Ji et al. 2015; Lee and Helmann 2006). However, the PerR repressor of *S. aureus* was hypersensitive to low H_2O_2 levels, which might explain its predominant Mn^{2+}-dependent repressor activity under aerobic conditions (Ji et al. 2015). This hypersensitivity of PerR towards H_2O_2 could be responsible for the higher basal expression of the PerR regulon genes, resulting in a high level of H_2O_2 resistance in *S. aureus*.

However, the PerR regulon was strongly induced under oxidative and electrophile stress, such as HOCl, diamide, MHQ, lapachol, allicin and AGXX® in *S. aureus* (Fritsch et al. 2019; Loi et al., 2018a,b, 2019; Linzner et al. 2020). Thus, PerR might sense various redox active compounds by a thiol switch mechanism in its structural Zn^{2+} site. In support of this hypothesis, a PerR intramolecular disulfide was identified by mass spectrometry in HOCl-treated *B. subtilis* cells (Chi et al. 2011). Thus, PerR might employ different redox sensing mechanisms to respond to H_2O_2 and other redox active compounds, that induce oxidative and electrophile stress responses.

The MgrA/SarZ/SarA-family of virulence and antibiotic regulators

S. aureus encodes several global MarR-type transcription factors, which control virulence, antibiotics and oxidative

stress resistance, including the Multiple gene regulator MgrA, SarZ and the Staphylococcal accessory regulator SarA (Ballal et al. 2009; Chen et al. 2009; Kaito et al. 2006; Poor et al. 2009; Truong-Bolduc et al. 2005, 2008). MgrA and SarZ belong to the MarR/OhrR-family, which sense ROS by thiol switch mechanisms via a conserved single Cys residue (Chen et al. 2011). MgrA is a global virulence regulator, which controls expression of >300 genes, involved in virulence, autolysis, antibiotics resistance, capsule and biofilm formation (Luong et al. 2006). Among the MgrA regulon genes are α–toxin (*hla*), coagulase (*coa*), protein A (*spa*), large surface proteins (*ebh, sraP* and *sasG*), fibrinogen-binding protein (*fnb*), extracellular protease, nuclease (*nuc*), autolysins (*lytM* and *lytN)*, multidrug efflux pumps (*norA*, *norB* and *tetAB)*, virulence regulators (*agr*, *lytRS*, *arlRS*, *sarS* and *sarV*) and capsule biosynthesis (*cap5*) (Ingavale et al. 2005; Kaatz et al. 2005; Luong et al. 2006; Truong-Bolduc et al. 2005, 2008). In DNase-I footprinting experiments, purified MgrA protein was shown to protect the conserved nucleotide sequence (A/T)GTTGT, which was repeated thrice upstream of the *sarV* promoter (Crosby et al. 2016; Manna et al. 2004). MgrA is important for virulence of *S. aureus* as shown in several infection models, including murine abscess, septic arthritis, sepsis, murine bacteremia and endocarditis models (Chen et al. 2006; Jonsson et al. 2008; Li et al. 2019).

The second MarR/OhrR-type regulator SarZ regulates a large regulon involved in virulence, antibiotic resistance, cellular metabolism and the *ohr* peroxiredoxin as main target gene (Kaito et al. 2006). Purified SarZ was shown to bind rather non-specific to the promoter regions of *hla*, *asp23* and *agr*, since a specific SarZ recognition motif could not be identified (Kaito et al. 2006). Both MgrA and SarZ sense oxidative stress by thiol switches, which involve the conserved redox sensing Cys13 residues. Thiol oxidation of MgrA and SarZ by organic hydroperoxides and H_2O_2 leads to formation of the Cys13-SOH (Chen et al., 2006,2009; Poor et al. 2009). Further oxidation of SarZ with the synthetic benzene thiol resulted in *S*-thiolated SarZ, which causes conformational changes in the HTH motif, leading to dissociation of SarZ from the promoter DNA as resolved in the crystal structure (Figure 4) (Poor et al. 2009).

The third redox sensing MarR-type regulator SarA controls genes involved in the oxidative stress defense (*sodB, trxB*) and virulence, including α- and ß-hemolysins (*hla, hlb*), toxic shock syndrome toxin 1 (*tst*), staphylococcal enterotoxin B (*entB*), protein A (*spa*) and fibronectin binding protein (*fnb*) (Chan and Foster 1998). SarA binds to a conserved operator sequence ATTTGTATTTAATATTTA-TATAATTG located upstream of the –35 promoter region of several SarA-regulated virulence genes (Chien et al. 1999). SarA contains a redox sensing Cys9 in its dimer interface and may use a thiol switch for regulation (Ballal and Manna 2009, 2010).

Furthermore, MgrA, SarZ and SarA were shown to be reversibly redox-controlled by Cys phosphorylation by the serine/threonine kinase (Stk1) and phosphatase (Stp1) (Sun et al. 2012). However, we identified the Cys13 peptides of MgrA and SarZ as HOCl-sensitive with >10% increased thiol oxidation under HOCl stress in *S. aureus* (Imber et al. 2018a). Furthermore, SarZ and MgrA were *S*-thioallylated under allicin stress in *S. aureus* (Loi et al. 2019). Thus, SarZ and MgrA might be redox-controlled by *S*-bacillithiolation under HOCl stress in *S. aureus*.

HypR as Rrf2-family redox sensor of HOCl stress

The Rrf2-family HypR repressor was identified as a novel redox sensor of HOCl stress in *S. aureus*, which uses a thiol switch mechanism (Loi et al. 2018b). Rrf2-family transcription factors are widespread in prokaryotes and regulate diverse functions, such as FeS cluster biogenesis (IscR), cysteine biosynthesis (CymR) and NO• detoxification (NsrR, RsrR) (Karlinsey et al. 2012; Mettert and Kiley 2015; Nakano et al. 2014; Partridge et al. 2009; Remes et al. 2015; Soutourina et al. 2009, 2010). HypR senses strong oxidants, such as HOCl, diamide, AGXX® and allicin stress (Loi et al. 2018a,b, 2019). HypR negatively controls expression of the *hypR-merA* operon, which was most strongly upregulated under HOCl stress in the transcriptome (Loi et al. 2018b) (Figure 7). In addition, the *hypR-merA* operon was highly induced in *S. aureus* during phagocytosis assays with neutrophils (Voyich et al. 2005), suggesting important functions as HOCl defense mechanism. The HypR repressor was shown to bind to a 12-3-12 bp inverted repeat sequence TAATTGTAACTA-N_3-CAGTTACAATTA in the *hypR-merA* promoter region, which is conserved across staphylococci.

HypR controls the NADPH-dependent flavin disulfide reductase MerA, which confers resistance towards HOCl, allicin and AGXX® stress as well as under infection conditions inside murine macrophages J774A.1 (Loi et al. 2018a,b, 2019). We hypothesize that MerA could be involved in HOCl reduction to promote survival under infections inside macrophages and neutrophils. In addition, the coupled transcription in the *hypR-merA* operon suggests that MerA could be the redox partner of HypR to recycle oxidized HypR during recovery from oxidative stress. The detailed functional analyses of MerA in terms of HOCl resistance are important goals of our current research.

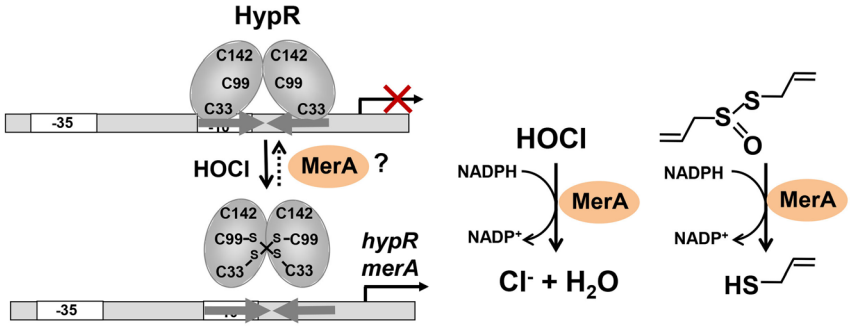

Figure 7: Redox regulation of the Rrf2-family repressor HypR under HOCl stress in *S. aureus*. HypR of *S. aureus* resembles a 2-Cys type redox regulator, which senses HOCl stress by intersubunit disulfide formation between Cys33 and Cys99′, leading to its inactivation and derepression of transcription of the *hypR-merA* operon (Loi et al. 2018b). The flavin disulfide reductase MerA is involved in HOCl and allicin detoxification and confers resistance to HOCl, allicin and infection conditions.

The HypR structure contains three Cys residues, including Cys33, Cys99 and Cys142, but only Cys99 is conserved in Rrf2 homologs (Loi et al. 2018b). While Cys33 is essential for redox sensing of HOCl stress, Cys99 is required for DNA binding activity of HypR in *S. aureus*. Under HOCl stress, HypR is oxidized to intersubunit disulfides between Cys33 and Cys99′ of opposing subunits in the HypR dimer, resulting in inhibition of its repressor activity and derepression of transcription of the *hypR-merA* operon (Loi et al. 2018b). Thus, the thiol switch model of HypR resembles that of a typical 2-Cys type redox sensing regulator. Future studies are directed to elucidate the structural changes of HypR upon oxidation, leading to its inactivation.

The MarR-family regulators QsrR and MhqR sense quinones

S. aureus and *B. subtilis* both encode two quinone-sensing MarR-type repressors, including the redox sensing MarR/DUF24-family regulator QsrR (or YodB) and the MarR-type repressor MhqR (Figure 8) (Chi et al. 2010; Fritsch et al. 2019; Ji et al. 2013; Leelakriangsak et al. 2008; Töwe et al. 2007). QsrR controls genes involved in the detoxification of quinones, such as ring-cleavage dioxygenases (*catE*, *catE2*), quinone and nitro reductases (*azoR1*, *frp*, and *yodC*) (Fritsch et al. 2019; Ji et al. 2013). A conserved inverted repeat with the consensus GTATAN$_5$TATAC was identified as the QsrR operator in the promoter region of the QsrR regulon genes (Ji et al. 2013). The MhqR repressor negatively controls transcription of the *mhqRED* operon, encoding the dioxygenase MhqE and the phospholipase/carboxylesterase MhqD. MhqR was shown to bind to a 9-9 bp inverted repeat sequence TATCTCGAA-aTCGAaATA in the promoter region upstream of *mhqR* (Fritsch et al. 2019). The quinone and nitroreductases (AzoR1, Frp, YodC) function in quinone reduction to redox stable hydroquinones (Figure 8) (Antelmann et al. 2008). Since benzoquinone alkylates protein and LMW thiols (Liebeke et al. 2008), the dioxygenases CatE, CatE2 and MhqE might catalyze ring cleavage of hydroquinones and quinone-*S*-adducts (Tam le et al. 2006).

In *S. aureus* and *B. subtilis*, the MhqR and QsrR regulons are most strongly upregulated by methylhydroquinone (MHQ) in the transcriptomes and contribute independently to quinone resistance (Fritsch et al. 2019; Ji et al. 2013; Leelakriangsak et al. 2008; Töwe et al. 2007). Interestingly, the MhqR and QsrR regulons of *S. aureus* confer resistance to antimicrobials with quinone elements, such as pyocyanin, rifampicin and ciprofloxacin (Fritsch et al. 2019; Ji et al. 2013). Pyocyanin is a toxic pigment produced by *P. aeruginosa*. Thus, MhqR and QsrR provide resistance of *S. aureus* towards pyocyanin to survive respiratory co-infections with *P. aeruginosa* in cystic fibrosis patients (Noto et al. 2017).

