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FORUM REVIEW ARTICLE

Redox Signaling in Human Pathogens

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Abstract

In recent studies of human bacterial pathogens, oxidation sensing and regulation have been shown to impact very diverse pathways that extend beyond inducing antioxidant genes in the bacteria. In fact, some redox-sensitive regulatory proteins act as major regulators of bacteria's adaptability to oxidative stress, an ability that originates from immune host response as well as antibiotic stress. Such proteins play particularly important roles in pathogenic bacteria *S. aureus*, *P. aeruginosa*, and *M. tuberculosis* in part because reactive oxygen species and reactive nitrogen species present significant challenges for pathogens during infection. Herein, we review recent progress toward the identification and understanding of oxidation sensing and regulation in human pathogens. The newly identified redox switches in pathogens are a focus of this review. We will cover several reactive oxygen species-sensing global regulators in both gram-positive and gram-negative pathogenic bacteria in detail. The following discussion of the mechanisms that these proteins employ to sense redox signals through covalent modification of redox active amino acid residues or associated metalloprotein centers will provide further understanding of bacteria pathogenesis, antibiotic resistance, and host–pathogen interaction. *Antioxid. Redox Signal.* 14, 1107–1118.

Introduction

Generation of reactive oxygen species (ROS) is one of the major defense mechanisms employed by host immune systems during bacterial infection. Oxidation-sensitive regulators and the associated antioxidant genes have been found in a variety of bacteria to survive oxidative stress [see review by Antelmann and Helmann in this issue (1a)]. Recent studies, however, have revealed that some of these oxidation-sensing regulators play more diverse roles in bacteria, which range from the regulation of virulence factors to the control of antibiotic-resistance genes. These redox-sensitive regulators are particularly important for pathogenic bacteria because they must cope with ROS challenges generated from both host immune response and antibiotic stress. Excellent reviews on bacterial redox sensors are available (18, 25); this article, however, does not intend to discuss all existing redox sensors. Rather, we discuss recent progress on the identification of oxidation-sensing regulators in pathogenic bacteria and the characterization of their roles during bacterial infection as well as other cellular activities. The focus is *Staphylococcus aureus* and *Pseudomonas aeruginosa* as representative of gram-positive and gram-negative pathogens, respectively. Several global regulators that employ a redox-switch mechanism in control of a broad spectrum of genes in these bacterial species will be discussed in detail. As *E. coli* OxyR and SoxR and their homologs are the two most extensively characterized and reviewed families of redox transcription factors (27, 55), they will also be briefly discussed in this review. To conclude, we review recent progress toward understanding the redox-sensitive transcription regulators in *Mycobacterium tuberculosis*, the causative agent of the deadly infectious disease Tuberculosis.

Redox Switches in *S. aureus*

MgrA, a major virulence determinant and global regulator, is a redox-sensing protein in S. aureus

Staphylococcus aureus is an important gram-positive pathogen responsible for a variety of human infections ranging from minor skin infections to life-threatening infectious diseases. In the study of the multiple antibiotic resistance (MarR) family proteins in this pathogen, MgrA was found to be an antibiotic-resistance regulator that also impacts other properties of *S. aureus* (Fig. 1) (8, 36, 48, 69). Results from several laboratories all indicated that MgrA regulates the resistance of *S. aureus* to a broad spectrum of antibiotics, including fluoroquinolones, vancomycin, tetracycline, and penicillin.

A further microarray study showed that MgrA controls expression of ~350 genes in *S. aureus* (47). These genes encode proteins that include a wide variety of virulence factors (*e.g.*, capsular polysaccharide, nuclease, alpha-toxin, coagulase, protease, and protein A), autolysins (*e.g.*, *lytM* and *lytN*), and

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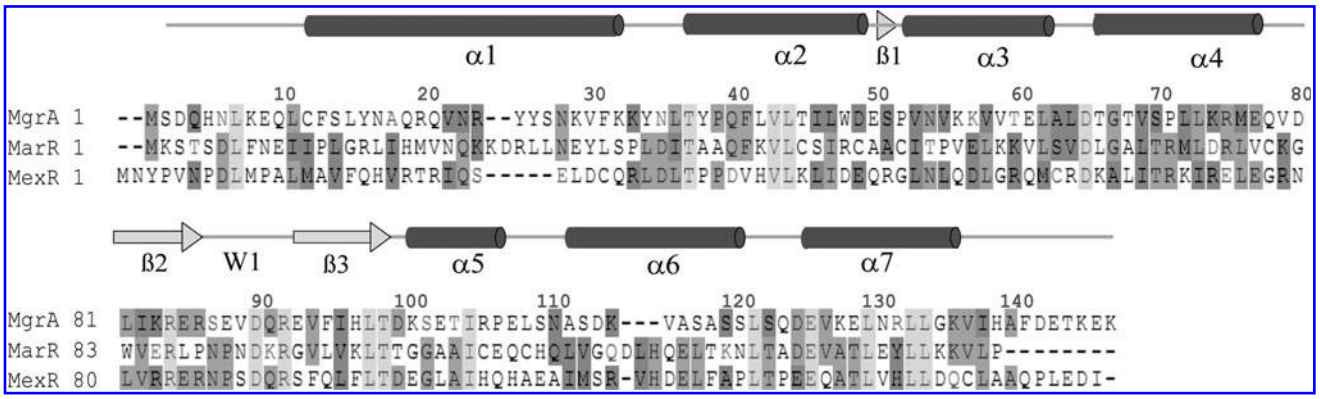

FIG. 1. Sequence alignment of the selected MarR family proteins with *S. aureus* MgrA, *E. coli* MarR, and *P. aeruginosa* MexR. Light highlights indicate identical aminoacids, dark highlights indicate conserved substitutions.

other global regulatory proteins (*e.g.*, *agr*, *lytRS*, *arlRS*, *sarS*, *sarV*, *sarA*, and *sarZ*) (Fig. 2), as well as proteins engaged in cell wall biosynthesis, membrane transporters, metabolism, and many other functions. Using a murine abscess model of infection, our laboratory showed that the *mgrA* mutant strain exhibits a 10,000-fold reduction of bacterial loading in the kidneys, and an over 100-fold reduction in the livers of tested mice compared to the isogenic parent, *S. aureus* strain Newman (8). Taken together, all these studies demonstrate that MgrA is a global regulator and a major virulence determinant in *S. aureus*.

In the hope of revealing the regulatory mechanism of MgrA, we solved the crystal structure of MgrA at 2.86 Å (8). Overall, the MgrA structure resembles those of *E. coli* MarR and its homologs (Fig. 3A). The dimer contains two winged helix-turn-helix DNA binding domains attached to a central dimerization domain in which helices α1, α6, and α7 from the two monomers are intertwined. The most noticeable difference between the two structures is the orientation of the two DNA-binding domains. When the N- and C-terminal dimerization helices of MgrA (α1 and α7) and the salicylate-bound MarR (α1 and α6) are overlaid, a dramatic difference is observed for the relative position of the two DNA-binding domains (Fig. 3B). The recognition helices (α4) of MgrA are poised to interact with the major groove of B-form DNA (Fig. 3C), whereas the same helices in MarR are twisted by ~43 degree and are nearly inline with each other. This conformation prevents the two α4 helices from interacting with the major grooves of a duplex DNA simultaneously in the salicylate-bound MarR.

