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FORUM REVIEW ARTICLE

Redox Active Thiol Sensors of Oxidative and Nitrosative Stress

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Abstract

Significance: The reactivity of the thiol in the side chain of cysteines is exploited by bacterial regulatory proteins that sense and respond to reactive oxygen and nitrogen species. ***Recent Advances:*** Charged residues and helix dipoles diminish the pKa of redox active cysteines, resulting in a thiolate that is stabilized by neighboring polar amino acids. The reaction of peroxides with thiolates generates a sulfenic acid (–SOH) intermediate that often gives rise to a reversible disulfide bond. Peroxide-induced intramolecular and intermolecular disulfides and intermolecular mixed disulfides modulate the signaling activity of members of the LysR/OxyR, MarR/OhrR, and RsrA family of transcriptional regulators. Thiol-dependent regulators also help bacteria resist the nitrosative and nitroxidative stress. −SOHs, mixed disulfides, and S-nitrosothiols are some of the post-translational modifications induced by nitrogen oxides in the thiol groups of OxyR and SsrB bacterial regulatory proteins. Sulfenylation, disulfide bond formation, S-thiolation, and S-nitrosylation are reversible modifications amenable to feedback regulation by antioxidant and antinitrosative repair systems. The structural and functional changes engaged in the thiol-dependent sensing of reactive species have been adopted by several regulators to foster bacterial virulence during exposure to products of NADPH phagocyte oxidase and inducible nitric oxide synthase. ***Critical Issues:*** Investigations with LysR/OxyR, MarR/OhrR, and RsrA family members have helped in an understanding of the mechanisms by which thiols in regulatory proteins react with reactive species, thereby activating antioxidant and antinitrosative gene expression. ***Future Directions:*** To define the determinants that provide selectivity of redox active thiolates for some reactive species but not others is an important challenge for future investigations. *Antioxid. Redox Signal.* 17, 1201–1214.

Introduction

THE SULFUR ATOM in cysteine is strategically positioned in some regulatory proteins to function as a sensor of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The oxidation states of cysteine vary from −2 to +6, making cysteine the most versatile redox sensor of all amino acids, and organic and inorganic cofactors (46). Thiols and thiolates, and the oxidized sulfenic, sulfinic, or disulfide derivatives often occur in biomolecules. The cysteine oxidation states are kept in equilibrium through enzymatically catalyzed oxidoreduction reactions. Signaling cascades in living organisms take advantage of both the reversibility of cysteine oxidation states and the structural changes associated with oxidized cysteines to coordinate adaptive responses to reactive species engendered by endogenous metabolic processes or exogenous enzymatic or chemical means.

By mediating electron donation, disulfide formation, hydrolysis, metal binding, and redox catalysis and sensing, the sulfur in cysteines can fulfill a wide variety of molecular functions (33, 46). Bacterial redox sensors of various reactive species utilize cysteines because of their ability to bind metals and adopt various oxidation states. Most of the regulators presented in this review rely on the redox-sensing activity of the free thiolate side chain of cysteines. This review also discusses the mechanisms by which redox active cysteines in zinc fingers help bacteria sense ROS. By analogy to other metalloproteins, the cysteines in zinc fingers provide a scaffold where a structural metal cation is assembled. In addition to serving a structural function, redox active cysteines in zinc fingers transmit signals in response to ROS. Free cysteine side chains and cysteines bound in zinc fingers are common strategies of thiol-dependent bacterial redox sensors of ROS and RNS. To learn how cysteines indirectly sense oxidative and nitrosative stress through ligation of redox active metal prosthetic groups, the reader is directed to the accompanying reviews in this forum.

The contribution of thiol-dependent signaling to the resistance of bacteria to oxidative stress was first hinted at in investigations with the frank pathogen *Salmonella enterica*

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serovar Typhimurium (20). Many investigations on thiol switches have been carried out in commensal *Escherichia coli* and saprophytic environmental bacteria of the species *Bacillus subtilis* and *Streptomyces coelicolor* [see (1, 77) for recent reviews]. A growing number of Gram-positive and Gram-negative pathogens are now known to apply the signaling properties of thiol switches in order to increase fitness during their associations with vertebrate hosts. The mechanisms by which thiol-dependent redox sensors respond to ROS and RNS can be broadly categorized within families of transcriptional regulators, irrespective of whether they are expressed by commensal, saprophytic, or pathogenic bacteria. Consequently, the mode of action by which active thiols sense reactive species is presented herein within the context of families of transcriptional regulators, and their contributions to bacterial pathogenesis are highlighted. A section in this review also discusses the structural and chemical determinants that impact the sensing activity of cysteines. The last section presents the dominant chemistry driving the interaction of ROS and RNS with redox active cysteines.

Sensing of Oxidative and Nitrosative Stress by Members of the LysR/OxyR Family of Transcriptional Regulators

The first indication that bacteria adapt to oxidative stress was the observation that the exposure of *Salmonella* to sublethal concentrations of hydrogen peroxide (H_2O_2) elicits hyperresistance to a subsequent challenge with high amounts of this ROS (20). OxyR was identified as a member of the LysR family of transcriptional activators after mapping of an interesting transposon mutant named *oxyR2* (21). The critical role that the *oxyR* locus plays in the adaptation of *E. coli* to oxidative stress was demonstrated by the fact that OxyR-deficient bacteria are readily killed by H_2O_2 (20, 21). In addition to regulating antioxidant resistance, OxyR helps coordinate the expression of antinitrosative defenses (38). OxyR is less responsive to nitrogen oxides than to H_2O_2, especially if the bacterial cytoplasm is rich in glutathione (GSH) (38, 111). Nonetheless, *E. coli oxyR* mutants are hypersusceptible to the bacteriostatic effects of nitric oxide (NO) (38). Why is it that a highly specialized H_2O_2 sensor such as OxyR can also be modulated by RNS? Nitroxidative stress may explain this conundrum. Most of the biological chemistry of NO is dominated by oxidative processes (61). Taking into consideration this notion, the OxyR-regulated GSH oxidoreductase, glutaredoxin (Grx), thioredoxin (Trx), catalase, and alkyl hydroperoxide reductase re-establish thiol homeostasis and detoxify a variety of peroxides (9, 45, 71), independently of whether the bacterial cells are exposed to oxidative or nitroxidative species.