Furthermore, the MhqR regulon conferred tolerance to lethal H_2O_2 stress and was important for long-term survival of *S. aureus* inside macrophages (Fritsch et al. 2019). Similarly, the QsrR regulon mediates resistance to killing by macrophages in infection assays, indicating a crucial role of quinone detoxification pathways for virulence and survival of *S. aureus* inside host cells (Ji et al. 2013). During infections, the QsrR and MhqR regulons could be involved in detoxification of host-derived catecholamines, which are produced in macrophages and neutrophils to enhance the inflammatory response (Flierl et al. 2007). Infection-relevant electrophilic quinones could further arise in neutrophils from MPO-catalyzed serotonin oxidation to tryptamine-4,5-dione, which might be detoxified by the QsrR and MhqR regulons to enhance survival of *S. aureus* (Ximenes et al. 2009). Phenotype analyses further revealed an increased respiratory chain activity and higher ATP levels in the *S. aureus mhqR* mutant, pointing to the physiological role of the MhqR regulon to maintain the respiratory menaquinones in their reduced state (Fritsch

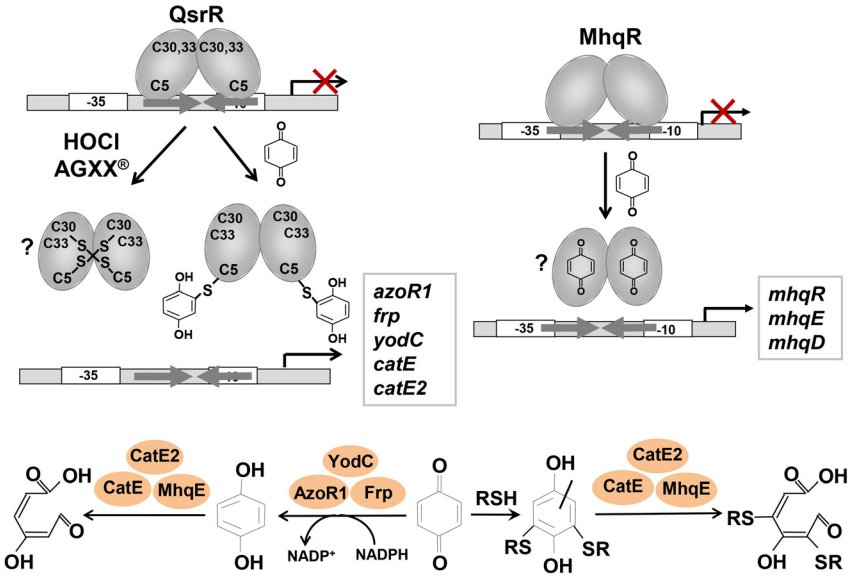

Figure 8: Redox sensing of quinones and antibiotics by the MarR-type repressors QsrR and MhqR in *S. aureus*. QsrR senses quinones by thiol-*S*-alkylation of Cys5, leading to derepression of dioxygenases (*catE, catE2*) and quinone reductases (*frp, azoR1, yodC*) (Ji et al. 2013). In addition, QsrR might sense oxidative stress by a thiol switch *via* intersubunit disulfide formation in *S. aureus* as revealed for the homologous YodB repressor in *B. subtilis* (Chi et al. 2010; Lee et al. 2016). Quinone sensing by MhqR involves most likely quinone binding to the conserved ligand pocket, resulting in upregulation of the *mhqRED* operon, which codes for the phospholipase/carboxylesterase MhqD and the dioxygenase MhqE (Fritsch et al. 2019). Since benzoquinone alkylates protein and LMW thiols in *B. subtilis* (Liebeke et al. 2008), we hypothesize that dioxygenases catalyze ring cleavage of hydroquinones and quinone-*S*-adducts. Both QsrR and MhqR confer independently resistance to quinones and the antimicrobials pyocyanin, ciprofloxacin and rifampicin.

et al. 2019). In support of this notion, the MhqR regulon of *B. subtilis* was important for the formation of cell-wall free L-forms, which requires reduced quinones for continued respiration and contributes to survival and resistance (Kawai et al. 2015).

The MhqR repressors of *B. subtilis* and *S. aureus* do not use thiol-based mechanisms for redox sensing of quinones (Fritsch et al. 2019; Töwe et al. 2007). Since many MarR--type regulators harbor conserved ligand binding pockets, MhqR might sense quinones by direct binding to a ligand pocket, leading to derepression of the *mhqRED* operon in *S. aureus* (Grove 2017; Perera and Grove 2010; Wilkinson and Grove 2006).

In contrast to MhqR, QsrR contains a conserved redox sensing Cys5 and two non-conserved Cys30 and Cys33 residues (Ji et al. 2013). The redox sensing mechanism of QsrR was shown to involve thiol-*S*-alkylation of the Cys5 residue, resulting in QsrR inactivation (Figure 8) (Ji et al. 2013). The detailed mechanism and structural changes of QsrR upon *S*-alkylation have been resolved for reduced and menadione-bound QsrR. QsrR alkylation leads to movement and rotation of the α4 and α4′ DNA binding helices from 106 to 117°, which is incompatible with DNA binding and causes dissociation of QsrR from the promoter (Ji et al. 2013). However, this Cys-alkylation model has been resolved with the QsrR Cys30,33 double mutant protein. The *B. subtilis* YodB repressor was previously shown to sense diamide and quinones via intersubunit disulfide bond formation between the conserved Cys6 and the C-terminal Cys101′ or Cys108′ of opposing subunits in the YodB dimer *in vivo* (Chi et al. 2010).

Recent crystal structure analyses have resolved two mechanisms for YodB inactivation, the *S*-alkylation of Cys6 by methyl-*p*-benzoquinone and the thiol switch between Cys6 and Cys101′ under diamide stress (Lee et al. 2016). Quinones cause *S*-alkylation at Cys6 with minor structural changes by 3 Å rotations of the α4 and α4′ domains, similar as shown for QsrR (Lee et al. 2016). In contrast, diamide leads to Cys6-Cys101′ intersubunit disulfide formation with large structural rearrangement, leading to complete dissociation from the DNA (Lee et al. 2016). Transcriptome analyses revealed that the QsrR regulon is strongly upregulated by quinones and other redox active compounds, such as MHQ, HOCl, AGXX®, allicin and lapachol in *S. aureus* (Fritsch et al. 2019; Linzner et al. 2020; Loi et al. 2018a,b, 2019). Thus, the two Cys QsrR repressor most likely senses quinones and strong oxidants by *S*-alkylation and disulfide formation, respectively, which remains to be elucidated.

It is possible that QsrR/YodB and MhqR sense different quinone-related redox signals, controlling the detoxification/reduction of oxidized quinones and ROS by QsrR/YodB as overarching thiol-based regulator, while MhqR could be additionally involved in trapping and regulation of ring cleavage of reduced and oxidized quinones. QsrR/YodB uses a thiol switch mechanism to respond to ROS,

which oxidize respiratory menaquinones and require quinone detoxification/reduction pathways to restore respiration. MhqR could rather sense accumulating quinones to induce additional quinone detoxification pathways when the QsrR/YodB regulon is overwhelmed.The crosstalk of both systems was confirmed by lower expression of QsrR/YodB regulons in the *mhqR* mutants and *vice versa* in *B. subtilis* and *S. aureus* (Fritsch et al. 2019; Leelakriangsak et al. 2008; Töwe et al. 2007). The investigation of the kinetics, specificities, crosstalks and reversibilities of both systems are interesting goals of future mechanistic studies.

The thiol-based redox sensor Spx and its YjbH adapter

The thiol-based redox sensor Spx belongs to the arsenate reductase (ArsC) family, representing an unusual transcriptional activator lacking the HTH motif (Nakano et al. 2003, 2005; Zuber 2009). In *S. aureus*, transcription of *spx* is positively regulated by the ArlRS two-component system (Crosby et al. 2020). Spx is activated in response to oxidative stress by thiol oxidation of its CXXC redox switch motif to an intramolecular disulfide (Nakano et al. 2005). The Spx disulfide interacts with the α-C-terminal domain of the RNA polymerase and activates transcription of the Spx regulon, which is involved in the thiol redox homeostasis and oxidative stress resistance, including thioredoxin and thioredoxin reductase (*trxA, trxB*) (Figure 9) (Nakano et al. 2003, 2005; Zuber 2004, 2009). Thus, the *spx* mutant shows increased susceptibility to oxidative, heat and salt stress in *S. aureus* (Pamp et al. 2006; Wang et al. 2010). Furthermore, Spx is involved in *pia*-dependent biofilm formation and controls expression of the *icaABCD* operon. Spx represses *cspA* transcription, which inhibits the activation of SigB leading indirectly to downregulation of aureolysin (*aur*) and staphyloxanthin biosynthesis (*crtOPQMN* operon) (Austin et al. 2019; Donegan et al. 2019).

The post-translational control involves the proteolytic degradation of Spx by the ClpXP system with the help of the YjbH adapter. Under control conditions, YjbH binds Spx, which is targeted to the ClpXP machinery resulting in Spx degradation (Engman et al. 2012; Garg et al. 2009; Pamp et al. 2006). Oxidative stress causes YjbH self-aggregation possibly due to thiol oxidation of its Zn^{2+} redox switch motif leading to Spx stabilization and increased Spx protein levels (Figure 9) (Engman and von Wachenfeldt 2015). Apart from proteolysis, YjbH controls antibiotic resistance in *S. aureus*. The *yjbH* mutant was resistant to ß-lactam antibiotics due to increased PBP4 expression and

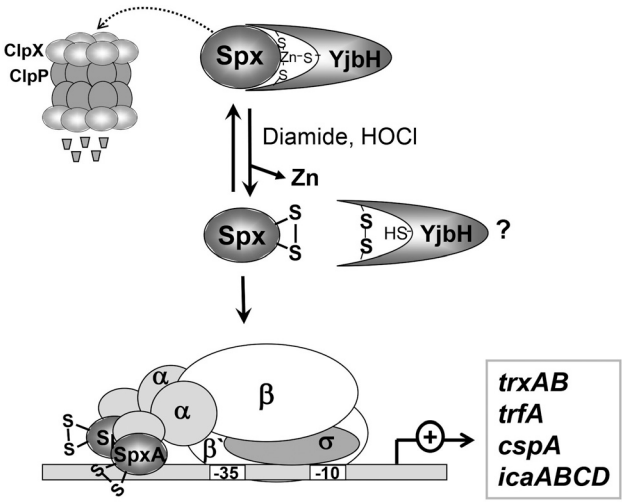

Figure 9: Redox control of the oxidative stress regulator Spx in *S. aureus*. Under control conditions, Spx is complexed by the YjbH adapter and targeted to the ClpXP machinery for proteolytic degradation. Oxidative stress causes possibly thiol oxidation and self-aggregation of YjbH, resulting in Spx stabilization (Engman and von Wachenfeldt 2015). Spx is activated by thiol oxidation to an intramolecular disulfide. Oxidized Spx binds to the αCTD of RNAP leading to activation of transcription of *trxA, trxB*, *trfA*, *cspA* and *icaABCD* in *S. aureus*, which impacts redox homeostasis, virulence factor expression, antibiotics resistance and biofilm formation. This figure is adapted from (Hillion and Antelmann 2015).

peptidoglycan cross-linking (Gohring et al. 2011). Spx controls the *mecA* homolog *trfA* in *S. aureus*. While the *trfA* mutant was susceptible to oxacillin and glycopeptide antibiotics, *trfA* upregulation in the *yjbH* mutant conferred antibiotics resistance (Jousselin et al. 2013). In addition, Spx activates the MazEF toxin-antitoxin system, which promotes dormancy and antibiotic tolerance (Panasenko et al. 2020). The lack of the extracellular proteases, such as aureolysin in the *yjbH* mutant led to hypervirulence in a systemic mouse infection model and enhanced colonization of the kidney and the spleen in a murine sepsis model (Austin et al. 2019; Kolar et al. 2013).

The TetR-family regulator and electrophile sensor GbaA

The TetR-family regulator GbaA (Glucose-induced biofilm accessory protein A) was characterized as a novel thiol switch and monothiol electrophile sensor (Ray et al. 2020). GbaA controls glucose-induced biofilm formation by the polysaccharide intracellular adhesion (PIA), which is composed of poly-*N*-acetyl glucosamine (PNAG) as biofilm matrix in *S. aureus* (Ray et al. 2020; You et al. 2014; Yu et al.

2017). GbaA negatively controls two operons of unknown functions, encoding a putative glyoxalase, the NAD^+ dependent epimerase/dehydratase NmrA, the short chain dehydrogenase/oxidoreductase GbaB, an amidohydrolase and an α, *ß*-fold hydrolase (Figure 10) (Ray et al. 2020; You et al. 2014; Yu et al. 2017). The GbaA repressor was shown to bind to a palindromic sequence AAACGGAGAGTTATCCGTTT in the upstream promoter region of *gbaA*. The *gbaA* mutant showed strongly enhanced biofilm formation in a super-biofilm elaborating clinical isolate *S. aureus* TF2758 (Yu et al. 2017), and biofilm formation was dependent on GbaB (Ray et al. 2020). GbaB was suggested to catalyze the oxidation of an alcohol to a sugar aldehyde in the PNAG biosynthesis pathway, but the physiological role and the inducer of the GbaA regulon are unknown (You et al. 2014; Yu et al. 2017).