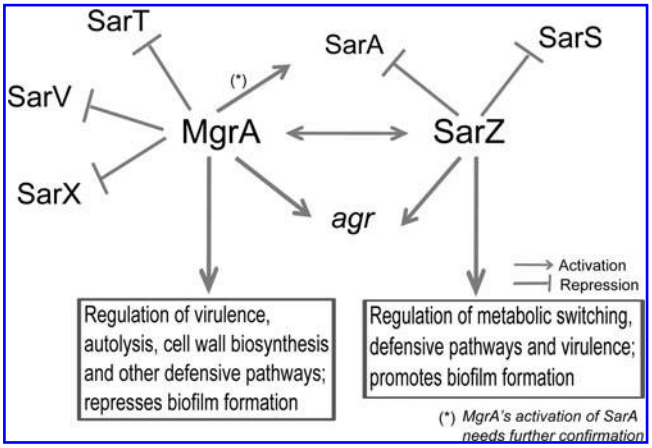

FIG. 2. MgrA, SarZ, and SarA regulatory network. MgrA, SarZ, and SarA are global regulatory proteins in *S. aureus.* MgrA and SarZ are redox switches, whereas SarA has been suggested to be redox active as well.

The structure of MgrA did not immediately reveal the signal that activates this regulator and regulatory mechanism used by the protein. It was only after a long period of research inquiry that we appreciated the significance of the single cysteine residue found in the MgrA sequence, Cys12. This lone cysteine is located in the N-terminal helix α1 in the dimerization domain. Each monomer presents the cysteine to be recognized through hydrogen bonding to Ser113 and Tyr38 from the other monomer (Fig. 3D). Close inspection of the organization of the dimer interface revealed striking similarities between MgrA and OhrR (32), a peroxide-sensing transcriptional factor that controls an organic hydroperoxide resistance gene (*ohr*) in *Bacillus subtilis* (22, 23), which is reviewed in this issue (1a). In the OhrR case, oxidation of Cys15 by hydrogen peroxide or organic hydroperoxide and subsequent modification leads to dissociation of the protein from the promoter DNA and derepression of the *ohr* gene to counter peroxide stress. Indeed, our subsequent studies had shown that ROS such as hydrogen peroxide, organic peroxide, and superoxide can efficiently oxidize the thiol group of Cys12 to form Cys-sulfenic acid *in vitro*, and that this oxidation leads to weakened affinity of MgrA to its cognate DNA (8). As will be discussed below for SarZ and OhrR, the sulfenic acid form of Cys12 is most likely further modified inside bacteria, which may lead to a further reduction of the protein's affinity to its cognate DNA. Bacterial whole cell assay further confirmed that peroxides serve as the signal that triggers transcriptional activation through the release of MgrA (derepression), which turns on transcription of a broad range of downstream genes that are engaged in various activity and properties of *S. aureus* (8).

Typically, bacteria employ the oxidation-sensing mechanism to counter challenges of ROS and reactive nitrogen species (RNS). The discovery that a global regulator that impacts the transcription of ~13% of all genes in *S. aureus* is actually a redox switch has significant implications. Since

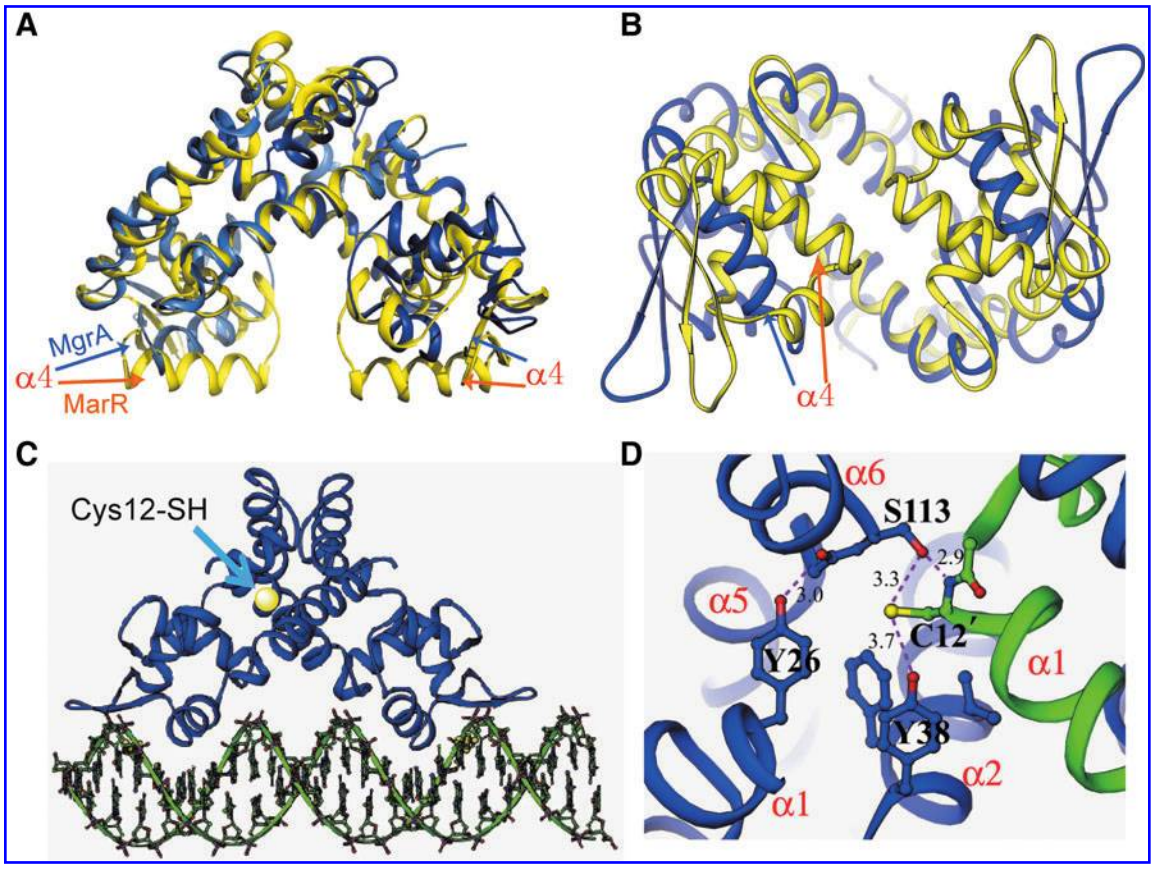

FIG. 3. Structural analysis of MgrA. (**A**) Overlay of the crystal structures of the reduced form of MgrA dimer (in blue) with the salicylate-bound form of MarR dimer (in yellow). The DNA-binding helix α4 is indicated by *arrows*. (**B**) View from the bottom of the structures of MgrA (blue) and MarR (yellow). (**C**) Structure of the reduced form of MgrA docked on a B-form duplex DNA. Cys12 on one monomer is shown as a yellow ball and indicated by an *arrow*. (**D**) Close-up of the active site Cys residue and the surrounding H-bond network in MgrA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

S. aureus and related human pathogens must cope with human immune response, oxidation signals must affect many different aspects of the bacterial physiology, including the tuning of its virulence. Clearly, MgrA adopts an oxidation-sensing mechanism in *S. aureus* to regulate much broader functions, particularly when compared to OhrR in *B. subtilis* (Fig. 4). This oxidation-sensing strategy enables the response of a single regulator, MgrA, to oxidative stress caused by various challenges and to globally regulate different defensive pathways.