OxyR is a redox active regulator that maintains thiol homeostasis

The exposure of *Salmonella* to sublethal concentrations of H_2O_2 induces the expression of about 30 proteins (20). Hydroperoxidase I, alkyl hydroperoxide reductase, the nonspecific DNA-binding protein Dps, Grx1, GSH oxidoreductase, Trx 2, and the DsbG disulfide isomerase are some of the gene products positively regulated by oxidized OxyR (21, 44, 111). The first two enzymes detoxify H_2O_2, organic hydroperoxides, and peroxynitrite ($ONOO^-$), while the iron-binding Dps protein protects DNA from Fe^{++}-catalyzed, H_2O_2-dependent Fenton chemistry (9, 37, 83). GSH oxidoreductase, Grx1, DsbG disulfide isomerase, and Trx 2 re-establish thiol/disulfide homeostasis and protect the cell against disulfide stress. Thiol/disulfide exchange systems exert feedback regulation over oxidized OxyR, as suggested by the fact that OxyR is more easily activated by ROS and RNS in *E. coli* strains deficient in GSH oxidoreductase or Grx1 (38, 111). Moreover, the intramolecular disulfide bond of OxyR is a substrate of thiol/disulfide exchange by Grx1 (111).

Mechanisms of sensing oxidative and nitrosative stress by OxyR redox active thiols

OxyR activates gene transcription after the intramolecular oxidation of strategically situated cysteines (Fig. 1). It was noted that oxidized OxyR drives *katG* transcription *in vitro* (90). Although both oxidized and reduced OxyR bind target genes, they do it with different conformations and outcomes. Oxidized, tetrameric OxyR cooperatively binds four adjacent major DNA grooves, whereas reduced OxyR binds two pairs of adjacent DNA grooves separated by a single helical turn (96). Oxidized OxyR binds the RNA polymerase and drives transcription; the reduced protein does neither.

Oxidation of the thiolate side chain of OxyR Cys^{199} to a $-SOH$ precedes the dramatic structural changes responsible for the activation of OxyR (111). The attack on Cys^{208} by the Cys^{199} sulfenic derivative generates an intramolecular disulfide that drives massive conformational changes in the protein (18, 63, 93, 111). Unexpectedly, mutational analysis indicates that Cys^{199} is more important than Cys^{208} for the activation of OxyR (111). The fact that a C208S substitution renders a partially constitutive active OxyR variant rationalizes the seemingly greater importance of Cys^{199} over Cys^{208} (63). Having a redox potential of -185 mV (111), Cys^{199} is maintained in the reduced form in the -280 mV potential of the cytoplasm of resting *E. coli*. However, these thermodynamic considerations cannot explain the rapid oxidation of OxyR in response to small rises in the intracytoplasmic concentration of H_2O_2. OxyR is oxidized within 30 s after *E. coli* is treated with 5 μM H_2O_2, a concentration of peroxide that does not appreciably change the ratio of the oxidized glutathione/GSH redox couple (2, 93). OxyR and H_2O_2 react with a second-order rate constant of about $10^5\ M^{-1}\ s^{-1}$ (2). Therefore, kinetics rather than thermodynamics drives the fast oxidation of OxyR by H_2O_2. On the other hand, re-reduction of OxyR is a slow process that takes about 5–10 min (2, 93). The delay in the re-reduction of OxyR allows for the transient expression of antioxidant defenses encoded in the regulon.

In addition to undergoing oxidation in response to H_2O_2, OxyR gets S-nitrosylated in *E. coli* exposed to RNS (38, 55). Similar to the oxidized protein (90), S-nitrosylated OxyR drives *katG* transcription *in vitro* (38). In addition to S-nitrosylating OxyR, nitrogen oxides modify the Cys^{199} thiol side chain to sulfenylated and S-glutathionylated derivatives (55). All these covalent modifications affect the secondary α-helical structure of OxyR. These RNS-modified OxyR products are transcriptionally active; however, they differ in cooperativity, structure, DNA-binding affinity, and promoter activity. One of the most salient conclusions that can be drawn from these investigations lies in the realization that a redox active regulator does not have to be a simple switch that alternates between "ON" and "OFF" positions, but one whose

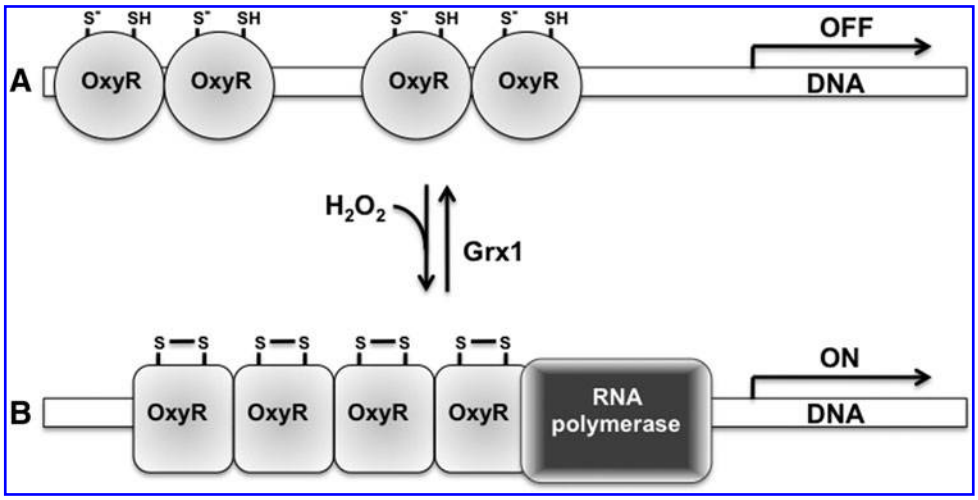

FIG. 1. Hydrogen peroxide (H_2O_2) sensing by OxyR. Reduced OxyR binds to DNA promoter regions of target genes, thereby preventing transcription **(A)**. Oxidation of the OxyR Cys^{199} thiolate group ($–S^-$) by H_2O_2 generates a sulfenic acid (–SOH) derivative that attacks the Cys^{208} thiol side chain. The resulting intramolecular disulfide bond drives the structural changes in OxyR that allow for the recruitment of RNA polymerase and the activation of transcription **(B)**. The Cys^{199}-Cys^{208} disulfide bond is reduced by glutaredoxin 1 (Grx1), a member of the OxyR regulon.

activity can be fine tuned according to the nature of its post-translational modifications.