Interestingly, the GbaA regulon was induced by different redox active compounds and antibiotics, including RNS, AGXX®, allicin, erythromycin, colistin, vancomycin and most strongly by the electrophiles methylglyoxal and N-ethylmaleimide (NEM) (Loi et al. 2018a, 2019; Mäder et al. 2016; Ray et al. 2020; Schlag et al. 2007Loi et al. 2018, 2019; Mäder et al. 2016; Ray et al. 2020; Schlag et al. 2007). GbaA was shown to function as a thiol switch and an electrophile sensor *in vitro*, which involves the conserved Cys55 and Cys104 residues in the DNA binding and regulatory domains (Ray et al. 2020). GbaA is oxidized to an intramolecular disulfide in response to diamide, BSSB, GSSG and GSNO *in vitro*, which does not affect its DNA binding activity (Figure 10). However, treatment of the single Cys55A or Cys104A mutant proteins with NEM and BSSB resulted in NEM-alkylation and *S*-bacillithiolation of the remaining Cys leading to inactivation of the GbaA repressor (Figure 10) (Ray et al. 2020). In addition, Cys104 was found more reactive and accessible towards electrophiles, indicating that Cys104 is the redox sensing Cys and most likely modified by electrophiles or BSSB *in vivo* (Ray et al. 2020). Thus, the redox sensing mechanism of GbaA differs from other electrophile sensors, such as YodB, which senses diamide and quinones via thiol switch and *S*-alkylation mechanisms, both leading to structural changes and inhibition of its DNA binding activity (Chi et al. 2010; Lee et al. 2016; Leelakriangsak et al. 2008). While GbaA resembles a 2-Cys type regulator, only *S*-thiolation or *S*-alkylation causes structural changes (Ray et al. 2020). However, the physiological electrophile and functions of the GbaA regulon members under oxidative and electrophile stress and biofilm formation are unknown.

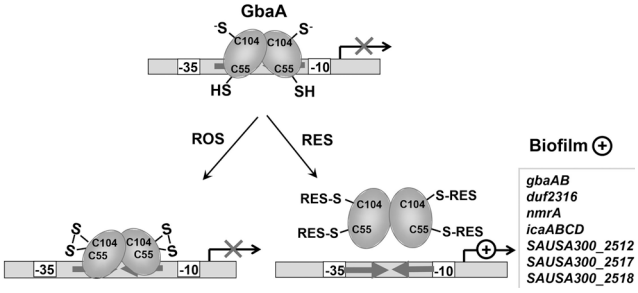

Figure 10: Redox sensing of electrophiles by the TetR-family biofilm regulator GbaA. The GbaA repressor is oxidized by different oxidants to the Cys55-Cys104 intramolecular disulfide, which does not affect the structure and DNA binding activity of GbaA. Alkylation of GbaA by electrophiles causes structural changes and loss of DNA binding, resulting in derepression of transcription of the GbaA regulon (Ray et al. 2020). GbaA controls two operons involved in biofilm formation, including a glyoxalase, the NAD^+-dependent epimerase/dehydratase NmrA, DUF2316, the short chain dehydrogenase/oxidoreductase GbaB, the GbaA repressor, an amidohydrolase and an *α*, *ß*-fold hydrolase.

Since the GbaA regulon is required for glucose-induced biofilm formation, host-derived oxidation products of glucose, such as the *α*,*ß*-dicarbonyls glucosone, glyoxal, methylglyoxal, glycolaldehydes, or other reactive glycolytic intermediates could be physiological electrophiles sensed by GbaA. These glucose oxidation products accumulate in human tissues, blood and activated neutrophils causing glycation of lysine, arginine and cysteine in proteins (Vetter 2015). In neutrophils, the MPO reaction product HOCl causes formation of chloramines, which are degraded to acrolein and *p*-hydroxyphenylacetaldehyde as further infection-related electrophiles (Hazen et al. 1998). Reactive aldehydes might increase in *S. aureus* during the switch to biofilm formation and anaerobic fermentation, which relies on high glycolytic activity for ATP production. In the next section, we discuss the SrrAB system, which senses oxygen levels and controls the switch to anaerobiose and biofilm formation via the SrrB redox switch (Tiwari et al. 2020; Yarwood and Schlievert 2000). Thus, GbaA and SrrAB might be responsible to sense different signals during biofilm formation and the switch from aerobic to anaerobic conditions to allow biofilm matrix synthesis, removal of toxic aldehydes and metabolic adaptation.

Another infection-relevant electrophile could be the major immunoregulatory metabolite itaconate, which inhibits the succinate dehydrogenase resulting in metabolic rewiring during inflammatory macrophage activation (Lampropoulou et al. 2016). Itaconate reacts as electrophile with GSH and induces electrophile stress in host cells (Bambouskova et al. 2018). In *P. aeruginosa* lung infections, host-derived itaconate reprograms bacterial metabolism and induces biofilm formation via enhanced exopolysaccharide matrix biosynthesis (Riquelme et al.

2020). Thus, itaconate could be a candidate electrophile to inactivate the GbaA repressor to promote PNAG synthesis and biofilm formation in *S. aureus*, which remains to be investigated.

Furthermore, GbaA belongs to the TetR-family regulators, which are often inactivated by direct binding of antibiotics as ligands to the C-terminal regulatory domain (Cuthbertson and Nodwell 2013). Since GbaA responds to various antibiotics and allicin (Loi et al. 2019; Mäder et al. 2016), a direct binding of antibiotics and electrophiles coupled with ROS-induced *S*-thiolation or *S*-alkylation of Cys104 could possibly inactivate GbaA *in vivo*. In summary, *S. aureus* can be exposed to a plethora of oxidants and electrophiles present during host-pathogen interactions that might be sensed by GbaA and other redox sensors, which enable host adaptation. Thus, future studies should shed light on the signals and redox chemistry of host-pathogen interactions.

The SrrAB two-component system as redox sensor of oxygen levels

The Staphylococcal respiratory regulator (SrrAB) two-component system consists of the sensor histidine kinase SrrB and the DNA-binding response regulator SrrA (Yarwood and Schlievert 2000). The SrrAB regulon is implicated in long-term biofilm stability, anaerobic growth, oxidative and nitrosative stress resistance and contributes to the virulence and protection against neutrophil killing in *S. aureus* (Kinkel et al. 2013; Mashruwala and Boyd 2017; Pragman et al. 2004; Ulrich et al. 2007). SrrAB regulon members include genes for the biogenesis of cytochromes and terminal oxidases (*ctaB*, *cydAB*, *qoxABCD*), heme biosynthesis (*hemACDX*), anaerobic fermentation (*pflAB*, *adhE*, *nrdDG*), and RNS resistance (*hmp*, *scdA*) (Figure 11) (Kinkel et al. 2013; Mashruwala and Boyd 2017; Oogai et al. 2016; Yarwood and Schlievert 2000). In addition, the SrrA response regulator binds to the *agr*, *spa*, *srrAB*, *icaA*, *RNAIII* and *tst* promoters and is involved in virulence regulation, directly and indirectly via Agr (Pragman et al. 2004; Ulrich et al. 2007). However, no consensus sequence of a potential SrrA recognition motif could be identified in the promoter regions of the target genes. Furthermore, SrrAB positively influences expression of *dps, scdA*, *ahpCF* and *katA*, which function in iron storage, FeS-cluster repair and H_2O_2 detoxification leading to an oxidative stress resistance (Mashruwala and Boyd 2017). The crosstalk between Nos and SrrAB mediates the switch from aerobic to anaerobic energy metabolism, since SrrAB co-regulates

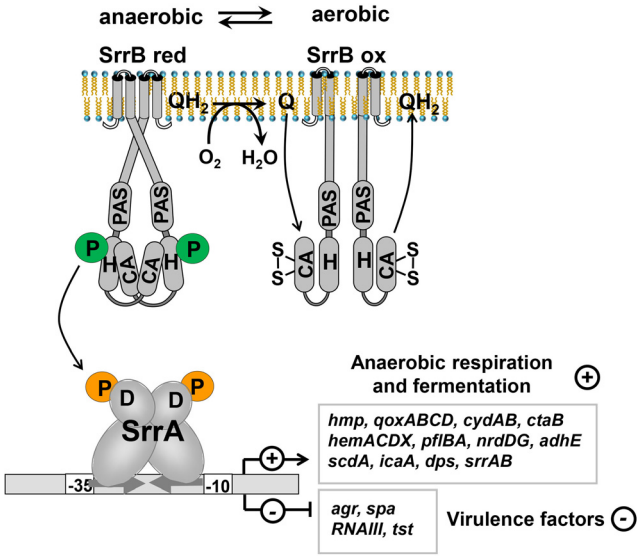

Figure 11: Sensing of oxygen by the SrrAB two-component system. SrrAB senses oxygen availability *via* the redox state of respiratory menaquinones and by a thiol switch in its sensor kinase SrrB (Tiwari et al. 2020). Aerobic conditions lead to menaquinone oxidation and intramolecular disulfide formation between Cys464 and Cys501 in SrrB, resulting in SrrB inactivation. During anaerobiosis, the two Cys residues in SrrB are reduced directly by reduced menaquinones or indirectly by a ligand binding to the PAS domain of SrrB. Reduced SrrB is active as kinase, leading to autophosphorylation and phosphorylation of the response regulator SrrA. SrrA-P positively controls biofilm formation *via* the *icaABCD* operon and represses *tst* gene transcription. Anaerobic respiration and fermentation are activated by SrrAB, while virulence factors are repressed as indicated.

narG and *nirB*, which are involved in nitrate respiration and nitrite transport (James et al. 2019).

S. aureus utilizes SrrAB for adaptation to lower oxygen availability during anaerobiosis and changes in the respiratory flux (Yarwood and Schlievert 2000). SrrB senses the reduced state of the respiratory menaquinone pool and acts as kinase and phosphatase via the PAS domain to regulate the phosphorylation state of the response regulator SrrA (Kinkel et al. 2013; Mashruwala et al. 2017; Tiwari et al. 2020). Two cysteines (Cys464 and Cys501) are present in the ATP-binding catalytic domain of SrrB, which are oxidized to an intramolecular disulfide under aerobic conditions inhibiting the autokinase activity of SrrB (Figure 11) (Tiwari et al. 2020). Under anaerobic conditions, the reduced menaquinone pool leads to reduction of the disulfide in SrrB, directly or indirectly via a PAS domain/ligand complex, leading to a 40% increase in autophosphorylation of SrrB, which activates SrrA, affecting biofilm formation and *tst* toxin expression (Tiwari et al. 2020). SrrAB senses indirectly increasing ROS levels during

aerobic growth and induces the oxidative stress response. Thus, SrrAB contributes to the protection of FeS cluster enzymes, such as aconitase and facilitates the growth under aerobic conditions in *S. aureus* (Mashruwala and Boyd 2017). In support of this notion, the SrrB disulfide under aerobic conditions was important for pathogenesis of *S. aureus* in an endocarditis model of infection (Tiwari et al. 2020).

Outlook for future research

In this review, we present an overview of thiol-based redox switches in the pathogen *S. aureus*, which sense different reactive species via conserved Cys residues and control specific enzymatic detoxification pathways. We provide new insights into the role of BSH and the Brx/BSH/YpdA/NADPH pathway for redox homeostasis under oxidative stress, infection conditions and redox active antimicrobials in *S. aureus*. Significant progress occurred in the physiological and biochemical characterization of the NADPH-dependent flavin disulfide reductase YpdA as a BSSB reductase, which established a functional Brx/BSH/YpdA/NADPH electron pathway for regeneration of *S*-bacillithiolated proteins. BSH and the Brx/BSH/YpdA/NADPH redox pathway are crucial for redox homeostasis in *S. aureus* during host-pathogen interactions and contribute to antibiotic resistance towards redox active antimicrobials. The novel Brx-roGFP2 biosensor revealed the impact of BSH and YpdA on redox homeostasis and the oxidative mode of action of the antimicrobials allicin, lapachol and AGXX®.

However, the functions of BSH as cofactor of redox enzymes for detoxification of electrophiles and oxidants are only beginning to emerge. While BSH-dependent und BSH-independent glyoxalases for methylglyoxal removal have been characterized in *B. subtilis* (Chandrangsu et al. 2014), nothing is known about related mechanisms in *S. aureus*, although methylglyoxal is the most conserved toxic electrophile in all organisms. In addition, *S. aureus* encodes so many peroxiredoxins, such as Tpx, Bcp, GpxA1 and GpxA2, but their functions, substrates and the roles of the Brx/BSH/YpdA/NADPH and Trx/TrxR/NADPH pathways for their recycling are completely unknown. These peroxiredoxins might be particularly important to prevent accumulation of host-derived fatty acid electrophiles during infections.