SarZ, a MgrA homolog in S. aureus, *is also a thiol-based redox switch*

Intrigued by the discovery of MgrA as a redox switch that exerts a global regulatory function in *S. aureus*, we asked the

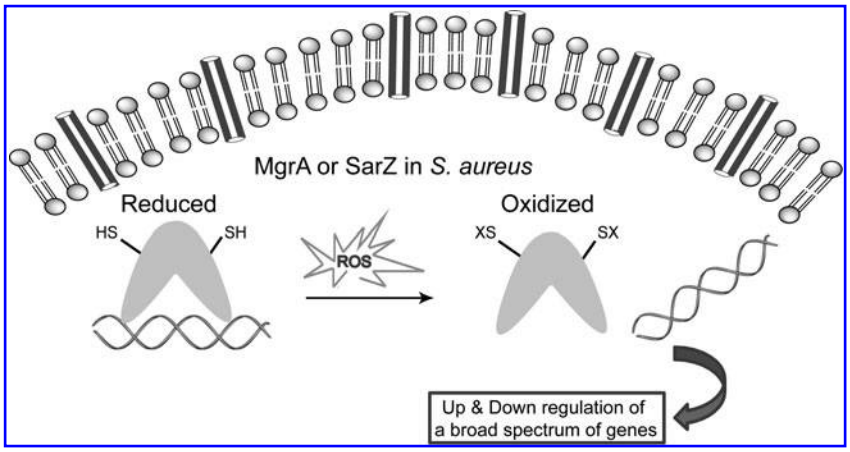

FIG. 4. Mechanism of MgrA and SarZ. An oxidation-sensing mechanism is employed by MgrA and SarZ for the regulation of a broad spectrum of genes in *S. aureus*. The character X refers to thiol modification, for example, SR for SarZ. ROS, reactive oxygen species.

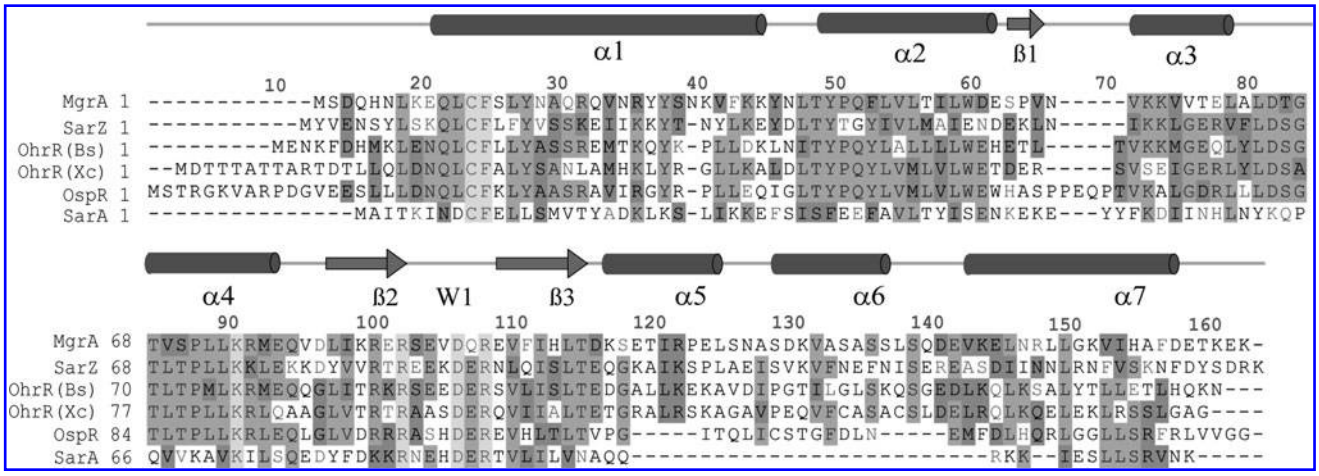

FIG. 5. Sequence alignment of the MarR-type of redox-sensitive regulators in *S. aureus* (MgrA, SarZ, and SarA), *P. aeruginosa* (OspR), and homologs in *B. subtilis* (OhrR-Bs) and *X. campestris* (OhrR-Xc). These proteins share a thiol-based, oxidative-sensing mechanism. Light highlights indicate identical aminoacids, dark highlights indicate conserved substitutions.

obvious question: Are there other homologs in *S. aureus* that also possess a redox-active Cys residue? Indeed, a sequence alignment showed that two additional regulatory proteins, SarA and SarZ, have a conserved Cys residue as does MgrA (Fig. 5). SarZ is a close homolog of MgrA with a 71% sequence identity. It affects expression of 87 genes that are involved in metabolism, autolysis, and defensive pathways (9). It also impacts bacterial virulence by controlling toxin secretion, protease production, and biofilm formation. Most interestingly, SarZ dramatically affects the activation of *pflA* and *pflB*, which encode enzymes of the first committed steps of anaerobic respiration. These two genes are known to be dramatically induced when *S. aureus* is under H_2O_2 stress. Thus, under oxidative stress, SarZ controls the metabolic switching to anaerobic energy production pathways, while the microbe transforms into a more inert state to protect itself (9). Bacteria in this state can presumably grow anaerobically with decreased nutrition and energy consumption, and are more resistant to environmental stresses such as ROS and antibiotics.

The conserved Cys residue in SarZ, Cys13, is redox active. It is readily oxidized by peroxides; oxidation and subsequent modifications then lead to the reduced affinity of the protein to the cognate DNA. As in the case for OhrR, sulfenic acid formation from Cys-oxidation is not sufficient for derepression. Hellman and coauthors have shown that cysteine modification, formation of a mixed disulfide or a cyclic sulfenamide, is necessary for the release of OhrR from DNA (30, 45). The formation of a mixed disulfide with small molecule thiols present in the bacterial cytoplasm can be the dominant biological modification inside bacteria (63). In fact, some other bacterial peroxide sensors employ the formation of intra- or intermolecular disulfide bonds to induce conformational changes necessary for gene activation (7, 51, 53, 72).