Contribution of LysR/OxyR regulators to bacterial pathogenesis

OxyR is dispensable for the pathogenesis of *Mycobacterium tuberculosis* and *Sal. enterica*. The *M. tuberculosis oxyR* gene has several inactivating nonsense and frameshift mutations, and *oxyR*-deficient *Salmonella* are completely virulent (25, 95). However, members of the OxyR regulon such as Dps are required for *Salmonella* virulence (37). *dps* and other members of the regulon are controlled by several regulators, which may explain the dispensability of OxyR in *Mycobacterium* and *Salmonella*. For example, the expression of *dps* in *Salmonella* is under the control of the ferric uptake regulator Fur (109), a gene product that is not only under OxyR regulation, but also essential for *Salmonella* pathogenesis (98, 104). One of the advantages of putting *dps* under the control of Fur is that its expression becomes responsive to iron levels as well as ROS and RNS (23, 94). Curiously, the OxyR-regulated KatG catalase capable of detoxifying high fluxes of H_2O_2 is dispensable for the survival of *Salmonella* in macrophages sustaining a respiratory burst (10). Several other catalases in the *Salmonella* genome may compensate for a lack of KatG (4).

In contrast to *Salmonella* and *M. tuberculosis*, OxyR augments the virulence potential of pathogenic microorganisms such as *E. coli* and *Pseudomonas aeruginosa* (47, 62). *E. coli* is an extraordinarily diverse species, encompassing common inhabitants of human and animal intestines as well as enterotoxigenic, enterohemorrhagic, and uropathogenic strains associated with high morbidity and mortality in humans and a variety of domestic animals. OxyR potentiates the ability of *E. coli* to cause ascending urinary tract infections (47). Curiously, OxyR-dependent antioxidant defenses do not antagonize the ROS produced by the NADPH phagocyte oxidase (47). Instead, OxyR may foster *E. coli* virulence by enhancing resistance to the reactive species generated during endogenous metabolic processes. According to this idea, alkyl hydroperoxide reductase, a member of the OxyR regulon, is quite effective at detoxifying low micromolar concentrations of H_2O_2, but it is inefficient against the large fluxes of H_2O_2 usually associated with inflammation (87). OxyR also augments the virulence of *P. aeruginosa* in rodent and insect models of infection (62). Future studies may reveal that other members of the growing LysR/OxyR family of transcriptional regulators do, in fact, foster colonization, invasion, and growth of many pathogenic microorganisms during their associations with vertebrate hosts. Recent evidence indicates that the redox-sensing activity of Cys^{235} in AphB, a member of the LysR/OxyR family of transcriptional regulators, is necessary for toxigenic *Vibrio cholerae* to colonize the anaerobic environment of the mammalian intestine (67).

Sensing of Oxidative Stress by Members of the MarA/OhrR Family of Transcriptional Regulators

The DNA binding domain in the MarA/OhrR family of transcriptional regulators has a characteristic winged helix-turn-helix. A growing number of regulators in the MarA/OhrR family have been noted as containing one or two redox-active cysteines. OhrR homologs have been described in *Staphylococcus aureus*, *Str. coelicolor* A3(2), *Xanthomonas campestris*, and *P. aeruginosa* (3, 15, 72, 75, 92). A great deal of what we know about OhrR homologs has been unmasked while studying OhrR from the soil saprophyte *B. subtilis*. Reduced OhrR binds to the operator regions of target genes *via* a winged helix-turn-helix (Fig. 2), and, thus, represses the transcription of antioxidant defenses (31). The exposure of *B. subtilis* to organic hydroperoxides increases antioxidant defenses through the oxidation of OhrR (30).

Mechanisms of sensing oxidative stress by MarA/OhrR thiol switches

In Gram-positive bacteria, members of the MarA/OhrR family of transcriptional regulators sense oxidative stress