While BSH plays an important role in FeS cluster assembly in *S. aureus*, the detailed mechanism awaits further studies. Similarly, the role of BSH in metal storage or buffering under conditions of nutritional immunity, such as Fe^{2+}, Cu^{2+} or Zn^{2+} starvation are important subjects in terms of host-pathogen interactions in *S. aureus*. Through mass spectrometry-based redox proteomics approaches, many new thiol switches, including *S*-bacillithiolated and *S*-CoAlated proteins have been discovered recently. While the role of *S*-bacillithiolation in thiol protection and redox regulation has been studied for GapDH in *S. aureus*, the implication of this redox modification and its reversal by the Brx/BSH/YpdA/NADPH pathway for cellular physiology during *S. aureus* infections are important tasks of future research. Furthermore, the physiological role of *S*-CoAlation and its reversal in *S. aureus* remain further subjects of investigations.

The described thiol-based redox regulators of *S. aureus* cover a wide spectrum of bacterial transcription factor families, including Fur (PerR), MarR (SarZ/MgrA/SarA/QsrR/MhqR), Rrf2 (HypR), ArsC (Spx), TetR (GbaA) and two-component systems (SrrAB). These redox regulators sense oxygen, ROS, HOCl, NEM, quinones, methylglyoxal or antibiotics and often promote survival during infections. The thiol switch mechanisms can be divided into 1-Cys type and 2-Cys type models, including intra- or intersubunit disulfide formation of the 2-Cys type regulators (e.g. QsrR, HypR, Spx, SrrAB) and *S*-thiolation, *S*-alkylation or *S*-phosphorylation of the 1-Cys type redox sensors (e.g. SarZ, MgrA, SarA, GbaA). Specific redox regulators may employ different regulatory mechanisms to sense different reactive species (e.g. PerR, QsrR, GbaA), which has yet to be explored mechanistically and for cellular physiology or pathophysiology.

Finally, future studies should be directed to investigate structural determinants of the specificity (or non-specificity) of regulatory thiol switches towards sensing of ROS, RES or HOCl. While there is evidence for an extensive cross reactivity of thiol switches towards various ROS, RES or HOCl (e.g. QsrR, MhqR, HypR, Spx, GbaA), the structural changes might be similar for some, but different for other redox sensors depending on the signals. The challenge is to find the physiological important signal and to dissect whether primary or secondary signals lead to inactivation or activation of the redox regulator. The functions of the specific regulon members might help to deduce the specific signals important for physiology or pathophysiology. For example, the HypR repressor responds to neutrophil infections, HOCl and the redox active antimicrobials AGXX®, allicin and lapachol by intersubunit disulfide formation leading to upregulation of the disulfide reductase MerA. However, in terms of pathophysiology, HOCl is the most important signal, since MerA provides protection during infections. Similar cross reactivities to RES and antimicrobials are observed for QsrR/YodB and MhqR, but their functions in quinone detoxification have been clearly established. Thus, the specificity

of the primary or secondary redox signal has to be revealed in concert with the functions of the regulon members in the physiology or pathophysiology of *S. aureus*. The discovery and characterization of thiol switches, which sense novel biochemical signals during host-pathogen interactions, remain exciting future challenges in the field of redox biology in microbial pathogens.

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References

Antelmann, H., Hecker, M., and Zuber, P. (2008). Proteomic signatures uncover thiol-specific electrophile resistance mechanisms in *Bacillus subtilis*. Expert Rev. Proteomics 5: 77–90.

Antelmann, H. and Helmann, J.D. (2011). Thiol-based redox switches and gene regulation. Antioxid. Redox. Signal 14: 1049–1063.

Arbach, M., Santana, T.M., Moxham, H., Tinson, R., Anwar, A., Groom, M., and Hamilton, C.J. (2019). Antimicrobial garlic-derived diallyl polysulfanes: interactions with biological thiols in *Bacillus subtilis*. Biochim. Biophys. Acta 1863: 1050–1058.

Archer, G.L. (1998). *Staphylococcus aureus*: a well-armed pathogen. Clin. Infect. Dis. 26: 1179–1181.

Argyrou, A. and Blanchard, J.S. (2004). Flavoprotein disulfide reductases: advances in chemistry and function. Prog. Nucleic Acid Res. Mol. Biol. 78: 89–142.

Austin, C.M., Garabaglu, S., Krute, C.N., Ridder, M.J., Seawell, N.A., Markiewicz, M.A., Boyd, J.M., and Bose, J.L. (2019). Contribution of YjbIH to virulence factor expression and host colonization in *Staphylococcus aureus*. Infect. Immun. 87: e00155-19.

Baek, Y., Kim, J., Ahn, J., Jo, I., Hong, S., Ryu, S., and Ha, N.C. (2020). Structure and function of the hypochlorous acid-induced flavoprotein RclA from *Escherichia coli*. J. Biol. Chem. 295: 3202–3212.

Ballal, A. and Manna, A.C. (2009). Regulation of superoxide dismutase (sod) genes by SarA in *Staphylococcus aureus*. J. Bacteriol. 191: 3301–3310.

Ballal, A., and Manna, A.C. (2010). Control of thioredoxin reductase gene (*trxB*) transcription by SarA in *Staphylococcus aureus*. J. Bacteriol. 192: 336–345.

Ballal, A., Ray, B., and Manna, A.C. (2009). *sarZ*, a SarA family gene, is transcriptionally activated by MgrA and is involved in the regulation of genes encoding exoproteins in *Staphylococcus aureus*. J. Bacteriol. 191: 1656–1665.

Bambouskova, M., Gorvel, L., Lampropoulou, V., Sergushichev, A., Loginicheva, E., Johnson, K., Korenfeld, D., Mathyer, M.E., Kim, H., Huang, L.H., et al. (2018). Electrophilic properties of itaconate and derivatives regulate the IkappaBzeta-ATF3 inflammatory axis. Nature 556: 501–504.

Barwinska-Sendra, A., Garcia, Y.M., Sendra, K.M., Basle, A., Mackenzie, E.S., Tarrant, E., Card, P., Tabares, L.C., Bicep, C., Un, S., et al. (2020). An evolutionary path to altered cofactor specificity in a metalloenzyme. Nat. Commun. 11: 2738.

Beavers, W.N., and Skaar, E.P. (2016). Neutrophil-generated oxidative stress and protein damage in *Staphylococcus aureus*. Pathog. Dis. 74: ftw060, https://doi.org/10.1093/femspd/ftw060.

Benchoam, D., Cuevasanta, E., Moller, M.N., and Alvarez, B. (2020). Persulfides, at the crossroads between hydrogen sulfide and thiols. Essays Biochem. 64: 155–168.

Booth, I.R., Ferguson, G.P., Miller, S., Li, C., Gunasekera, B., and Kinghorn, S. (2003). Bacterial production of methylglyoxal: a survival strategy or death by misadventure? Biochem. Soc. Trans. 31: 1406–1408.

Borlinghaus, J., Albrecht, F., Gruhlke, M.C., Nwachukwu, I.D., and Slusarenko, A.J. (2014). Allicin: chemistry and biological properties. Molecules 19: 12591–12618.

Boucher, H.W. and Corey, G.R. (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. 46(Suppl. 5): S344–S349.

Bragg, P.D., Glavas, N.A., and Hou, C. (1997). Mutation of conserved residues in the NADP(H)-binding domain of the proton translocating pyridine nucleotide transhydrogenase of *Escherichia coli*. Arch. Biochem. Biophys. 338: 57–66.

Brunmark, A. and Cadenas, E. (1989). Redox and addition chemistry of quinoid compounds and its biological implications. Free Radic. Biol. Med. 7: 435–477.

Chambers, H.F. and Deleo, F.R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol. 7: 629–641.

Chan, P.F. and Foster, S.J. (1998). Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*. J. Bacteriol. 180: 6232–6241.

Chandrangsu, P., Dusi, R., Hamilton, C.J., and Helmann, J.D. (2014). Methylglyoxal resistance in *Bacillus subtilis*: contributions of bacillithiol-dependent and independent pathways. Mol. Microbiol. 91: 706–715.

Chandrangsu, P., Loi, V.V., Antelmann, H., and Helmann, J.D. (2018). The role of bacillithiol in Gram-positive firmicutes. Antioxid. Redox. Signal 28: 445–462.

Chen, P.R., Bae, T., Williams, W.A., Duguid, E.M., Rice, P.A., Schneewind, O., and He, C. (2006). An oxidation-sensing mechanism is used by the global regulator MgrA in *Staphylococcus aureus*. Nat. Chem. Biol. 2: 591–595.

Chen, P.R., Brugarolas, P., and He, C. (2011). Redox signaling in human pathogens. Antioxid. Redox. Signal 14: 1107–1118.

Chen, P.R., Nishida, S., Poor, C.B., Cheng, A., Bae, T., Kuechenmeister, L., Dunman, P.M., Missiakas, D., and He, C. (2009). A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in *Staphylococcus aureus*. Mol. Microbiol. 71: 198–211.

Chi, B.K., Albrecht, D., Gronau, K., Becher, D., Hecker, M., and Antelmann, H. (2010). The redox-sensing regulator YodB senses quinones and diamide via a thiol-disulfide switch in *Bacillus subtilis*. Proteomics 10: 3155–3164.

Chi, B.K., Gronau, K., Mäder, U., Hessling, B., Becher, D., and Antelmann, H. (2011). *S*-bacillithiolation protects against hypochlorite stress in *Bacillus subtilis* as revealed by transcriptomics and redox proteomics. Mol. Cell. Proteomics 10: M111–009506.

Chi, B.K., Huyen, N.T.T., Loi, V.V., Gruhlke, M.C.H., Schaffer, M., Mäder, U., Maass, S., Becher, D., Bernhardt, J., and Arbach, M., et al (2019). The disulfide stress response and protein S-thioallylation caused by allicin and diallyl polysulfanes in *Bacillus subtilis* as revealed by transcriptomics and proteomics. Antioxidants 8: 605.

Chi, B.K., Roberts, A.A., Huyen, T.T., Basell, K., Becher, D., Albrecht, D., Hamilton, C.J., and Antelmann, H. (2013). *S*-bacillithiolation protects conserved and essential proteins against hypochlorite stress in firmicutes bacteria. Antioxid. Redox. Signal 18: 1273–1295.

Chien, Y., Manna, A.C., Projan, S.J., and Cheung, A.L. (1999). SarA, a global regulator of virulence determinants in *Staphylococcus aureus*, binds to a conserved motif essential for *sar*-dependent gene regulation. J. Biol. Chem. 274: 37169–37176.

Chirgadze, Y.N., Boshkova, E.A., Battaile, K.P., Mendes, V.G., Lam, R., Chan, T.S.Y., Romanov, V., Pai, E.F., and Chirgadze, N.Y. (2018). Crystal structure of *Staphylococcus aureus* Zn-glyoxalase I: new subfamily of glyoxalase I family. J. Biomol. Struct. Dyn. 36: 376–386.

Clauss-Lendzian, E., Vaishampayan, A., de Jong, A., Landau, U., Meyer, C., Kok, J., and Grohmann, E. (2018). Stress response of a clinical *Enterococcus faecalis* isolate subjected to a novel antimicrobial surface coating. Microbiol. Res. 207: 53–64.

Clements, M.O., Watson, S.P., and Foster, S.J. (1999). Characterization of the major superoxide dismutase of *Staphylococcus aureus* and its role in starvation survival, stress resistance, and pathogenicity. J. Bacteriol. 181: 3898–3903.

Cosgrove, K., Coutts, G., Jonsson, I.M., Tarkowski, A., Kokai-Kun, J.F., Mond, J.J., and Foster, S.J. (2007). Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. J. Bacteriol. 189: 1025–1035.

Crosby, H.A., Schlievert, P.M., Merriman, J.A., King, J.M., Salgado-Pabon, W., and Horswill, A.R. (2016). The *Staphylococcus aureus* global regulator MgrA modulates clumping and virulence by controlling surface protein expression. PloS Pathog. 12: e1005604.

Crosby, H.A., Tiwari, N., Kwiecinski, J.M., Xu, Z., Dykstra, A., Jenul, C., Fuentes, E.J., and Horswill, A.R. (2020). The *Staphylococcus aureus* ArlRS two-component system regulates virulence factor expression through MgrA. Mol. Microbiol. 113: 103–122.