The crystal structures of SarZ in three different states—reduced, sulfenic acid form, and disulfide-modified with an external small molecule thiol—were solved recently in an effort to understand the full picture of the activation mechanism of SarZ (57). Overall, very few differences manifest between the reduced and the sulfenic-acid modified structures, but a large conformational shift in the DNA-binding domains and the hydrogen-binding network surrounding Cys13 were observed when SarZ was further modified to the mixed disulfide form (Fig. 6). Superimposition of the structure of reduced SarZ and the sulfenic-acid modified form results in a root mean square deviation (rmsd) of only 1.16 Å. In the reduced SarZ, Cys13 is surrounded by hydrogen-bonding residues from the other monomer just like that in MgrA. Upon oxidation to the sulfenic acid form, the same hydrogen bonds are formed with Oδ instead of Sγ. The spacing between the DNA-binding helices α4 and α4′ is 32 Å and 30 Å for the reduced and sulfenic acid-modified SarZ, respectively, and is compatible with the binding of the recognition helices to consecutive major grooves of B-DNA. This structural observation coincides with DNA-binding results, which demonstrate that reduced SarZ and sulfenic-acid modified SarZ have similar affinities to DNA. In contrast, a large conformational shift in the DNA-binding domains is observed when SarZ is further modified to the mixed disulfide form (Fig. 6C). The formation of the disulfide bond disrupts both the hydrogen bond network and the organization of residues in the cysteine pocket. The addition of benzene thiol to the cysteine pocket also induces changes through steric interactions. A combination of losing the interactions of the hydrogen bonds that surround the reactive Cys residue and the additionally added sterics from the mixed disulfide contributes to an allosteric conformational change, which in turn leads to a 7-Å translational shift of the DNA-binding helices that induces sterics with the duplex DNA and gives rise to a decreased affinity of the disulfide-modified SarZ to DNA (57).

This detailed study of SarZ revealed a more complete mechanistic picture for the single Cys-containing MgrA/OhrR type of regulatory proteins. The nature of the small molecule thiol *in vivo* is still unclear, as well as whether or not the disulfide-modified proteins return to a reduced state inside these bacteria in the absence of oxidation stress. Possibly, the disulfide-modified proteins can be reduced back to the native form through thioredoxin or other thiol-based reductants.

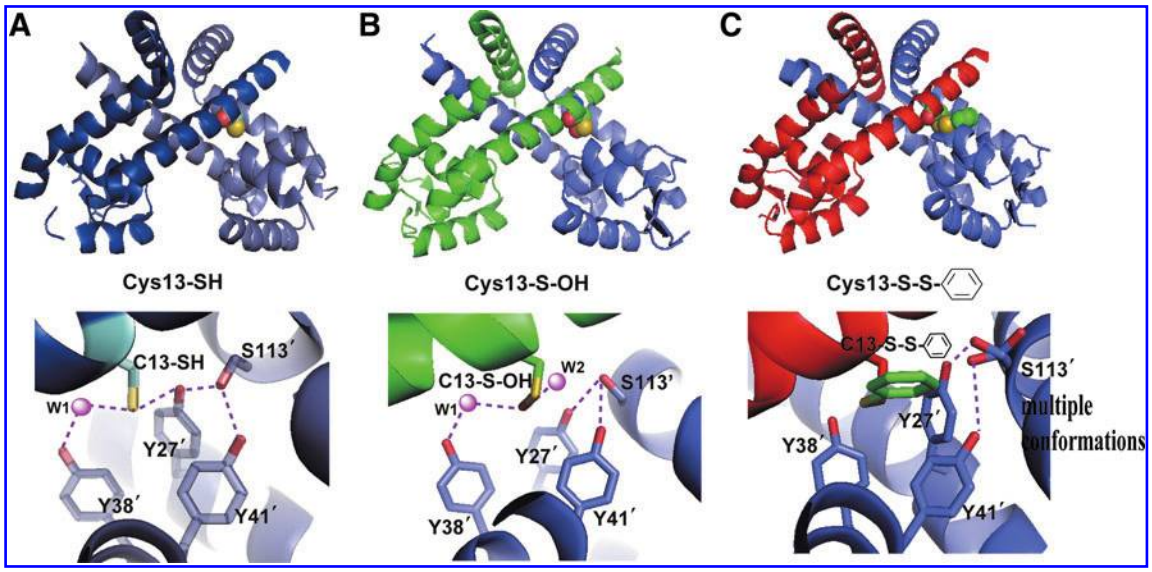

FIG. 6. Structural analysis of SarZ. Structures of reduced (**A**), sulfenic acid (**B**), and disulfide-modified (**C**) forms of SarZ. Close-up of the active site Cys residue and the surrounding H-bond networks on SarZ are shown below (PDB IDs: 3HSE, 3HRM, and 3HSR). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

SarA has been proposed to act as a redox switch in S. aureus

SarA is one the first transcriptional regulators discovered in *S. aureus* (10). SarA controls approximately 120 genes that encode proteins performing diverse functions in *S. aureus*. Sequence alignment with MgrA and SarZ indicates that SarA has a conserved Cys residue, Cys9. Cys9 is also the only Cys residue in the entire sequence of SarA. The crystal structure of SarA also indicates the presence of a Cys-recognition pocket at the protein dimerization interface (46). Cys9 is not very sensitive to oxidation, however, and requires millimolar levels of peroxides to oxidize this Cys residue (3, 4, 24, and He, unpublished). Instead, this Cys in SarA is very sensitive to alkylation (He unpublished). Recent results show that SarA controls *sod* (encodes superoxide dismutase) and *trxB* (encodes thioredoxin reductase) in *S. aureus* (3, 4). Thus, SarA is involved in redox balance in *S. aureus*. The exact sensing and regulatory mechanism employed by SarA may need further studies. Direct Cys9 oxidation is an attractive proposal (3, 4, 24). Redox signals may also be relayed through a different pathway.

PerR is a metal-dependent oxidative stress regulator in S. aureus

PerR is a metalloprotein first identified from Gram-positive bacterium *Bacillus subtilis*. PerR belongs to the Fur (ferric uptake regulator) family of transcription factors and is a major regulator of the hydrogen peroxide stress response in *B. subtilis*. The homolog of PerR had also been found in various gram-positive pathogens, including *S. aureus*. PerR and OxyR control similar regulatory pathways, including peroxide scavenging and iron metabolism, and generally present in different organisms. In *S. aureus*, PerR may act as a redox-sentinel protein during infection (33). The pathogenesis of *perR* mutant strain MJH001 was investigated through a murine subcutaneous skin abscess model of infection, which exhibited a $\sim$10-fold reduction of virulence ($p < 0.005$) and produced smaller lesions ($0.237 \pm .0.143$ g) compared to the wild-type strain 8325–4 (0.528 ± 0.173g). Although catalase has been previously proposed as an important virulence determinant in *S. aureus* (38, 50), the same model of infection on *katA* mutant strain ST16 showed no attenuation of virulence ($p = 0.125$) with similar lesion size (0.582 ± 0.217) compared to 8325–4. PerR had also been shown to regulate expression of a broad spectrum of oxidative stress resistance genes in *S. aureus*, including catalase (*katA*), alkyl hydroperoxide reductase (*ahpCF*), and thioredoxin reductase (*trxB*). Besides sensing oxidative stress, *S. aureus* PerR is also responsible for iron balance within the cell by controlling the iron homeostasis regulator Fur and a few iron storage and uptake genes such as ferritin (*ftn*) and *mrgA* (33, 34).