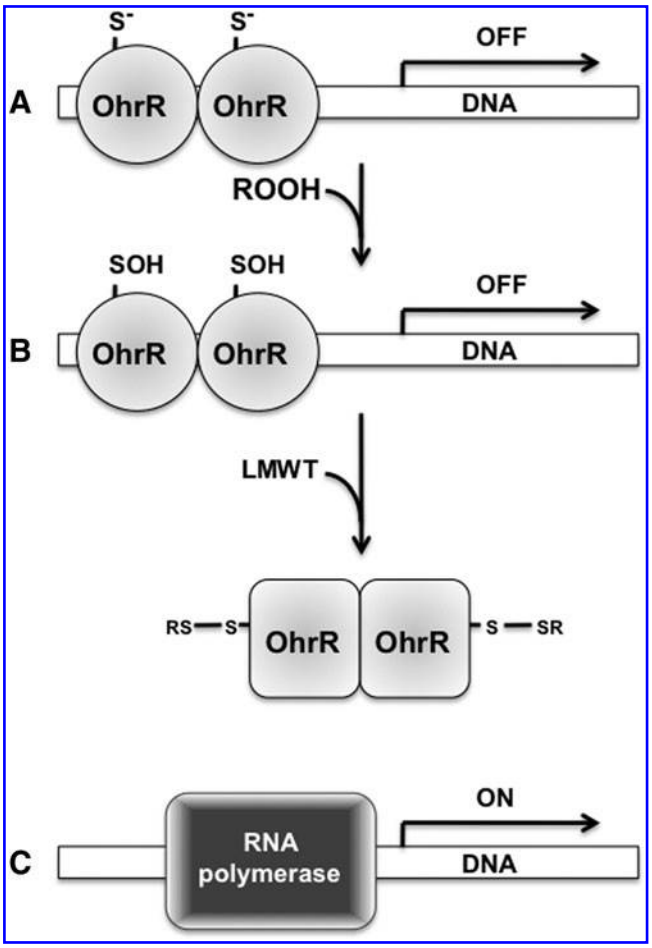

FIG. 2. Regulation of gene transcription by the peroxide sensor OhrR. The dimeric, reduced OhrR protein represses the transcription of target genes in *Bacillus subtilis* **(A)**. Organic hydroperoxides oxidize the OhrR Cys^{15} $-S^-$ to yield a remarkably stable $-SOH$ **(B)**. Sulfenylated OhrR binds to the operator regions of target genes; however, the $-SOH$ is S-thiolated by low-molecular-weight thiols (LMWTs), driving the conformational changes associated with the dissociation of OhrR from DNA **(C)**. The dissociation of oxidized OhrR allows the recruitment of RNA polymerase to the promoters of several antioxidant genes, including thioredoxins (Trxs) and Trx reductase involved in thiol/disulfide exchange reactions. *Staphylococcus aureus* MgrA and SarZ regulators are homologs of *B. subtilis* OhrR. Gram-negative *Xanthomonas campestris* and *Pseudomonas aeruginosa* also express OhrR-like repressors; however, these regulators use two redox-active cysteines to sense oxidative stress (not shown).

through a lone cysteine residue, whereas their counterparts in Gram-negative bacteria involve two cysteines. A lone cysteine in *B. subtilis* OhrR, *Sta. aureus* SarZ, and *Sta. aureus* MgrA is oxidized by organic hydroperoxides to a remarkably stable $-SOH$ (15, 31, 84). The formation of a $-SOH$ is necessary but not sufficient to derepress transcription. In fact, reduced and sulfenylated *B. subtilis* OhrR and *Sta. aureus* SarZ bind with apparently similar affinities to the operator regions of target genes (64, 84). In *B. subtilis*, sulfenamide generated from the reaction of sulfenylated Cys^{15} with the amide group of the adjacent Phe^{16} may trigger the conformational changes required for the dissociation of OhrR from the operator regions (64). However, the OhrR sulfenamide derivative is far less abundant than an S-thiolated, mixed disulfide-bonded derivative (64). The $-SOH$ in oxidized OhrR is prone to nucleophilic substitution with endogenous, low-molecular-weight thiols (LMWTs) (64, 84). Both mixed disulfides and sulfenamide are reversible modifications and, hence, the DNA binding activity of OhrR-like proteins can be restored on rereduction. Together, these data unequivocally identify the redox-active thiol in members of the OhrR family of transcriptional regulators as a genuine signaling switch.

As just mentioned, the OhrR homologs in Gram-negative bacteria have two redox-active cysteines (13, 14, 80). In *X. campestris*, OhrR Cys^{22} is oxidized by organic hydroperoxides to $-SOH$, which rapidly reacts with Cys^{127} to form an intermolecular disulfide in the homodimer (80). As expected, disulfide-linked dimer formation drives the dissociation of *X. campestris* OhrR from DNA. *P. aeruginosa* MexR also has two redox-active cysteines (13). Organic hydroperoxides trigger the oxidation of MexR Cys^{30} and Cys^{62} to form two intermolecular disulfides with $Cys^{62'}$ and $Cys^{30'}$ of the homodimer (14). Disulfide formation is the driving force behind the depression of the *mexAB-oprN* drug efflux operon, and it increases the resistance of *P. aeruginosa* to antibiotics (14).

Contribution of the redox-sensing activity of the MarA/OhrR family members to bacterial pathogenesis

The contribution of the MarR/OhrR members to bacterial pathogenesis has been investigated in *Sta. aureus* and *P. aeruginosa*. A *Sta. aureus sarZ* mutant is attenuated in both silkworms and a 24-h mouse model of infection, but regains full virulence at later times of the infection (50). The transitory contribution of SarZ to *Sta. aureus* virulence probably reflects the complex SarZ regulatory network. SarZ regulates the expression of 87 genes in *Sta. aureus* (6, 16), including the repression of *sarS* and the induction of genes encoding exoproteins and cell wall biosynthesis. Decreased expression of these genes could contribute to the initial attenuation of *sarZ*-deficient *Sta. aureus*. However, by derepressing antioxidant defenses, the lack of *sarZ* increases the resistance of *Sta. aureus* to organic hydroperoxides and H_2O_2 (16). Moreover, an *sarZ* mutant forms denser biofilms, another aspect that may also foster *Sta. aureus* pathogenesis (92). In comparison to SarZ, MgrA plays a much greater and more durable role in *Sta. aureus* virulence, as measured by bacterial growth in viscera and the degree of sinovitis and arthritis affecting infected animals (48). The predominant role of MgrA over SarZ in the pathogenesis of *Sta. aureus* is not surprising, as MgrA regulates *sarZ* as well as more than 300 other genes (48, 69) such as those encoding α-hemolysin, α-toxin, capsular polysaccharide biosynthesis, coagulase, efflux pumps, leukotoxins, serine proteases, and sortase A.

In analogy to *Sta. aureus*, the opportunistic pathogen *P. aeruginosa* expresses two members of the MarR/OhrR family of transcriptional regulators, OhrR and OspR. A *P. aeruginosa* strain lacking *ospR* is hypervirulent in an acute pneumonia model of infection, because the derepression of GSH peroxidase enhances resistance to H_2O_2 (60). However, *P. aeruginosa* OhrR mutants are hyperresistant to organic hydroperoxides, but attenuated in a *Caenorhabditis elegans* model of infection (3). These findings indicate that the resistance to

oxidative stress does not always equate to enhanced virulence. The overproduction of antioxidant defenses may, in turn, elicit reductive stress that leads to detrimental changes in gene expression.