Cuthbertson, L. and Nodwell, J.R. (2013). The TetR family of regulators. Microbiol. Mol. Biol. Rev. 77: 440–475.

Davies, M.J. (2011). Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. J. Clin. Biochem. Nutr. 48: 8–19.

delCardayre, S.B. and Davies, J.E. (1998). *Staphylococcus aureus* coenzyme A disulfide reductase, a new subfamily of pyridine nucleotide-disulfide oxidoreductase. Sequence, expression, and analysis of cdr. J. Biol. Chem. 273: 5752–5757.

delCardayré, S.B., Stock, K.P., Newton, G.L., Fahey, R.C., and Davies, J.E. (1998). Coenzyme A disulfide reductase, the primary low molecular weight disulfide reductase from *Staphylococcus aureus*. Purification and characterization of the native enzyme. J. Biol. Chem. 273: 5744–5751.

Delmastro-Greenwood, M., Freeman, B.A., and Wendell, S.G. (2014). Redox-dependent anti-inflammatory signaling actions of unsaturated fatty acids. Annu. Rev. Physiol. 76: 79–105.

Deng, X., Weerapana, E., Ulanovskaya, O., Sun, F., Liang, H., Ji, Q., Ye, Y., Fu, Y., Zhou, L., Li, J., et al. (2013). Proteome-wide quantification and characterization of oxidation-sensitive cysteines in pathogenic bacteria. Cell Host Microbe. 13: 358–370.

Derewenda, U., Boczek, T., Gorres, K.L., Yu, M., Hung, L.W., Cooper, D., Joachimiak, A., Raines, R.T., and Derewenda, Z.S. (2009). Structure and function of *Bacillus subtilis* YphP, a prokaryotic disulfide isomerase with a CXC catalytic motif. Biochemistry 48: 8664–8671.

Dickerhof, N., Paton, L., and Kettle, A.J. (2020). Oxidation of bacillithiol by myeloperoxidase-derived oxidants. Free Radic. Biol. Med. 158: 74–83.

Donegan, N.P., Manna, A.C., Tseng, C.W., Liu, G.Y., and Cheung, A.L. (2019). CspA regulation of *Staphylococcus aureus* carotenoid levels and sigma (B) activity is controlled by YjbH and Spx. Mol. Microbiol. 112: 532–551.

Dooley, C.T., Dore, T.M., Hanson, G.T., Jackson, W.C., Remington, S.J., and Tsien, R.Y. (2004). Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. J. Biol. Chem. 279: 22284–22293.

Duarte, V. and Latour, J.M. (2010). PerR versus OhrR: selective peroxide sensing in *Bacillus subtilis*. Mol. Biosyst. 6: 316–323.

Dubbs, J.M. and Mongkolsuk, S. (2007). Peroxiredoxins in bacterial antioxidant defense. Subcell. Biochem. 44: 143–193.

Engman, J., Rogstam, A., Frees, D., Ingmer, H., and von Wachenfeldt, C. (2012). The YjbH adaptor protein enhances proteolysis of the transcriptional regulator Spx in *Staphylococcus aureus*. J. Bacteriol. 194: 1186–1194.

Engman, J. and von Wachenfeldt, C. (2015). Regulated protein aggregation: a mechanism to control the activity of the ClpXP adaptor protein YjbH. Mol. Microbiol. 95: 51–63.

Faulkner, M.J. and Helmann, J.D. (2011). Peroxide stress elicits adaptive changes in bacterial metal ion homeostasis. Antioxid. Redox. Signal 15: 175–189.

Favazzo, L.J., Gill, A.L., Farnsworth, C.W., Mooney, R.A., and Gill, S.R. (2019). The response of *nor* and *nos* contributes to *Staphylococcus aureus* virulence and metabolism. J. Bacteriol. 201: e00107-19.

Ferguson, G.P., Totemeyer, S., MacLean, M.J., and Booth, I.R. (1998). Methylglyoxal production in bacteria: suicide or survival? Arch. Microbiol. 170: 209–218.

Fernando, V., Zheng, X., Walia, Y., Sharma, V., Letson, J., and Furuta, S. (2019). *S-nitrosylation: an emerging paradigm of redox signaling*-nitrosylation: an emerging paradigm of redox signaling. Antioxidants 8: 404.

Flierl, M.A., Rittirsch, D., Nadeau, B.A., Chen, A.J., Sarma, J.V., Zetoune, F.S., McGuire, S.R., List, R.P., Day, D.E., Hoesel, L.M., et al. (2007). Phagocyte-derived catecholamines enhance acute inflammatory injury. Nature 449: 721–725.

Forman, H.J., and Torres, M. (2001). Redox signaling in macrophages. Mol. Aspect. Med. 22: 189–216.

Foster, M.W., McMahon, T.J., and Stamler, J.S. (2003). *S*-nitrosylation in health and disease. Trends Mol. Med. 9: 160–168.

Foster, T.J. (2004). The *Staphylococcus aureus* "superbug". J. Clin. Invest. 114: 1693–1696.

Fritsch, V.N., Loi, V.V., Busche, T., Sommer, A., Tedin, K., Nürnberg, D.J., Kalinowski, J., Bernhardt, J., Fulde, M., and Antelmann, H. (2019). The MarR-type repressor MhqR confers quinone and antimicrobial resistance in *Staphylococcus aureus*. Antioxid. Redox. Signal 31: 1235–1252.

Fuller, J.R., Vitko, N.P., Perkowski, E.F., Scott, E., Khatri, D., Spontak, J.S., Thurlow, L.R., and Richardson, A.R. (2011). Identification of a lactate-quinone oxidoreductase in *Staphylococcus aureus* that is essential for virulence. Front. Cell Infect. Microbiol. 1: 19.

Gaballa, A., Chi, B.K., Roberts, A.A., Becher, D., Hamilton, C.J., Antelmann, H., and Helmann, J.D. (2014). Redox regulation in *Bacillus subtilis*: the bacilliredoxins BrxA(YphP) and BrxB(YqiW) function in de-bacillithiolation of *S*-bacillithiolated OhrR and MetE. Antioxid. Redox. Signal 21: 357–367.

Gaballa, A., Newton, G.L., Antelmann, H., Parsonage, D., Upton, H., Rawat, M., Claiborne, A., Fahey, R.C., and Helmann, J.D. (2010). Biosynthesis and functions of bacillithiol, a major low-molecular-weight thiol in Bacilli. Proc. Natl. Acad. Sci. USA 107: 6482–6486.

Garcia, Y.M., Barwinska-Sendra, A., Tarrant, E., Skaar, E.P., Waldron, K.J., and Kehl-Fie, T.E. (2017). A superoxide dismutase capable of functioning with iron or manganese promotes the resistance of *Staphylococcus aureus* to calprotectin and nutritional immunity. PloS Pathog. 13: e1006125.

Garg, S.K., Kommineni, S., Henslee, L., Zhang, Y., and Zuber, P. (2009). The YjbH protein of *Bacillus subtilis* enhances ClpXP-catalyzed proteolysis of Spx. J. Bacteriol. 191: 1268–1277.

Gaupp, R., Ledala, N., and Somerville, G.A. (2012). Staphylococcal response to oxidative stress. Front. Cell Infect. Microbiol. 2: 33.

Gohring, N., Fedtke, I., Xia, G., Jorge, A.M., Pinho, M.G., Bertsche, U., and Peschel, A. (2011). New role of the disulfide stress effector YjbH in beta-lactam susceptibility of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 55: 5452–5458.

Goncalves, V.L., Nobre, L.S., Vicente, J.B., Teixeira, M., and Saraiva, L.M. (2006). Flavohemoglobin requires microaerophilic conditions for nitrosative protection of *Staphylococcus aureus*. FEBS Lett. 580: 1817–1821.

Gout, I. (2019). Coenzyme A: a protective thiol in bacterial antioxidant defence. Biochem. Soc. Trans. 47: 469–476.

Green, J.N., Chapman, A.L.P., Bishop, C.J., Winterbourn, C.C., and Kettle, A.J. (2017). Neutrophil granule proteins generate bactericidal ammonia chloramine on reaction with hydrogen peroxide. Free Radic. Biol. Med. 113: 363–371.

Grosser, M.R., Weiss, A., Shaw, L.N., and Richardson, A.R. (2016). Regulatory requirements for *Staphylococcus aureus* nitric oxide resistance. J. Bacteriol. 198: 2043–2055.

Grove, A. (2017). Regulation of metabolic pathways by MarR family transcription factors. Comput. Struct. Biotechnol. J. 15: 366–371.

Gupta, A., Matsui, K., Lo, J.F., and Silver, S. (1999). Molecular basis for resistance to silver cations in *Salmonella*. Nat. Med. 5: 183–188.

Guridi, A., Diederich, A.K., Aguila-Arcos, S., Garcia-Moreno, M., Blasi, R., Broszat, M., Schmieder, W., Clauss-Lendzian, E., Sakinc-Gueler, T., Andrade, R., et al. (2015). New antimicrobial contact catalyst killing antibiotic resistant clinical and waterborne pathogens. Mater. Sci. Eng. C Mater. Biol. Appl. 50: 1–11.

Hawkins, C.L., Pattison, D.I., and Davies, M.J. (2003). Hypochlorite-induced oxidation of amino acids, peptides and proteins. Amino acids 25: 259–274.

Hazen, S.L., d'Avignon, A., Anderson, M.M., Hsu, F.F., and Heinecke, J.W. (1998). Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to oxidize alpha-amino acids to a family of reactive aldehydes. Mechanistic studies identifying labile intermediates along the reaction pathway. J. Biol. Chem. 273: 4997–5005.

Heiss, A., Freisinger, B., and Held-Fohn, E. (2017). Enhanced antibacterial activity of silver-ruthenium coated hollow microparticles. Biointerphases 12: 05G608.

Higgins, K.A., Peng, H., Luebke, J.L., Chang, F.M., and Giedroc, D.P. (2015). Conformational analysis and chemical reactivity of the multidomain sulfurtransferase, *Staphylococcus aureus* CstA. Biochemistry 54: 2385–2398.

Hillion, M. and Antelmann, H. (2015). Thiol-based redox switches in prokaryotes. Biol. Chem. 396: 415–444.

Hiras, J., Sharma, S.V., Raman, V., Tinson, R.A.J., Arbach, M., Rodrigues, D.F., Norambuena, J., Hamilton, C.J., and Hanson, T.E. (2018). Physiological studies of *Chlorobiaceae* suggest that bacillithiol derivatives are the most widespread thiols in bacteria. mBio. 9: e01603-18.

Horsburgh, M.J., Clements, M.O., Crossley, H., Ingham, E., and Foster, S.J. (2001a). PerR controls oxidative stress resistance and iron storage proteins and is required for virulence in *Staphylococcus aureus*. Infect. Immun. 69: 3744–3754.

Horsburgh, M.J., Ingham, E., and Foster, S.J. (2001b). In *Staphylococcus aureus, Fur* is an interactive regulator with PerR, contributes to virulence, and is necessary for oxidative stress resistance through positive regulation of catalase and iron homeostasis. J. Bacteriol. 183: 468–475.

Imber, M., Huyen, N.T.T., Pietrzyk-Brzezinska, A.J., Loi, V.V., Hillion, M., Bernhardt, J., Thärichen, L., Kolsek, K., Saleh, M., Hamilton, C.J., et al. (2018a). Protein S-bacillithiolation functions in thiol protection and redox regulation of the glyceraldehyde-3-phosphate dehydrogenase gap in *Staphylococcus aureus* under hypochlorite stress. Antioxid. Redox. Signal 28: 410–430.

Imber, M., Loi, V.V., Reznikov, S., Fritsch, V.N., Pietrzyk-Brzezinska, A.J., Prehn, J., Hamilton, C., Wahl, M.C., Bronowska, A.K., and Antelmann, H. (2018b). The aldehyde dehydrogenase AldA contributes to the hypochlorite defense and is redox-controlled by protein S-bacillithiolation in *Staphylococcus aureus*. Redox. Biol. 15: 557–568.

Imber, M., Pietrzyk-Brzezinska, A.J., and Antelmann, H. (2019). Redox regulation by reversible protein *S*-thiolation in Gram-positive bacteria. Redox. Biol. 20: 130–145.

Imlay, J.A. (2003). Pathways of oxidative damage. Annu. Rev. Microbiol. 57: 395–418.