B. subtilis PerR, the prototype of this widespread family of iron-containing oxidative stress regulators, possesses a unique oxidation-sensing mechanism: it contains a nonheme iron center that lacks reactive Cys residues. It has been demonstrated that the two His residues involved in chelating iron(II) in the metal-binding site can be oxidized to 2-oxo-His through an iron-catalyzed hydrogen peroxide reduction (44). This reaction releases iron and PerR loses its DNA-binding ability (Fig. 7). PerR seems to be not only sensitive to H_2O_2, but also responsive to its toxic potential, which correlates with the iron-catalyzed Fenton reaction. The PerR's activation mechanism provides great peroxide specificity given that only these compounds can react with the active-site iron to induce His oxidation in PerR. PerR is an autoregulator and its activation mechanism turns out to be sacrificial: in contrast to the rich chemistry in thiol's reversible oxidation and reduction, no oxo-His reduction mechanism is currently known. This is a unique feature that distinguishes PerR from cysteine-based regulators.

Redox Switches in *P. aeruginosa*

OspR, a redox-sensitive global regulator in P. aeruginosa

Besides *S. aureus*, *Pseudomonas aeruginosa* is another human pathogen responsible for a variety of infectious diseases, most

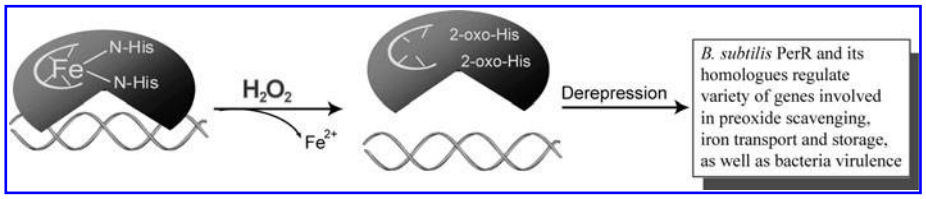

FIG. 7. Mechanism of PerR. *B. subtilis* PerR is an iron-containing oxidative stress regulator that is a functional analog of *E. coli* OxyR. It senses H_2O_2 by its two His residues that are converted to 2-oxo-His and lose their Fe(II)-binding ability upon oxidation. This His oxidation leads to the dissociation of PerR from its cognate DNA and turn on the derepression pathway.

notably in those afflicted with cystic fibrosis or individuals with compromised immune systems. As a gram-negative pathogen, *P. aeruginosa* must also overcome the oxidative stress response generated by phagocytic cells for successful infection. To counter ROS challenges, *P. aeruginosa* possesses a multifaceted regulatory system that responds to host immune response and other ROS-generation processes.

A BLAST search of MgrA's homologs in the whole genome of *P. aeruginosa* identifies two close homologs. Annotation indicated that one of them, PA2849, is very likely to be OhrR in *P. aeruginosa* since it seems to control antioxidation genes (42). A recent result confirmed this protein as a thiol-based redox switch sensitive to organic hydroperoxides (2). The other one, PA2825, is a global regulator that controls the expression of genes involved in oxidative stress response, quorum sensing, tyrosine metabolism, β-lactam resistance, and the dissemination of *P. aeruginosa* during infection. It is also an essential regulator involved in pigment production; therefore, it was named OspR (oxidative stress response and pigment production regulator). OspR is a homolog of the bacterial OhrR/MgrA family of oxidative-stress sensing and regulatory proteins (Fig. 5). It binds to the promoter of PA2826, which encodes a glutathione peroxidase homolog, and represses expression of PA2826 and itself (Fig. 8). OspR may recognize many additional sites in the *P. aeruginosa* genome and its regulatory function is redox sensitive because the addition of oxidants dissociates OspR from its cognate DNA. OspR uses a cysteine residue, Cys24, to sense potential oxidative stress. Different from MgrA and SarZ, a second Cys, Cys134′ from the other monomer, attacks the oxidized sulfenic acid form of Cys24 to form an intermonomer disulfide, which presumably leads to conformational change of OspR upon oxidation. This is similar to the oxidation-sensing mechanism employed by the 2-Cys subfamily of OhrR proteins such as *Xanthomonas campestris* OhrR (Xc-OhrR), which utilizes a reactive cysteine residue (Cys22) that upon oxidation by organic hydroperoxides forms an intersubunit disulfide bond with residue Cys127′ from the other monomer (51, 53). OspR responds to millimolar levels of peroxides. Besides Cys-oxidation, other signaling pathways through OspR may also exist, for example, other posttranslational modifications such as phosphorylation of specific amino acid residues of OspR.

An interesting feature that makes OspR unique from other redox regulators such as MgrA and SarZ is that it controls bacterial pigment production. *Pseudomonas* species are well known to produce multiple-colored phenazine pigments. These molecules can undergo redox cycling to produce toxic superoxide and H_2O_2, and thereby affect bacterial virulence and redox balance. OspR affects expression of phenazine-modifying genes as well as the pyomelanin pigment production gene, both of which contribute to the observed pigment phenotypes. The other unique feature of OspR is its regulatory role on bacterial quorum-sensing, which had not been observed for other members of the OhrR/MgrA family of redox-active regulatory proteins. OspR regulates expression of *phzM* and *phzS*, two well-known quorum-sensing-regulated genes and PA1897, a gene controlled by QscR, which is a modulator of quorum-sensing signal synthesis and virulence. *P. aeruginosa* quorum sensing is known to control expression of catalase and superoxide dismutase genes. Additional links between oxidative response and quorum sensing through OspR exist in *P. aeruginosa*. Bacterial infections have long been thought to involve cooperative bacterial activities facilitated by quorum-sensing systems. In cystic fibrosis patients, bacteria routinely reach very high densities