Sensing of Oxidative Stress by Redox Active Thiols That Coordinate Zinc

Most of the characterization of RsrA and the σ^R regulon has been done in the soil saprophyte *Str. coelicolor* A3(2). The σ^R regulon coordinates the response of *Streptomyces* to disulfide and oxidative stress associated with diamide, H_2O_2, and the superoxide ($O_2^{\bullet -}$) generator plumbagin (78), suggesting that members in this regulatory pathway are prone to redox control. The σ^R protein lacks cysteines. In contrast, its cognate antisigma factor RsrA has several cysteines, raising the possibility that redox active thiols in RsrA could mediate the response of the σ^R regulon to oxidative challenges (Fig. 3).

Regulation of antioxidant defenses by members of the σ^R family of extracytoplasmic function sigma factors

A functional σ^R is necessary for the proper expression of disulfide reductase activity in *Str. coelicolor* (78). Further analysis revealed that σ^R in fact regulates transcription of the *trxAB* operon encoding Trx 1 and Trx reductase, and the *trxC*-encoded Trx 2 (78, 79). A search of the *Str. coelicolor* genome for conserved σ^R binding sequences identified a total of 27 σ^R targets (79). These include the aforementioned *trxC*, and genes in the cysteine and molybdepterin biosynthetic pathways. σ^R also regulates the expression of *msrA* and *msrB* methionine sulphoxide reductase genes, the *mshA*-encoded mycothiol biosynthesis gene, and the *mca*-endoced amidase (81). The expression of these genes is consistent with the fact that the oxidation of RsrA derepresses σ^R-dependent antioxidant defenses. Oxidation of the cell also induces the accumulation of unfolded proteins in cytoplasmic aggregates (73). It is, therefore, not surprising that σ^R directly regulates Clp and Lon ATP-dependent AAA(+) proteases in order to eliminate damaged proteins (51). *M. tuberculosis* expresses σ^H, a σ^R homolog. Detailed transcriptional analysis indicates that σ^H regulates 31 genes in *M. tuberculosis*, including genes involved in Trx recycling, a Grx-like protein, ferredoxin, chaperones, and universal stress proteins (53, 70, 85). Therefore, it is becoming clear that actinomycetes use σ^R-like extracytoplasmic function sigma factors which regulate the transcription of classical antioxidant defenses and stress responses to unfolded proteins.

Mechanisms of sensing oxidative stress by the RsrA antisigma factor

The metalloprotein RsrA binds a single zinc cation with an affinity of $10^{17}\ M^{-1}$ (5, 76). The zinc metal is ligated by His^{37}, Cys^{41}, and Cys^{44} that form a characteristic HX_3CX_2C zinc-containing antisigma (ZAS) motif (49, 76, 110). Cys^{11} completes the tetrahedral coordination of zinc by RsrA (110). Genetic studies indicate that Cys^{11}, Cys^{41}, and Cys^{44} are essential for the antisigma activity of RsrA (76). Cys^{11} is the triggering residue that responds to oxidative stress, most likely by forming a $-SOH$ intermediate that attacks the neighboring thiol Cys^{44} (5, 66, 76). The importance of Cys^{11} as the triggering residue responsive to oxidative stress is circumstantially supported by the fact that members of the ZAS family of transcriptional regulators that lack a cysteine at this position are indifferent to oxidative stress (66). In addition to Cys^{11}-Cys^{44}, a second disulfide forms between Cys^{41} and Cys^{61} (5). Disulfide formation releases zinc from RsrA (66). Nonetheless, disulfide bond formation, rather than the dissociation of zinc from the protein, increases the α-helical structure of RsrA that releases σ^R and allows it to transcriptionally activate target genes (66).

Similar to its RsrA homolog in *Str. coelicolor*, the *M. tuberculosis* RshA antisigma factor is responsive to oxidative stress (89). The binding of RshA to σ^H can also be modulated in response to metabolic changes in the cell. Thr^{94} is phosphorylated by the eukaryotic-like serine/threonine protein kinase PknB (36, 82). By analogy to the oxidized protein, phosphorylated RshA binds less efficiently to σ^H (82). The oxidation of thiols in the RshA zinc finger and phosphorylation of RshA Thr^{94} may facilitate the graded regulation of *M. tuberculosis* gene transcription in response to shifting redox and metabolic challenges.

RsrA is redox active

The oxidative modifications that mediate redox signaling are often reversible. Strains of *Str. coelicolor* deficient in mycothiol biosynthesis exhibit increased expression of σ^R,

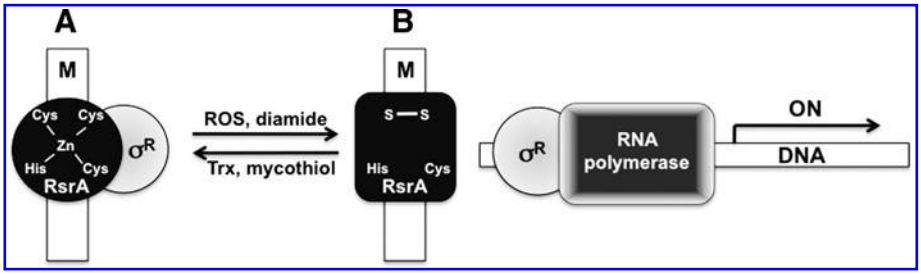

FIG. 3. Regulation of antioxidant defenses by thiols in zinc-finger-containing antisigma factors. Cys^{11}, Cys^{41}, Cys^{44}, and His^{37} coordinate a zinc finger in the antisigma factor RsrA of the soil saprophyte *Streptomyces coelicolor* **(A)**. Zinc-bound RsrA sequesters its cognate σ^R sigma factor to the vicinity of the bacterial membrane (M). Disulfide stress and several reactive oxygen species (ROS) oxidize the triggering Cys^{11}, which forms a disulfide bond with Cys^{44}. The disulfide bond releases the zinc cation, and promotes structural changes that drive dissociation of the RsrA-σ^R complex **(B)**. The liberated σ^R recruits the RNA polymerase to the promoters of genes encoding antioxidant defenses. Similar to OhrR, Trx-mediated thiol/disulfide exchange exerts feedback regulation over the system. *Mycobacterium tuberculosis* RshA is a homolog of the *Str. coelicolor* RsrA protein.

suggesting that decreases in intracellular mycothiol content could be the natural signal that drives the oxidation of RsrA and the consequent activation of the σ^R regulon (81). Both Trx and mycothiol limit RsrA disulfide bond formation, thereby stimulating the binding of this antisigma factor to σ^R (52, 81). On the other hand, σ^R activates the transcription of Trxs and the mycothiol biosynthetic pathway. These findings demonstrate that RsrA is a *bona fide* redox switch subjected to feedback regulation by members of the regulon.