Imlay, J.A. (2008). Cellular defenses against superoxide and hydrogen peroxide. Annu. Rev. Biochem. 77: 755–776.

Ingavale, S., van Wamel, W., Luong, T.T., Lee, C.Y., and Cheung, A.L. (2005). Rat/MgrA, a regulator of autolysis, is a regulator of virulence genes in *Staphylococcus aureus*. Infect. Immun. 73: 1423–1431.

Jackowski, S. and Rock, C.O. (1986). Consequences of reduced intracellular coenzyme A content in *Escherichia coli*. J. Bacteriol. 166: 866–871.

James, K.L., Mogen, A.B., Brandwein, J.N., Orsini, S.S., Ridder, M.J., Markiewicz, M.A., Bose, J.L., and Rice, K.C. (2019). Interplay of

nitric oxide synthase (NOS) and SrrAB in modulation of *Staphylococcus aureus* metabolism and virulence. Infect. Immun. 87: e00570-18.

Ji, C.J., Kim, J.H., Won, Y.B., Lee, Y.E., Choi, T.W., Ju, S.Y., Youn, H., Helmann, J.D., and Lee, J.W. (2015). *Staphylococcus aureus* PerR is a hypersensitive hydrogen peroxide sensor using iron-mediated histidine oxidation. J. Biol. Chem. 290: 20374–20386.

Ji, Q., Zhang, L., Jones, M.B., Sun, F., Deng, X., Liang, H., Cho, H., Brugarolas, P., Gao, Y.N., Peterson, S.N., et al. (2013). Molecular mechanism of quinone signaling mediated through *S*-quinonization of a YodB family repressor QsrR. Proc. Natl. Acad. Sci. USA 110: 5010–5015.

Jonsson, I.M., Lindholm, C., Luong, T.T., Lee, C.Y., and Tarkowski, A. (2008). MgrA regulates staphylococcal virulence important for induction and progression of septic arthritis and sepsis. Microb. Infect. 10: 1229–1235.

Jousselin, A., Kelley, W.L., Barras, C., Lew, D.P., and Renzoni, A. (2013). The *Staphylococcus aureus* thiol/oxidative stress global regulator Spx controls *trfA*, a gene implicated in cell wall antibiotic resistance. Antimicrob. Agents Chemother. 57: 3283–3292.

Kaatz, G.W., Thyagarajan, R.V., and Seo, S.M. (2005). Effect of promoter region mutations and *mgrA* overexpression on transcription of *norA*, which encodes a *Staphylococcus aureus* multidrug efflux transporter. Antimicrob. Agents Chemother. 49: 161–169.

Kaito, C., Morishita, D., Matsumoto, Y., Kurokawa, K., and Sekimizu, K. (2006). Novel DNA binding protein SarZ contributes to virulence in *Staphylococcus aureus*. Mol. Microbiol. 62: 1601–1617.

Kalapos, M.P. (2008). The tandem of free radicals and methylglyoxal. Chem. Biol. Interact. 171: 251–271.

Karavolos, M.H., Horsburgh, M.J., Ingham, E., and Foster, S.J. (2003). Role and regulation of the superoxide dismutases of *Staphylococcus aureus*. Microbiology 149: 2749–2758.

Karlinsey, J.E., Bang, I.S., Becker, L.A., Frawley, E.R., Porwollik, S., Robbins, H.F., Thomas, V.C., Urbano, R., McClelland, M., and Fang, F.C. (2012). The NsrR regulon in nitrosative stress resistance of *Salmonella enterica* serovar Typhimurium. Mol. Microbiol. 85: 1179–1193.

Kawai, Y., Mercier, R., Wu, L.J., Dominguez-Cuevas, P., Oshima, T., and Errington, J. (2015). Cell growth of wall-free L-form bacteria is limited by oxidative damage. Curr. Biol. 25: 1613–1618.

Kehl-Fie, T.E., Chitayat, S., Hood, M.I., Damo, S., Restrepo, N., Garcia, C., Munro, K.A., Chazin, W.J., and Skaar, E.P. (2011). Nutrient metal sequestration by calprotectin inhibits bacterial superoxide defense, enhancing neutrophil killing of *Staphylococcus aureus*. Cell Host Microbe. 10: 158–164.

Kim, H.J., Lee, K.Y., Kwon, A.R., and Lee, B.J. (2017). Structural and functional studies of SAV0551 from *Staphylococcus aureus* as a chaperone and glyoxalase III. Biosci. Rep. 37: BSR20171106.

Kinkel, T.L., Roux, C.M., Dunman, P.M., and Fang, F.C. (2013). The *Staphylococcus aureus* SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. mBio. 4: e00696–e00613.

Kolar, S.L., Ibarra, J.A., Rivera, F.E., Mootz, J.M., Davenport, J.E., Stevens, S.M., Horswill, A.R., and Shaw, L.N. (2013). Extracellular proteases are key mediators of *Staphylococcus aureus* virulence via the global modulation of virulence-determinant stability. Microbiologyopen 2: 18–34.

Lalaouna, D., Baude, J., Wu, Z., Tomasini, A., Chicher, J., Marzi, S., Vandenesch, F., Romby, P., Caldelari, I., and Moreau, K. (2019). RsaC sRNA modulates the oxidative stress response of *Staphylococcus aureus* during manganese starvation. Nucleic Acids Res. 47: 9871–9887.

Lampropoulou, V., Sergushichev, A., Bambouskova, M., Nair, S., Vincent, E.E., Loginicheva, E., Cervantes-Barragan, L., Ma, X., Huang, S.C., Griss, T., et al. (2016). Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metabol. 24: 158–166.

Lee, J.W. and Helmann, J.D. (2006). The PerR transcription factor senses H_2O_2 by metal-catalysed histidine oxidation. Nature 440: 363–367.

Lee, J.W., Soonsanga, S., and Helmann, J.D. (2007). A complex thiolate switch regulates the *Bacillus subtilis* organic peroxide sensor. OhrR. Proc Natl Acad Sci USA 104: 8743–8748.

Lee, S.J., Lee, I.G., Lee, K.Y., Kim, D.G., Eun, H.J., Yoon, H.J., Chae, S., Song, S.H., Kang, S.O., Seo, M.D., et al. (2016). Two distinct mechanisms of transcriptional regulation by the redox sensor YodB. Proc. Natl. Acad. Sci. USA. 113: E5202–E5211.

Leelakriangsak, M., Huyen, N.T., Towe, S., van Duy, N., Becher, D., Hecker, M., Antelmann, H., and Zuber, P. (2008). Regulation of quinone detoxification by the thiol stress sensing DUF24/MarR-like repressor, YodB in *Bacillus subtilis*. Mol. Microbiol. 67: 1108–1124.

Lewis, A.M., Matzdorf, S.S., Endres, J.L., Windham, I.H., Bayles, K.W., and Rice, K.C. (2015). Examination of the *Staphylococcus aureus* nitric oxide reductase (saNOR) reveals its contribution to modulating intracellular NO levels and cellular respiration. Mol. Microbiol. 96: 651–669.

Li, L., Wang, G., Cheung, A., Abdelhady, W., Seidl, K., and Xiong, Y.Q. (2019). MgrA governs adherence, host cell interaction, and virulence in a murine model of bacteremia due to *Staphylococcus aureus*. J. Infect. Dis. 220: 1019–1028.

Liebeke, M., Pöther, D.C., van Duy, N., Albrecht, D., Becher, D., Hochgräfe, F., Lalk, M., Hecker, M., and Antelmann, H. (2008). Depletion of thiol-containing proteins in response to quinones in *Bacillus subtilis*. Mol. Microbiol. 69: 1513–1529.

Lill, R. (2020). From the discovery to molecular understanding of cellular iron-sulfur protein biogenesis. Biol. Chem. 401: 855–876.

Linzner, N., Fritsch, V.N., Busche, T., Tung, Q.N., Van Loi, V., Bernhardt, J., Kalinowski, J., and Antelmann, H. (2020). The plant-derived naphthoquinone lapachol causes an oxidative stress response in *Staphylococcus aureus*. Free Radic. Biol. Med. 158: 126–136.

Linzner, N., Loi, V.V., Fritsch, V.N., Tung, Q.N., Stenzel, S., Wirtz, M., Hell, R., Hamilton, C.J., Tedin, K., Fulde, M., et al. (2019). *Staphylococcus aureus* uses the bacilliredoxin (BrxAB)/bacillithiol disulfide reductase (YpdA) redox pathway to defend against oxidative stress under infections. Front. Microbiol. 10: 1355.

Loi, V.V., and Antelmann, H. (2020). Method for measurement of bacillithiol redox potential changes using the Brx-roGFP2 redox biosensor in *Staphylococcus aureus*. MethodsX 7: 100900.

Loi, V.V., Busche, T., Preuss, T., Kalinowski, J., Bernhardt, J., and Antelmann, H. (2018a). The AGXX antimicrobial coating causes a thiol-specific oxidative stress response and protein *S*-bacillithiolation in *Staphylococcus aureus*. Front. Microbiol. 9: 3037.

Loi, V.V., Busche, T., Tedin, K., Bernhardt, J., Wollenhaupt, J., Huyen, N.T.T., Weise, C., Kalinowski, J., Wahl, M.C., Fulde, M., et al. (2018b). Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*. Antioxid. Redox. Signal 29: 615–636.

Loi, V.V., Harms, M., Müller, M., Huyen, N.T.T., Hamilton, C.J., Hochgräfe, F., Pane-Farre, J., and Antelmann, H. (2017). Real-time imaging of the bacillithiol redox potential in the human pathogen *Staphylococcus aureus* using a genetically encoded bacilliredoxin-fused redox biosensor. Antioxid. Redox. Signal 26: 835–848.

Loi, V.V., Huyen, N.T.T., Busche, T., Tung, Q.N., Gruhlke, M.C.H., Kalinowski, J., Bernhardt, J., Slusarenko, A.J., and Antelmann, H. (2019). *Staphylococcus aureus* responds to allicin by global *S*-thioallylation – role of the Brx/BSH/YpdA pathway and the disulfide reductase MerA to overcome allicin stress. Free Radic. Biol. Med. 139: 55–69.

Loi, V.V., Rossius, M., and Antelmann, H. (2015). Redox regulation by reversible protein *S*-thiolation in bacteria. Front. Microbiol. 6: 187.

Lowy, F.D. (1998). *Staphylococcus aureus* infections. N. Engl. J. Med. 339: 520–532.

Luebke, J.L., Shen, J., Bruce, K.E., Kehl-Fie, T.E., Peng, H., Skaar, E.P., and Giedroc, D.P. (2014). The CsoR-like sulfurtransferase repressor (CstR) is a persulfide sensor in *Staphylococcus aureus*. Mol. Microbiol. 94: 1343–1360.

Luong, T.T., Dunman, P.M., Murphy, E., Projan, S.J., and Lee, C.Y. (2006). Transcription profiling of the *mgrA* regulon in *Staphylococcus aureus*. J. Bacteriol. 188: 1899–1910.

Mäder, U., Nicolas, P., Depke, M., Pane-Farre, J., Debarbouille, M., van der Kooi-Pol, M.M., Guerin, C., Derozier, S., Hiron, A., Jarmer, H., et al. (2016). *Staphylococcus aureus* transcriptome architecture: from laboratory to infection-mimicking conditions. PloS Genet. 12: e1005962.

Manna, A.C., Ingavale, S.S., Maloney, M., van Wamel, W., and Cheung, A.L. (2004). Identification of sarV (SA2062), a new transcriptional regulator, is repressed by SarA and MgrA (SA0641) and involved in the regulation of autolysis in *Staphylococcus aureus*. J. Bacteriol. 186: 5267–5280.

Marchetti, M., De Bei, O., Bettati, S., Campanini, B., Kovachka, S., Gianquinto, E., Spyrakis, F., and Ronda, L. (2020). Iron metabolism at the interface between host and pathogen: from nutritional immunity to antibacterial development. Int. J. Mol. Sci. 21: 2145.

Maresso, A.W. and Schneewind, O. (2006). Iron acquisition and transport in *Staphylococcus aureus*. Biometals 19: 193–203.

Marnett, L.J., Riggins, J.N., and West, J.D. (2003). Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J. Clin. Invest. 111: 583–593.

Mashruwala, A.A. and Boyd, J.M. (2017). The *Staphylococcus aureus* SrrAB regulatory system modulates hydrogen peroxide resistance factors, which imparts protection to aconitase during aerobic growth. PloS One 12: e0170283.