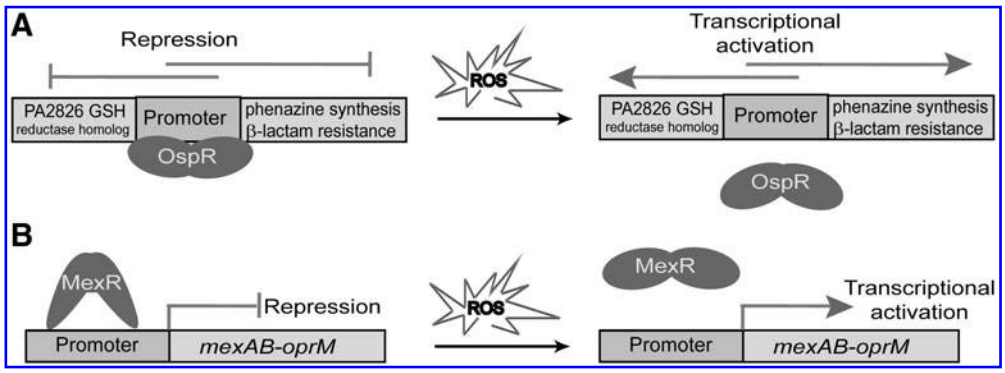

FIG. 8. Mechanisms of OspR and MexR. (**A**) OspR and (**B**) MexR are thiol-based redox-sensitive regulators in *P. aeruginosa*.

within respiratory secretions and their infections are thought to be coordinated by quorum sensing. An interesting future research direction is to reveal detailed cross-talk between oxidative stress sensing and quorum sensing for *P. aeruginosa* to cope with changes in the host environment. Lastly, another noticeable feature of OspR is its role in the virulence of *P. aeruginosa*. While activation of MgrA and SarZ in *S. aureus* leads to reduced bacterial virulence inside the host, which may suggest that the bacterium transfers to an inert state when challenged with oxidation, activation of OspR in *P. aeruginosa* seems to induce bacterial virulence in a lung infection model. Perhaps ROS is not the major signal for OspR, and is used to convey other signals yet to be identified. There might be other global redox active switches that perform similar functions as MgrA, or *P. aeruginosa* could respond very differently to ROS challenge as compared to *S. aureus*. Detailed analysis of OspR-based transcriptional regulation and exploration of other redox-sensitive regulatory pathways are required to answer these questions.

MexR, a multidrug efflux regulator uses an oxidation-sensing mechanism

P. aeruginosa has a complex genome compared to *S. aureus*. It possesses multiple drug efflux pumps that give the microbe intrinsic resistance to a variety of antimicrobial agents. A major tripartite pump system encoded by the *mexAB-oprM* operon has been extensively studied (56, 65, 74). This drug efflux system couples the inner and outer membranes for extrusion of a range of antibiotics, including β-lactams, tetracycline, chloramphenicol, quinolones, and novobiocin. The MexR protein is a negative regulator of *mexAB-oprM* and itself. It has been suggested that this regulation has a link to bacterial virulence in *P. aeruginosa*.

MexR is a member of the MarR-type transcriptional regulators. This family of regulators had been shown to be sensitive to various environmental stresses, whereas the real activation signal for MarR, the archetype of this family of proteins, remains poorly understood. The commonly accepted mechanism for the MarR family proteins involves binding of a small molecule drug to the dimer protein, which leads to the dissociation of the protein from DNA, but the low binding affinity measured between MarR and salicylate type molecules, as well as the high concentration of salicylate required for the MarR-salicylate complex crystal, cast doubts on the physiological link between the direct small molecule binding and MarR's transcriptional derepression (1, 14, 67, 70). The *mar* regulon also responds to chloraphenicol, tetracycline, fluoroquinolones, and various other agents in *E. coli*, similar to MexR, which seems able to sense the presence of a diverse range of different antibiotics in *P. aeruginosa*. It is hard to envision that a simple protein like MarR (142 aa) or MexR (147 aa) is able to recognize such a diverse range of structures under physiological conditions. Other antibiotic-resistance regulators such as QacR and BmrR have been conclusively shown to directly bind a particular group of antibiotics (28, 59). These proteins all possess well-defined, hydrophobic, small molecule-binding pockets that are missing in MexR and MarR. Then, how are small regulatory proteins such as MexR and MarR capable of recognizing very different structures? The work on MgrA and SarZ suggests that secondary signals exist to relay the information. Bactericidal antibiotics are known to generate oxidative stress (7, 8, 20, 40). Thus, ROS represents a secondary signal for the antibiotic stress, which can be sensed by regulatory proteins to cover the presence of a range of different antibiotics. Indeed, recent work showed that two Cys residues in MexR (Cys32 and Cys60) are redox active and form intermonomer disulfide bonds under mild oxidation conditions. The disulfide-linked MexR dissociates from promoter DNA, which activates the *mexAB-oprM* operon. To conclusively support this mechanism a structure of the oxidized MexR has also been obtained (He unpublished).

OxyR and SoxR in P. aeruginosa

OxyR and SoxR are two key oxidative stress regulators of bacteria (73). The regulation and posttranslational activation of these two proteins had been extensively studied in *E. coli*. OxyR activates expression of several antioxidant genes in response to elevated levels of H_2O_2 (13). The *E. coli oxyR* knockout strain is sensitive to H_2O_2 and has a higher incidence of spontaneous mutations. OxyR forms a tetramer in solution. Oxidation by peroxide has been shown to lead to the formation of a disulfide bond in OxyR, which triggers the activation of this transcription factor (72). The disulfide bond formation and reduction between Cys199 and Cys208 tune OxyR's activation and deactivation states (12). In *P. aeruginosa*, an array of essential oxidation defense genes, including *katB-ankB*, *ahpB*, and *ahpC-ahpF*, are regulated by OxyR (52). The *oxyR* mutant strain of *P. aeruginosa* was hypersusceptible to ROS reagents, including H_2O_2 and paraquat. A unique feature of OxyR in *P. aeruginosa* is its involvement in the regulation of DNA repair function, which previously had not been observed in *E. coli* OxyR. OxyR was found to be located upstream of *recG*, a putative DNA repair enzyme in *P. aeruginosa*. Results from recent investigations provided evidence that the *oxyR-recG* locus is critical for oxidative stress defense as well as DNA repair activity of *P. aeruginosa*. Animal model study also demonstrated that OxyR is required for full virulence of *P. aeruginosa* during infection and is a major factor for the resistance to human neutrophils (43).

SoxR is a redox-based transcriptional regulator that controls oxidative stress response in *E. coli*. Activation of SoxR led to the upregulation of a single gene, *soxS*, which then turns on a regulon containing many oxidative-stress response genes (55). In addition to protecting against oxidative damage, SoxR had been shown to control the resistance to antibiotics, organic solvents, and heavy metals. Upregulation of SoxR/S pathway is also crucial for resistance to nitric oxide-generating macrophages. SoxR belongs to the mercury resistance regulator family of transcription factors, the prototype of which is mercury resistance regulator found in an *E. coli* transposon. It forms a homodimer with the N-terminal containing a helix-turn-helix DNA-binding motif. A cysteine-rich domain exists at the C-terminal of SoxR, which was later found to form a redox-active iron-sulfur cluster [2Fe-2S] (29). The two [2Fe-2S] clusters that each dimer contains are essential for the transcriptional activity of SoxR *in vitro* and *in vivo*. The redox potential of the [2Fe-2S] clusters in *P. aeruginosa* SoxR is -290 mV, a value very similar to *E. coli* SoxR (-285 mV) (50). However, the regulatory activity of SoxR in *P. aeruginosa* is dramatically different from its role in *E. coli*. As SoxS was absent in the whole genome of *P. aeruginosa*, SoxR had been shown to act as a direct transcriptional activator of a

hypothetical protein PA2274. Although the function of this protein is currently unknown, it is not a SoxS homolog, nor is it likely to be a transcription factor. Past studies suggest that SoxR may play multiple regulatory roles in addition to its oxidative stress protection. It bears investigating whether an unknown SoxS-equivalent protein exists in *P. aeruginosa* or SoxR exerts its regulatory role by direct binding to the promoter regions of various genes other than PA2274 (39).