Role of redox-active antisigma factors in bacterial pathogenesis

σ^H defends the human pathogen *M. tuberculosis* against both RNS generated from acidified nitrite, and ROS such as organic hydroperoxides, H_2O_2, and $O_2^{\bullet -}$ (24, 28, 70, 85). σ^H is expressed by *M. tuberculosis* inside macrophages (34). However, this extracytoplasmic function sigma factor does not increase the intracellular fitness of mycobacteria (70), and *M. tuberculosis* σ^H mutants grow and persist with apparent normality in murine models of infection (53). Nonetheless, σ^H contributes to *M. tuberculosis* pathogenesis, and its expression affords *M. tuberculosis* the ability to recruit $CD4^+$ and $CD8^+$ T lymphocytes to well-formed granulomas in the lungs of infected mice (53). It remains to be investigated whether the immune response triggered by *M. tuberculosis* is related to the expression of σ^H-dependent antioxidant defenses.

Sensing of Nitrosative Stress by Members of the NarL/SsrB Family of Transcriptional Regulators

The response regulator SsrB is the only member of the NarL/FixL family of transcriptional regulators that has so far been shown as possessing a redox-active cysteine. In contrast to the thiol switches just described, SsrB is a regulator of genes dedicated to bacterial pathogenesis. SsrB and its cognate SsrA sensor kinase form a two-component regulatory system essential for the intracellular replication of *Salmonella* (22). By analogy to other two-component regulatory systems, the SsrA sensor kinase phosphorylates Asp^{56} in the receiver domain of SsrB, unfolding the DNA binding domain for activation of gene transcription. SsrB binds to a flexible AT-rich palindrome sequence in ancestral and horizontally acquired genes (11). The phosphorylated SsrB protein relieves the gene silencing imposed by polymerized H-NS nucleoid protein on target DNA (105). SsrB partially binds to the predicted σ^{70} −35 region and activates cyclic AMP receptor protein-type class II promoters independently of the αCTD subunit of the RNA polymerase (105).

More than 100 loci are regulated by SsrB, including the 25 kb *Salmonella* pathogenicity island 2 (SPI2) that encodes a secretion apparatus, chaperones, translocon, the SsrA/SsrB two-component regulatory system, and effector proteins (39, 97, 108). SPI2 effectors translocated into the host cell cytoplasm help remodel *Salmonella* phagosomes and *Salmonella*-containing vacuoles. SsrB increases antioxidant and antinitrosative defenses (7, 12, 32, 91, 100, 101, 103); however, it does it in dramatically different ways from other regulators discussed in this review (Fig. 4). OxyR, OhrR, and RshA regulate the expression of classical enzymatic detoxification systems, whereas SsrB lessens oxidative and nitrosative stress of intracellular *Salmonella* by activating the expression of the SPI2 type III secretion system that decreases contact of phagosomes with incoming NADPH phagocyte oxidase- and inducible NO synthase-containing vesicles. In addition, the SPI2 type III secretion system prevents phagolysosome