Mashruwala, A.A., Guchte, A.V., and Boyd, J.M. (2017). Impaired respiration elicits SrrAB-dependent programmed cell lysis and biofilm formation in *Staphylococcus aureus*. Elife 6: e23845.

Mettert, E.L. and Kiley, P.J. (2015). Fe-S proteins that regulate gene expression. Biochim. Biophys. Acta 1853: 1284–1293.

Meyer, A.J. and Dick, T.P. (2010). Fluorescent protein-based redox probes. Antioxid. Redox. Signal 13: 621–650.

Mikheyeva, I.V., Thomas, J.M., Kolar, S.L., Corvaglia, A.R., Gaiotaa, N., Leo, S., Francois, P., Liu, G.Y., Rawat, M., and Cheung, A.L. (2019). YpdA, a putative bacillithiol disulfide reductase, contributes to cellular redox homeostasis and virulence in *Staphylococcus aureus*. Mol. Microbiol. 111: 1039–1056.

Mironov, A., Seregina, T., Nagornykh, M., Luhachack, L.G., Korolkova, N., Lopes, L.E., Kotova, V., Zavilgelsky, G., Shakulov, R., Shatalin, K., et al. (2017). Mechanism of H_2S-mediated protection against oxidative stress in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 114: 6022–6027.

Mishra, S. and Imlay, J. (2012). Why do bacteria use so many enzymes to scavenge hydrogen peroxide? Arch. Biochem. Biophys. 525: 145–160.

Mongkolsuk, S. and Helmann, J.D. (2002). Regulation of inducible peroxide stress responses. Mol. Microbiol. 45: 9–15.

Monks, T.J., Hanzlik, R.P., Cohen, G.M., Ross, D., and Graham, D.G. (1992). Quinone chemistry and toxicity. Toxicol. Appl. Pharmacol. 112: 2–16.

Morgan, B., Ezerina, D., Amoako, T.N., Riemer, J., Seedorf, M., and Dick, T.P. (2013). Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat. Chem. Biol. 9: 119–125.

Morgan, B., Sobotta, M.C., and Dick, T.P. (2011). Measuring E(GSH) and H_2O_2 with roGFP2-based redox probes. Free Radic. Biol. Med. 51: 1943–1951.

Müller, A., Langklotz, S., Lupilova, N., Kuhlmann, K., Bandow, J.E., and Leichert, L.I. (2014). Activation of RidA chaperone function by N-chlorination. Nat. Commun. 5: 5804.

Münchberg, U., Anwar, A., Mecklenburg, S., and Jacob, C. (2007). Polysulfides as biologically active ingredients of garlic. Org. Biomol. Chem. 5: 1505–1518.

Nakano, M.M., Kominos-Marvell, W., Sane, B., Nader, Y.M., Barendt, S.M., Jones, M.B., and Zuber, P. (2014). *spxA2*, encoding a regulator of stress resistance in *Bacillus anthracis*, is controlled by SaiR, a new member of the Rrf2 protein family. Mol. Microbiol. 94: 815–827.

Nakano, S., Erwin, K.N., Ralle, M., and Zuber, P. (2005). Redox-sensitive transcriptional control by a thiol/disulphide switch in the global regulator, Spx. Mol. Microbiol. 55: 498–510.

Nakano, S., Kuster-Schock, E., Grossman, A.D., and Zuber, P. (2003). Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA 100: 13603–13608.

Newton, G.L., Fahey, R.C., and Rawat, M. (2012). Detoxification of toxins by bacillithiol in *Staphylococcus aureus*. Microbiology 158: 1117–1126.

Newton, G.L., Leung, S.S., Wakabayashi, J.I., Rawat, M., and Fahey, R.C. (2011). The DinB superfamily includes novel mycothiol, bacillithiol, and glutathione *S*-transferases. Biochemistry 50: 10751–10760.

Newton, G.L., and Rawat, M. (2019). N-methyl-bacillithiol, a novel thiol from anaerobic bacteria. mBio. 10: e02634-18.

Newton, G.L., Rawat, M., La Clair, J.J., Jothivasan, V.K., Budiarto, T., Hamilton, C.J., Claiborne, A., Helmann, J.D., and Fahey, R.C. (2009). Bacillithiol is an antioxidant thiol produced in bacilli. Nat. Chem. Biol. 5: 625–627.

Nontaleerak, B., Duang-Nkern, J., Wongsaroj, L., Trinachartvanit, W., Romsang, A., and Mongkolsuk, S. (2020). Roles of the AhpD-family protein RcsA in reactive chlorine stress resistance and

virulence in *Pseudomonas aeruginosa*. Appl. Environ. Microbiol 86: e01480-20.

Norambuena, J., Wang, Y., Hanson, T., Boyd, J.M., and Barkay, T. (2018). Low-molecular-weight thiols and thioredoxins are important players in Hg(II) resistance in *Thermus thermophilus* HB27. Appl. Environ. Microbiol. 84: e01931-17.

Noto, M.J., Burns, W.J., Beavers, W.N., and Skaar, E.P. (2017). Mechanisms of pyocyanin toxicity and genetic determinants of resistance in *Staphylococcus aureus*. J. Bacteriol. 199: e00221-17.

O'Brien, P.J. (1991). Molecular mechanisms of quinone cytotoxicity. Chem. Biol. Interact. 80: 1–41.

Oogai, Y., Kawada-Matsuo, M., and Komatsuzawa, H. (2016). *Staphylococcus aureus* SrrAB affects susceptibility to hydrogen peroxide and co-existence with *Streptococcus sanguinis*. PloS One 11: e0159768.

Palazzolo-Ballance, A.M., Reniere, M.L., Braughton, K.R., Sturdevant, D.E., Otto, M., Kreiswirth, B.N., Skaar, E.P., and DeLeo, F.R. (2008). Neutrophil microbicides induce a pathogen survival response in community-associated methicillin-resistant *Staphylococcus aureus*. J. Immunol. 180: 500–509.

Pamp, S.J., Frees, D., Engelmann, S., Hecker, M., and Ingmer, H. (2006). Spx is a global effector impacting stress tolerance and biofilm formation in *Staphylococcus aureus*. J. Bacteriol. 188: 4861–4870.

Panasenko, O.O., Bezrukov, F., Komarynets, O., and Renzoni, A. (2020). YjbH solubility controls Spx in *Staphylococcus aureus*: implication for MazEF toxin-antitoxin system regulation. Front. Microbiol. 11: 113.

Pandey, S., Sahukhal, G.S., and Elasri, M.O. (2019). The *msaABCR* operon regulates the response to oxidative stress in *Staphylococcus aureus*. J. Bacteriol. 201: e00417-19.

Parker, B.W., Schwessinger, E.A., Jakob, U., and Gray, M.J. (2013). The RclR protein is a reactive chlorine-specific transcription factor in *Escherichia coli*. J. Biol. Chem. 288: 32574–32584.

Parsonage, D., Karplus, P.A., and Poole, L.B. (2008). Substrate specificity and redox potential of AhpC, a bacterial peroxiredoxin. Proc. Natl. Acad. Sci. USA 105: 8209–8214.

Parsonage, D., Nelson, K.J., Ferrer-Sueta, G., Alley, S., Karplus, P.A., Furdui, C.M., and Poole, L.B. (2015). Dissecting peroxiredoxin catalysis: separating binding, peroxidation, and resolution for a bacterial AhpC. Biochemistry 54: 1567–1575.

Partridge, J.D., Bodenmiller, D.M., Humphrys, M.S., and Spiro, S. (2009). NsrR targets in the *Escherichia coli* genome: new insights into DNA sequence requirements for binding and a role for NsrR in the regulation of motility. Mol. Microbiol. 73: 680–694.

Peng, H., Shen, J., Edmonds, K.A., Luebke, J.L., Hickey, A.K., Palmer, L.D., Chang, F.J., Bruce, K.A., Kehl-Fie, T.E., and Skaar, E.P., et al (2017a). Sulfide homeostasis and nitroxyl intersect via formation of reactive sulfur species in *Staphylococcus aureus*. mSphere 2: e00082-17.

Peng, H., Zhang, Y., Palmer, L.D., Kehl-Fie, T.E., Skaar, E.P., Trinidad, J.C., and Giedroc, D.P. (2017b). Hydrogen sulfide and reactive sulfur species impact proteome *S*-sulfhydration and global virulence regulation in *Staphylococcus aureus*. ACS Infect. Dis. 3: 744–755.

Perera, I.C. and Grove, A. (2010). Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. J. Mol. Cell Biol. 2: 243–254.

Perera, V.R., Newton, G.L., Parnell, J.M., Komives, E.A., and Pogliano, K. (2014). Purification and characterization of the *Staphylococcus aureus* bacillithiol transferase BstA. Biochim. Biophys. Acta 1840: 2851–2861.

Pinochet-Barros, A. and Helmann, J.D. (2018). Redox sensing by Fe(2+) in bacterial fur family metalloregulators. Antioxid. Redox. Signal 29: 1858–1871.

Poole, L.B. and Ellis, H.R. (1996). Flavin-dependent alkyl hydroperoxide reductase from *Salmonella typhimurium*. 1. Purification and enzymatic activities of overexpressed AhpF and AhpC proteins. Biochemistry 35: 56–64.

Poole, L.B., Hall, A., and Nelson, K.J. (2011). Overview of peroxiredoxins in oxidant defense and redox regulation. Curr. Protoc. Toxicol., Chapter 7: Unit7.9, https://doi.org/10.1002/0471140856.tx0709s49.

Poor, C.B., Chen, P.R., Duguid, E., Rice, P.A., and He, C. (2009). Crystal structures of the reduced, sulfenic acid, and mixed disulfide forms of SarZ, a redox active global regulator in *Staphylococcus aureus*. J. Biol. Chem. 284: 23517–23524.

Posada, A.C., Kolar, S.L., Dusi, R.G., Francois, P., Roberts, A.A., Hamilton, C.J., Liu, G.Y., and Cheung, A. (2014). Importance of bacillithiol in the oxidative stress response of *Staphylococcus aureus*. Infect. Immun. 82: 316–332.

Pöther, D.C., Gierok, P., Harms, M., Mostertz, J., Hochgräfe, F., Antelmann, H., Hamilton, C.J., Borovok, I., Lalk, M., Aharonowitz, Y., et al. (2013). Distribution and infection-related functions of bacillithiol in *Staphylococcus aureus*. Int. J. Med. Microbiol. 303: 114–123.

Pragman, A.A., Yarwood, J.M., Tripp, T.J., and Schlievert, P.M. (2004). Characterization of virulence factor regulation by SrrAB, a two-component system in *Staphylococcus aureus*. J. Bacteriol. 186: 2430–2438.

Radi, R. (2018). Oxygen radicals, nitric oxide, and peroxynitrite: redox pathways in molecular medicine. Proc. Natl. Acad. Sci. USA 115: 5839–5848.

Rajkarnikar, A., Strankman, A., Duran, S., Vargas, D., Roberts, A.A., Barretto, K., Upton, H., Hamilton, C.J., and Rawat, M. (2013). Analysis of mutants disrupted in bacillithiol metabolism in *Staphylococcus aureus*. Biochem. Biophys. Res. Commun. 436: 128–133.

Rawat, M., and Maupin-Furlow, J.A. (2020). Redox and thiols in archaea. Antioxidants 9: 381, https://doi.org/10.3390/antiox9050381.

Ray, A., Edmonds, K.A., Palmer, L.D., Skaar, E.P., and Giedroc, D.P. (2020). *Staphylococcus aureus* glucose-induced biofilm accessory protein A (GbaA) is a monothiol-dependent electrophile sensor. Biochemistry 59: 2882–2895.

Remes, B., Eisenhardt, B.D., Srinivasan, V., and Klug, G. (2015). IscR of *Rhodobacter sphaeroides* functions as repressor of genes for iron-sulfur metabolism and represents a new type of iron-sulfur-binding protein. Microbiologyopen 4: 790–802.

Riquelme, S.A., Liimatta, K., Wong Fok Lung, T., Fields, B., Ahn, D., Chen, D., Lozano, C., Saenz, Y., Uhlemann, A.C., Kahl, B.C., et al. (2020). *Pseudomonas aeruginosa* utilizes host-derived itaconate to redirect its metabolism to promote biofilm formation. Cell Metabol. 31: 1091–1106, e1096.