Redox Switches in *M. tuberculosis*

Mycobacterium tuberculosis, one of the world's most devastating human pathogens, kills ~1.8 million people annually. Current estimates indicate that one-third of the world's population is host to this pathogen and 10 million new infections occur every year (71). Current treatments are expensive and long, which reduces treatment efficacy. A better understanding of the bacterial physiology is instrumental to the discovery of new therapies and the eradication of the disease. In recent years interest toward understanding redox regulation in *Mycobacterium tuberculosis* and its role in virulence has developed. It bears elucidating whether the activation of the bacterium inside of the host (transition from a nonreplicating nonpathogenic state to a replicating and highly pathogenic state) is due to random fluctuations in the bacterial physiology or is a direct response to sensing a more favorable environment (*i.e.*, less oxidative environment) by the bacterium (21).

Mycobacterium tuberculosis is an obligate aerobe that can survive for many years inside of the host under hypoxic conditions in a nonreplicating state. *M. tuberculosis* resides inside macrophages and must cope with ROS and RNS produced by the host to combat the infection (5). To withstand ROS and RNS, *M. tuberculosis* has several antioxidant defenses: (a) a thick and semiimpermeable cell wall containing mycolic acids, glycolipids, and polyketides (60); (b) intracellular millimolar concentrations of small molecule thiols like mycothiol (18); (c) protective enzymes like KatG, AhpC, and Tpx (2); and (d) reduced coenzymes like $F_{420}H_2$ (58). Additionally, *M. tuberculosis* has several redox-sensitive regulators, which will be introduced in this section.

DosT, DosS, and DosR: dual two-component system involved in gas and redox sensing

DosT, DosS, and DosR, also known as DevT, DevS, and DevR, form an extensively studied dual two-component system (TCS) composed of two sensor kinases (DosT and DosS) and a transcriptional regulator (DosR). Both DosT and DosS are necessary for the induction of the *dosR* regulon (a set of 48 genes induced during latency). Full induction of these genes is necessary for survival during anaerobiosis (31). DosT and DosS are membrane-associated histidine kinases that contain a heme-bound GAF domain in the N-terminal-sensing domain where autophosphorylation occurs, and a histidine kinase ATPase domain. The heme ferrous iron can coordinate O_2, NO, and CO. In the O_2-bound and the ferric forms of the enzyme, the autophosphorylation is turned off. The enzyme is on in the unbound or NO- or CO-bound states. Initially, DosT and DosS were reported to be oxygen-sensing kinases (64). Later, based on *in vitro* results it was proposed that DosT functions as a hypoxia sensor and DosS as a redox sensor (64). DosS was reported to be readily oxidized *in vitro* and a crystal structure of the ferric form was published supporting the redox-sensing hypothesis (11, 41). Soon after, a study measured the half-life of the oxidation of iron(II) under different *in vitro* conditions similar to biological conditions and concluded that the iron(II) form of the enzyme is very stable under biologically relevant conditions and that DosS is a gas sensor-like DosT (37). DosT and DosS have different affinities to O_2, CO, and NO *in vitro*, which indicates that they may serve the bacterium to finely monitor the environment (DosT $K_d = 26$ mM to O_2, $K_d = 0.94$ mM to CO, and $K_d = 5$ nM to NO; DosS has affinities of $K_d = 3$ mM to O_2, $K_d = 36$ nM to CO, and $K_d = 20$ nM to NO) (64). To our knowledge, studies looking at the redox state of the iron *in vivo* or the phosphorylation status of the kinase *in vivo* in the presence of an oxidant environment have not yet been carried out. Therefore, the possibility exists that the bacterium uses DosS to sense gas and redox changes.

Once DosS and DosT are phosphorylated, they transfer a phosphate group to DosR, which activates the *dosR* regulon. DosR is a transcriptional regulator divided into two domains: an N-terminal receiver domain and a C-terminal DNA-binding domain (17). DosR binds to several DNA elements upstream of the *dosR*. DosR is thought to be required to enter, maintain, and exit latency; further, it was shown that aerobic transcription of *dosR* regulon is DosR-independent, but induction under hypoxic conditions was DosR dependent (6).

Different studies have observed different effects of *dosR/S/T* mutants in virulence. A study reported that mice infected with a *dosR* gene deletion mutant strain died faster than those infected with wild type (54). Subsequently, a *dosR* null mutant was found to be less virulent in guinea pigs (49). Still a third study reported that a *dosT-dosR* gene deletion mutant exhibited a growth defect in mice and guinea pigs, but showed no differences in rabbits (15). These differences are likely to due to different strains and different models used. Nonetheless, they indicate an important role for *dosR/S/T* in pathogenesis.

RshA and RslA, zinc-associated antisigma factors in M. tuberculosis, *are redox active*

Sigma factors regulate numerous cellular processes in prokaryotes that include stress response, transport, and cell growth. Antisigma factors bind to sigma factors and inhibit their transcriptional activity. RshA and RslA are zinc-associated antisigma factors of SigH and SigL, which are alternative sigma factors of *M. tuberculosis* that regulate the response to different stress conditions. Binding of RshA to SigH was demonstrated *in vitro* and *in vivo* using a two-hybrid system. The *in vitro* binding was shown to be disrupted under oxidation conditions or heat stress. *In vitro* transcription was disrupted by oxidative stress as well (62). RslA was identified on a bacterial two-hybrid assay and reported to interact with SigL (26). Subsequently, RslA was shown to interact with SigL *in vitro* (16). More recently, structural information was reported showing that zinc(II) is coordinated in a tetrahedral fashion by two His and two Cys residues. One His and two Cys residues are from a HXXXCXXC motif, which is conserved in all zinc-associated antisigma factors (68). The Cys residues are solvent accessible and it was proposed that these Cys residues are sensitive to oxidation. Accordingly, the zinc binding is disrupted upon incubation with 10 mM H_2O_2,

suggesting that Cys oxidation releases the zinc ion. A recent study indicated that the absence of zinc decreases the affinity of RslA to SigL (Fig. 9A) (68). Finally, an rslA-sigL mutant strain was attenuated in a mouse model of infection (16).

These regulators tend to be less reactive to ROS as compared to other ROS sensors mentioned above. For example, Hsp33 from *E. coli*, which also has a similar Cys-zinc motif, can only be activated by simultaneous heat and oxidative shock from H_2O_2, but not by either stress alone (35). The reaction rate of Hsp33 toward H_2O_2 oxidation at elevated temperature is 10^5–10^6 times slower than the reaction rates of OxyR and PerR toward oxidation.