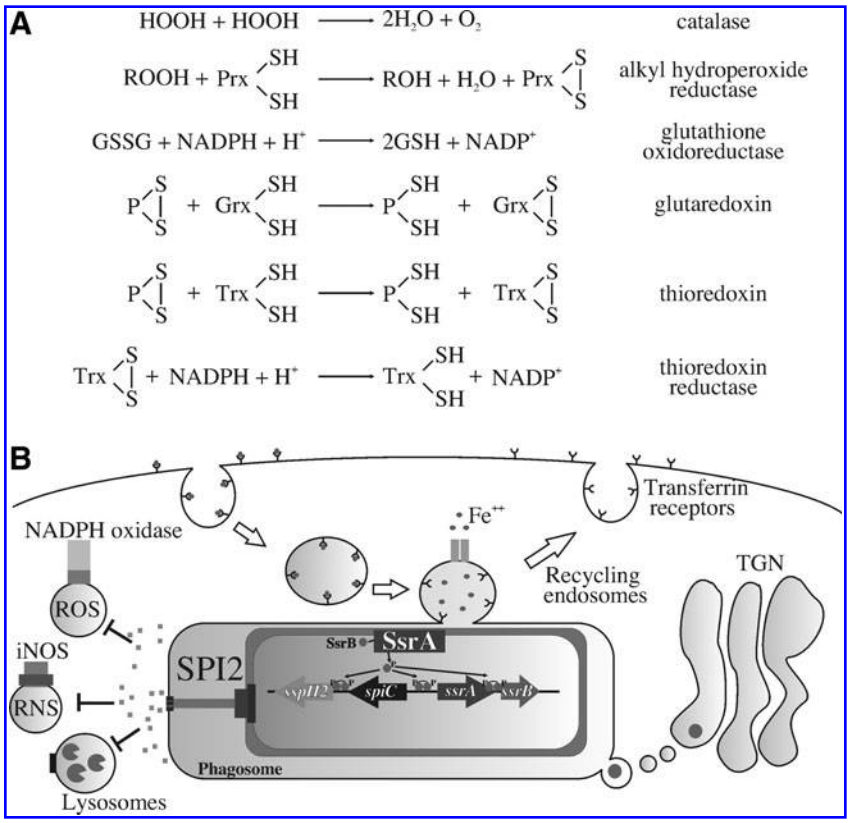

FIG. 4. Strategies of antioxidant defenses regulated by thiol-based regulators. Regulatory proteins bearing thiol switches induce the expression of classical antioxidant defenses for the detoxification of peroxides or repair intramolecular or intermolecular disulfides **(A)**. P in the glutaredoxin and thioredoxin reactions denotes any protein. OxyR disulfide bond is a substrate of Grx1. Trx reductase catalyses the reduction of a disulfide bond in Trxs using the reducing power of NADPH. The *Salmonella* pathogenicity island 2 (SPI2) type III secretion system regulated by the SsrB response regulator minimizes the contact of *Salmonella* phagosomes with vesicles containing NADPH phagocyte oxidase or inducible nitric oxide synthase (iNOS) and lysosomes, while helping establish contact with the trans-Golgi network (TGN) **(B)**. By doing so, the SPI2 type III secretion system lessens the contact of *Salmonella* with a variety of ROS and reactive nitrogen species (RNS), thereby indirectly fostering antioxidant and antinitrosative defenses.

formation, while promoting the interactions of *Salmonella*-containing vesicles with nutrient-rich vacuoles in the exocytic pathway (58, 86, 99). As a consequence of the vacuolar remodeling, the SPI2 type III secretion system allows *Salmonella* to survive and grow in a variety of epithelial cells and mononuclear phagocytes, thereby playing a vital role in the course of systemic infection (74, 88). SsrB and the SPI2 type III secretion system are essential for *Salmonella* pathogenesis, as *Salmonella* strains lacking a functional SPI2 type III secretion system are attenuated at least five orders of magnitude (88).

As it will become clear next, SsrB Cys^{203} is modified by RNS (43). A *Salmonella* strain expressing the SsrB C203S variant exhibits wild-type virulence in an acute model of infection, which is consistent with the fact that this allele is transcriptionally active (11, 43). Nonetheless, a strain of *Salmonella* bearing the SsrB C203S variant is attenuated in the context of nitrogen oxides produced in an $Nramp^R$ murine model of oral infection (43). These findings suggest that Cys^{203} in the C-terminal dimerization domain of the SsrB response regulator is a sensor of RNS produced in the course of a gastrointestinal infection.

Regulation of SsrB function by the redox-active Cys^{203}

As is the case for other response regulators, phosphorylation of a key aspartate in the receiver domain triggers the binding of SsrB to cognate promoters (11, 27). Recent investigations indicate that in addition to classical regulation by phosphorylation, two other signals influence SsrB's *modus operandi*. The $EIIA^{Ntr}$ component of the nitrogen-metabolic phosphotransferase system prevents the detrimental hyperactivation of SPI2 transcription by post-translationally precluding the association of SsrB to target promoters (19). The DNA-binding activity of SsrB is also subject to regulation by RNS (43). Cys^{203} in the $\alpha 4$ helix of the dimerization domain is susceptible to S-nitrosylation and oxidation by RNS such as $ONOO^-$, and products of the reaction of NO and oxygen (O_2). $ONOO^-$ oxidizes the thiol group of Cys^{203} to a mixture of sulfenic, sulfinic, and sulfonic oxidative products (43). C203D or C203E substitutions mimicking the negative charge of high-order oxidative products of $ONOO^-$-treated SsrB block dimerization and the transcriptional activity of SsrB (11, 43). The side chain of Cys^{203} interacts with the hydrophobic residue Leu^{192} located in the loop between the DNA-binding H3 helix and the dimerization H4 helix (11). Sulfinylation or sulfonylation of Cys^{203} would disrupt this interaction, and, thus, alter the secondary structure required for homodimerization of SsrB, DNA binding, and transcription. Accordingly, the electronegative charge introduced by C203D or C203E substitutions inhibits transcriptional activity (11, 43). S-nitrosylation would not be expected to alter the interactions between Cys^{203} and Leu^{192}. However, S-nitrosylation is not likely to be a terminal modification, but a precursor of further oxidations and/or S-thiolation. The extent to which these modifications prevent the formation of SsrB homodimers and affect gene transcription awaits further investigation.

Determinants That Define the Redox Activity of Cysteines

Cysteines are abundant in proteins, but only a few of them are modified by ROS and RNS. What makes a cysteine become redox active? A comprehensive review by Stamler and collaborators, and elegant recent work by the Ischiropoulos' group coincide to identify electrostatic interactions that affect pKa, hydrophobicity, and allosteric constraints as important determinants for the S-nitrosylation of selected thiols (26, 35, 40). These principles also dictate the reactivity of thiols toward ROS. Thiol-based bacterial redox sensors discussed in this review nicely illustrate the physical and chemical determinants that affect the reaction of redox-active cysteines with peroxides and nitrogen oxides.

Redox mechanism for the formation of thiolate in OxyR

Cys^{199} is located in a hydrophobic pocket between two α/β domains surrounded by acidic and basic residues (18). His^{130}, Glu^{151}, His^{198}, Arg^{201}, Asp^{202}, and Arg^{266} may influence the reactivity of OxyR with H_2O_2 (18, 59). Arg^{266} has been shown to lower the pKa of Cys^{199} (18). The positive effect of Arg^{266} on Cys^{199} redox capacity is demonstrated by the fact that a strain of *E. coli* expressing the *oxyR* R266A variant is both hypersusceptible to oxidative stress and defective in *oxyS* expression in response to H_2O_2 (18). Dipoles from neighboring α-helices may also influence the ionization of Cys^{199} thiol (18). The Cys^{199} thiolate is likely stabilized by hydrophobic interactions with Leu^{200}, Leu^{224}, and Pro^{241} (18). The reaction of the thiolate with H_2O_2 forms a $-SOH$ that forces Cys^{199} out of the hydrophobic pocket. This, in turn, induces flexibility of the region between 205 and 216 residues where Cys^{203} resides, thereby increasing the probability of collision between sulfenylated Cys^{199} and reduced Cys^{208} to form a disulfide bond (63).