Roberts, A.A., Sharma, S.V., Strankman, A.W., Duran, S.R., Rawat, M., and Hamilton, C.J. (2013). Mechanistic studies of FosB: a divalent-metal-dependent bacillithiol-*S*-transferase that mediates fosfomycin resistance in *Staphylococcus aureus*. Biochem. J. 451: 69–79.

Rosario-Cruz, Z. and Boyd, J.M. (2016). Physiological roles of bacillithiol in intracellular metal processing. Curr. Genet. 62: 59–65.

Rosario-Cruz, Z., Chahal, H.K., Mike, L.A., Skaar, E.P., and Boyd, J.M. (2015). Bacillithiol has a role in Fe-S cluster biogenesis in *Staphylococcus aureus*. Mol. Microbiol. 98: 218–242.

Rosen, H., Klebanoff, S.J., Wang, Y., Brot, N., Heinecke, J.W., and Fu, X. (2009). Methionine oxidation contributes to bacterial killing by the myeloperoxidase system of neutrophils. Proc. Natl. Acad. Sci. USA 106: 18686–18691.

Schlag, S., Nerz, C., Birkenstock, T.A., Altenberend, F., and Gotz, F. (2007). Inhibition of staphylococcal biofilm formation by nitrite. J. Bacteriol. 189: 7911–7919.

Schwarzländer, M., Dick, T.P., Meyer, A.J., and Morgan, B. (2016). Dissecting redox biology using fluorescent protein sensors. Antioxid. Redox. Signal 24: 680–712.

Sharma, S.V., Arbach, M., Roberts, A.A., Macdonald, C.J., Groom, M., and Hamilton, C.J. (2013). Biophysical features of bacillithiol, the glutathione surrogate of *Bacillus subtilis* and other firmicutes. Chembiochem 14: 2160–2168.

Shatalin, K., Shatalina, E., Mironov, A., and Nudler, E. (2011). H_2S: a universal defense against antibiotics in bacteria. Science 334: 986–990.

Shen, J., Keithly, M.E., Armstrong, R.N., Higgins, K.A., Edmonds, K.A., and Giedroc, D.P. (2015). *Staphylococcus aureus* CstB is a novel multidomain persulfide dioxygenase-sulfurtransferase involved in hydrogen sulfide detoxification. Biochemistry 54: 4542–4554.

Shen, J., Peng, H., Zhang, Y., Trinidad, J.C., and Giedroc, D.P. (2016). *Staphylococcus aureus sqr* encodes a type II sulfide: quinone oxidoreductase and impacts reactive sulfur speciation in cells. Biochemistry 55: 6524–6534.

Smith, M.T. (1985). Quinones as mutagens, carcinogens, and anticancer agents: introduction and overview. J. Toxicol. Environ. Health 16: 665–672.

Soutourina, O., Dubrac, S., Poupel, O., Msadek, T., and Martin-Verstraete, I. (2010). The pleiotropic CymR regulator of *Staphylococcus aureus* plays an important role in virulence and stress response. PLoS Pathog. 6: e1000894.

Soutourina, O., Poupel, O., Coppee, J.Y., Danchin, A., Msadek, T., and Martin-Verstraete, I. (2009). CymR, the master regulator of cysteine metabolism in *Staphylococcus aureus*, controls host sulphur source utilization and plays a role in biofilm formation. Mol. Microbiol. 73: 194–211.

Stomberski, C.T., Hess, D.T., and Stamler, J.S. (2019). Protein S-nitrosylation: determinants of specificity and enzymatic regulation of S-nitrosothiol-based signaling. Antioxid. Redox. Signal 30: 1331–1351.

Storkey, C., Davies, M.J., and Pattison, D.I. (2014). Reevaluation of the rate constants for the reaction of hypochlorous acid (HOCl) with cysteine, methionine, and peptide derivatives using a new competition kinetic approach. Free Radic. Biol. Med. 73: 60–66.

Sun, F., Ding, Y., Ji, Q., Liang, Z., Deng, X., Wong, C.C., Yi, C., Zhang, L., Xie, S., Alvarez, S., et al. (2012). Protein cysteine phosphorylation of SarA/MgrA family transcriptional regulators mediates bacterial virulence and antibiotic resistance. Proc. Natl. Acad. Sci. USA 109: 15461–15466.

Tam, K. and Torres, V.J. (2019). *Staphylococcus aureus* secreted toxins and extracellular enzymes. Microbiol. Spectr. 7, https://doi.org/10.1128/microbiolspec.gpp3-0039-2018.

Tam le, T., Eymann, C., Albrecht, D., Sietmann, R., Schauer, F., Hecker, M., and Antelmann, H. (2006). Differential gene expression in response to phenol and catechol reveals different metabolic activities for the degradation of aromatic compounds in *Bacillus subtilis*. Environ. Microbiol. 8: 1408–1427.

Tiwari, N., Lopez-Redondo, M., Miguel-Romero, L., Kulhankova, K., Cahill, M.P., Tran, P.M., Kinney, K.J., Kilgore, S.H., Al-Tameemi, H., Herfst, C.A., et al. (2020). The SrrAB two-component system regulates *Staphylococcus aureus* pathogenicity through redox sensitive cysteines. Proc. Natl. Acad. Sci. USA 117: 10989–10999.

Tocmo, R., Wu, Y., Liang, D., Fogliano, V., and Huang, D. (2017). Boiling enriches the linear polysulfides and the hydrogen sulfide-releasing activity of garlic. Food Chem. 221: 1867–1873.

Toliver-Kinsky, T., Cui, W., Toro, G., Lee, S.J., Shatalin, K., Nudler, E., and Szabo, C. (2019). H_2S, a bacterial defense mechanism against the host immune response. Infect. Immun. 87: e00272-18.

Töwe, S., Leelakriangsak, M., Kobayashi, K., Van Duy, N., Hecker, M., Zuber, P., and Antelmann, H. (2007). The MarR-type repressor MhqR (YkvE) regulates multiple dioxygenases/glyoxalases and an azoreductase which confer resistance to 2-methylhydroquinone and catechol in *Bacillus subtilis*. Mol. Microbiol. 66: 40–54.

Treffon, J., Block, D., Moche, M., Reiss, S., Fuchs, S., Engelmann, S., Becher, D., Langhanki, L., Mellmann, A., Peters, G., et al. (2018). Adaptation of *Staphylococcus aureus* to airway environments in patients with cystic fibrosis by upregulation of superoxide dismutase M and iron-scavenging proteins. J. Infect. Dis. 217: 1453–1461.

Truong-Bolduc, Q.C., Ding, Y., and Hooper, D.C. (2008). Posttranslational modification influences the effects of MgrA on *norA* expression in *Staphylococcus aureus*. J. Bacteriol. 190: 7375–7381.

Truong-Bolduc, Q.C., Dunman, P.M., Strahilevitz, J., Projan, S.J., and Hooper, D.C. (2005). MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. J. Bacteriol. 187: 2395–2405.

Tsuchiya, Y., Zhyvoloup, A., Bakovic, J., Thomas, N., Yu, B.Y.K., Das, S., Orengo, C., Newell, C., Ward, J., and Saladino, G., et al (2018). Protein CoAlation and antioxidant function of coenzyme A in prokaryotic cells. Biochem. J. 475: 1909–1937.

Ulfig, A. and Leichert, L.I. (2020). The effects of neutrophil-generated hypochlorous acid and other hypohalous acids on host and pathogens. Cell. Mol. Life Sci., https://doi.org/10.1007/s00018-020-03591-y.

Ulrich, M., Bastian, M., Cramton, S.E., Ziegler, K., Pragman, A.A., Bragonzi, A., Memmi, G., Wolz, C., Schlievert, P.M., Cheung, A., et al. (2007). The staphylococcal respiratory response regulator SrrAB induces ica gene transcription and polysaccharide intercellular adhesin expression, protecting *Staphylococcus aureus* from neutrophil killing under anaerobic growth conditions. Mol. Microbiol. 65: 1276–1287.

Vaishampayan, A., de Jong, A., Wight, D.J., Kok, J., and Grohmann, E. (2018). A novel antimicrobial coating represses biofilm and virulence-related genes in methicillin-resistant *Staphylococcus aureus*. Front. Microbiol. 9: 221.

Valderas, M.W., Gatson, J.W., Wreyford, N., and Hart, M.E. (2002). The superoxide dismutase gene *sodM* is unique to *Staphylococcus aureus*: absence of *sodM* in coagulase-negative staphylococci. J. Bacteriol. 184: 2465–2472.

Valderas, M.W. and Hart, M.E. (2001). Identification and characterization of a second superoxide dismutase gene (*sodM*) from *Staphylococcus aureus*. J. Bacteriol. 183: 3399–3407.

Van Laer, K., Hamilton, C.J., and Messens, J. (2013). Low-molecular-weight thiols in thiol-disulfide exchange. Antioxid. Redox. Signal 18: 1642–1653.

Vazquez-Torres, A. (2012). Redox active thiol sensors of oxidative and nitrosative stress. Antioxid. Redox. Signal 17: 1201–1214.

Vestergaard, M., Frees, D., and Ingmer, H. (2019). Antibiotic resistance and the MRSA problem. Microbiol. Spectr. 7, https://doi.org/10.1128/microbiolspec.gpp3-0057-2018.

Vetter, S.W. (2015). Glycated serum albumin and AGE receptors. Adv. Clin. Chem. 72: 205–275.

Voyich, J.M., Braughton, K.R., Sturdevant, D.E., Whitney, A.R., Said-Salim, B., Porcella, S.F., Long, R.D., Dorward, D.W., Gardner, D.J., Kreiswirth, B.N., et al. (2005). Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. J. Immunol. 175: 3907–3919.

Walsh, B.J.C. and Giedroc, D.P. (2020). H_2S and reactive sulfur signaling at the host-bacterial pathogen interface. J. Biol. Chem. 295: 13150–13168.

Wang, C., Fan, J., Niu, C., Wang, C., Villaruz, A.E., Otto, M., and Gao, Q. (2010). Role of spx in biofilm formation of *Staphylococcus epidermidis*. FEMS Immunol. Med. Microbiol. 59: 152–160.

Weber, H., Engelmann, S., Becher, D., and Hecker, M. (2004). Oxidative stress triggers thiol oxidation in the glyceraldehyde-3-phosphate dehydrogenase of *Staphylococcus aureus*. Mol. Microbiol. 52: 133–140.

Wilkinson, S.P. and Grove, A. (2006). Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. Curr. Issues Mol. Biol. 8: 51–62.

Winterbourn, C.C., Hampton, M.B., Livesey, J.H., and Kettle, A.J. (2006). Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. J. Biol. Chem. 281: 39860–39869.

Winterbourn, C.C. and Kettle, A.J. (2013). Redox reactions and microbial killing in the neutrophil phagosome. Antioxid. Redox. Signal 18: 642–660.

Winterbourn, C.C., Kettle, A.J., and Hampton, M.B. (2016). Reactive oxygen species and neutrophil function. Annu. Rev. Biochem. 85: 765–792.

Winterbourn, C.C. and Metodiewa, D. (1999). Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. Free Radic. Biol. Med. 27: 322–328.

Ximenes, V.F., Maghzal, G.J., Turner, R., Kato, Y., Winterbourn, C.C., and Kettle, A.J. (2009). Serotonin as a physiological substrate for myeloperoxidase and its superoxide-dependent oxidation to cytotoxic tryptamine-4,5-dione. Biochem. J. 425: 285–293.

Yarwood, J.M. and Schlievert, P.M. (2000). Oxygen and carbon dioxide regulation of toxic shock syndrome toxin 1 production by *Staphylococcus aureus* MN8. J. Clin. Microbiol. 38: 1797–1803.

You, Y., Xue, T., Cao, L., Zhao, L., Sun, H., and Sun, B. (2014). *Staphylococcus aureus* glucose-induced biofilm accessory proteins, GbaAB, influence biofilm formation in a PIA-dependent manner. Int. J. Med. Microbiol. 304: 603–612.

Yu, L., Hisatsune, J., Hayashi, I., Tatsukawa, N., Sato'o, Y., Mizumachi, E., Kato, F., Hirakawa, H., Pier, G.B., and Sugai, M. (2017). A novel repressor of the *ica* Locus discovered in clinically isolated super-biofilm-elaborating *Staphylococcus aureus*. mBio. 8: e02282-16.

Zuber, P. (2004). Spx-RNA polymerase interaction and global transcriptional control during oxidative stress. J. Bacteriol. 186: 1911–1918.

Zuber, P. (2009). Management of oxidative stress in *Bacillus*. Annu. Rev. Microbiol. 63: 575–597.