WhiB3 integrates lipid metabolism and redox signaling

WhiB3 is an iron–sulfur cluster protein that functions as a redox active center to maintain redox homeostasis. WhiB3 was reported to respond to O_2 and NO through its iron–sulfur cluster and integrate environmental signals with core intermediary metabolism (61). While the redox state of the [4Fe-4S] cluster did not affect DNA binding, reduction of the Cys thiols of the apo form abolished DNA binding, whereas oxidation stimulated it (Fig. 9B). It was reported that WhiB3 directly controls the expression of polyketide biosynthetic genes involved in oxidative defense and virulence (60). The catabolism of highly reducing host fatty acids causes reductive stress, which in turn reduces thiols in WhiB3, causing the protein to dissociate from DNA, thus enabling the transcriptional activation of lipid anabolism genes. This reductive stress is likely to be found during latent infection in which O_2, CO, and NO concentrations are low and host fatty acids are present. Under these conditions WhiB3 is proposed to neutralize reducing equivalents and upregulate lipid anabolism. Growth of a whiB3 mutant strain of *M. bovis* was impaired in guinea pigs, but no difference was observed in *M. tuberculosis* (66).

Other redox sensors in M. tuberculosis

Lastly, OxyR, a model regulatory system for oxidative stress in bacteria, was found to be inactivated in *M. tuberculosis* with numerous frameshifts and deletions (19). To our knowledge, no SoxR homologs have been studied in *M. tuberculosis*, and an OhrR/MgrA homolog in the genome of *M. tuberculosis* has not been characterized.

Summary and Perspective

Human immune systems produce high concentrations of reactive oxygen and nitrogen species in response to bacterial infections. It is no surprise that pathogens such as *S. aureus*, *P. aeruginosa*, and *M. tuberculosis* possess extensive oxidation-sensing capacity to cope with the host immune response and regulate global changes of their properties. These changes not only include activation of defensive systems to reduce the ROS threat, but may also involve a major shift of life forms of these pathogens such as dramatically reducing or modulating the virulence and switching on and off key metabolic pathways. Thus, these bacteria could adopt either a latent form or a virulent form inside a human host. For instance, one could envision that in healthy individuals with strong immune systems a pathogen residing in or near host immune cells may stay in an inert form that completely turns off its virulence. When the host immune system is compromised, the bacteria become highly virulent and cause diseases. Redox active switches play key roles during the transformation, as demonstrated for MgrA in *S. aureus* (8, 9). The redox sensing also plays important roles in antibiotic resistance regulation. Recent studies have shown that ROS are generated in most cases when bacteria are under antibiotic stress (1, 70). Instead of detecting the presence of various antibiotics, the structures of which are quite different, bacteria can sense ROS as a simple, secondary signal (7).

Future research should (a) discover and characterize new, global redox regulators in human pathogens that have to cope with human immune response; (b) understand the molecular level signal(s) and signaling mechanism; and (c) develop small molecules that may modulate the regulatory functions of these key switches. Indeed, we face many challenges. New tools and methods need to be developed and applied to discover redox-sensitive proteins that are not homologous to existing ones. For instance, chemical probes may be synthesized to profile all reactive Cys residues in the proteome, and

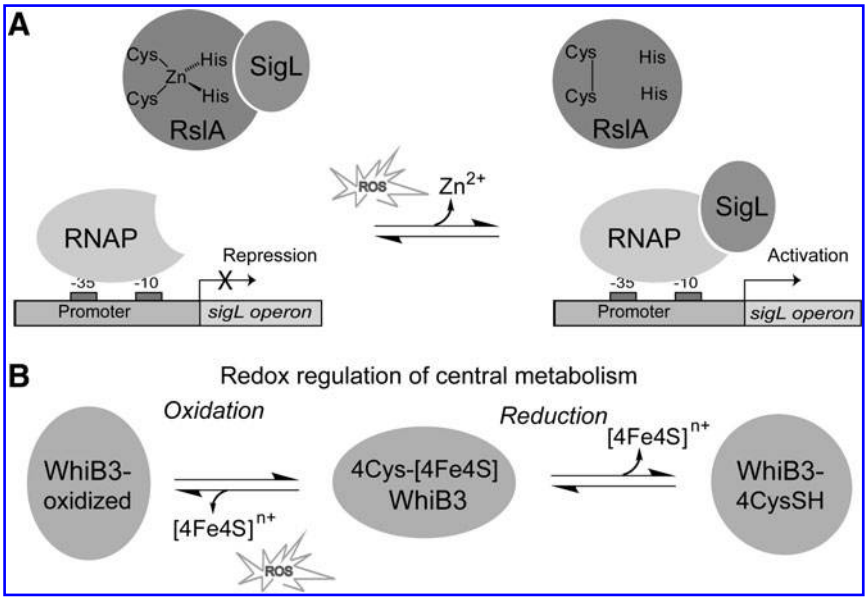

FIG. 9. Redox sensitive metalloswitches in *M. tuberculosis*. (**A**) RslA regulation of the *sigL* operon. RslA binds to SigL under reducing conditions. Upon oxidation the two Cys residues in RslA form a disulfide bond. The oxidized RslA releases SigL, which binds to RNA polymerase to activate transcription. (**B**) WhiB3 works as a redox-active regulator to maintain redox homeostasis in *M. tuberculosis*. The reduced form of apo-WhiB3 does not bind DNA, which derepresses transcription of fatty acid metabolism genes. The oxidized apo-WhiB3 binds strongly to DNA to repress transcription. The holo forms of WhiB3 bind to DNA weakly.

redox-active proteins can be identified and characterized. Redox-sensitive regulatory proteins could utilize metallocenters. Genetic tools in combination with expertise to study metalloproteins are required to identify and characterize these proteins. In addition, the TCSs are major signaling components to mediate communications between bacteria and bacteria, bacteria and host, and bacteria and environment. Molecular-level signals for most of the TCSs are currently unknown. Unsurprisingly, many of these signals are involved in or affected by redox sensing and regulation, which requires further elucidation. Lastly, redox sensing and regulation can very well be integrated into networks of regulations that involve all types of signaling, which collectively impact bacterial physiology. A system biology-level approach that globally matches transcriptional regulator with promoter activity can reveal a clearer picture.

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Abbreviations Used

agr = accessory gene regulator
MarR = multiple antibiotic resistance regulator
MexR = multidrug efflux regulator
MgrA = multiple global regulator A
OhrR = organic hydroperoxide sensing regulator
OspR = Oxidative stress response and pigment production regulator
OxyR = oxygen sensing regulator
PerR = peroxide response regulator
RNS = reactive nitrogen species
ROS = reactive oxygen species
SarZ = *Staphylococcus* accessory regulator Z
SoxR = superoxide response regulator
TCS = two-component system

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