Sensing by MarR/OhrR family members

The proximity of charged residues within 6 Å of a thiol group increases its potential to be ionized (26). No basic residues are found within a 7-Å distance from the OhrR Cys^{15} side chain (42). Instead, a positively charged macrodipole in helix 1 decreases the *B. subtilis* OhrR Cys^{15} pKa value to 5.2 (42). Therefore, this cysteine is kept in the ionized, thiolated form under physiological conditions, ready to react with incoming organic hydroperoxides. The negative charge of the thiolate in helix $\alpha 1$ is stabilized by hydrogen bonding with the side chains of Tyr^{29} and Tyr^{40} in helix $\alpha 2$ (42). The mutations of Tyr^{29} and Tyr^{40} have shown that hydrogen bonding to the Cys^{15} thiolate is important for *B. subtilis* OhrR sensing of organic hydroperoxides (42). The residues that form hydrogen bonding with the redox active thiolate and a hydrophobic pocket near it are conserved between *B. subtilis* OhrR and *Sta. aureus* SarZ and MgrA repressors (15, 42, 84). Hydrogen bonding is also important for the stabilization of the sulfenic modifications of SarZ Cys^{13} and MgrA Cys^{12}. S-thiolation disrupts hydrogen bonding, thereby triggering a conformational change that lowers the affinity of these repressors for DNA.

Activation of thiols in the zinc-binding RsrA antisigma factor

Zinc can increase the reactivity of cysteine by lowering the pKa of the coordinating thiol side chains. However, similar to the situation described for the eukaryotic protein Sp1 (56), zinc protects the redox-active cysteines of RsrA from

oxidative stress (66). Since zinc actually lowers the reactivity of the triggering thiolate in the finger, other determinants should activate the critical cysteine(s). The electronegative charge provided by glutamate residues flanking the HX_3CX_2C ZAS motif serves as an important redox determinant for the reactivity of RsrA (49). It remains to be investigated to what extent these glutamate residues influence the pKa and reactivity of the Cys^{11} triggering switch.

Activation of SsrB Cys^{203}

The fact that Cys^{203} reacts with nitrogen oxides and forms a disulfide bond with $Cys^{203'}$ of the homodimer (11, 43) indicates that the thiol group of this cysteine is redox active. The determinants that make SsrB Cys^{203} redox active have yet to be identified. However, lessons learned with the bacterial regulators just mentioned and well-characterized redox active cysteines in a variety of proteins help make some informed predictions on how the environment surrounding SsrB Cys^{203} may dictate the redox activity of this response regulator (Fig. 5). As predicted by the fact that most reactive cysteines are overrepresented in α-helices (26), Cys^{203} is located in the SsrB's H4 α-helix. Basic residues at position 2 and acid residues at positions −3 and −4 influence the pKa of thiol groups (35). The analysis of the flanking residues around Cys^{203} indicate the presence of both positive and negative residues, raising the possibility that Cys^{203} is ionized in the cytoplasm of *Salmonella*. Of note, the guanidine group of Arg^{206} is 4.1 Å from the Cys^{203} thiol side chain. Moreover, the carboxyl group of Glu^{199} is 6.1 Å from Cys^{203}. This configuration raises the possibility that the Cys^{203} side chain is, in fact, in the thiolate form. The hydrophobicity of the H4 helix and Leu^{192} located only 4.2 Å away could stabilize the Cys^{203} thiolate. These interesting scenarios await further investigation.

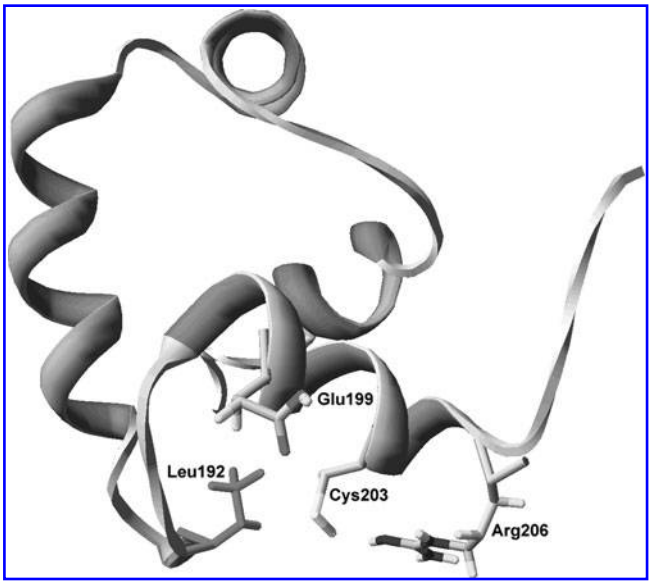

FIG. 5. Model for the activation of the thiol side chain in SsrB Cys^{203}. The redox active, RNS-modifiable Cys^{203} is located in the H4 α-helix dimerization domain of SsrB (11). Analysis of the nuclear magnetic resonance structure of the C-terminus domain of SsrB reveals that the sulfur atom of Cys^{203} is 4.1 Å from the positively charged guanidine group of Arg^{206}. The proximity of Arg^{206} is likely to draw the proton from Cys^{203}, keeping this cysteine in the ionic, redox-active, thiolated state. The carboxyl group of Glu^{199} likely influences the pKa of the Cys^{203} thiol group located 6.1 Å away. The Cys^{203} $-S^-$ could be stabilized through hydrophobic interactions with Leu^{192} located 4.2 Å away.

Chemistry Underlying the Modification of Redox-Active Thiols by ROS and RNS

Redox active thiol groups involved in signaling react selectively with ROS and RNS. The resulting oxidative or nitrosative modifications are often reversible. The mechanism by which thiol-dependent bacterial redox sensors react with peroxides is well understood (Fig. 6); however, several models could explain the reactivity of thiol switches with RNS for the formation of S-nitrosothiols.

Selectivity of the reaction of thiols and ROS

The rate constants of hydroxyl radical, $O_2^{\bullet -}$, and H_2O_2 with LMWTs are 10^{10}, 30–1000, and 20 M^{-1} s^{-1}, respectively (106, 107). Although it has high affinity for thiols, the hydroxyl radical reacts nonspecifically with nearly all biomolecules,

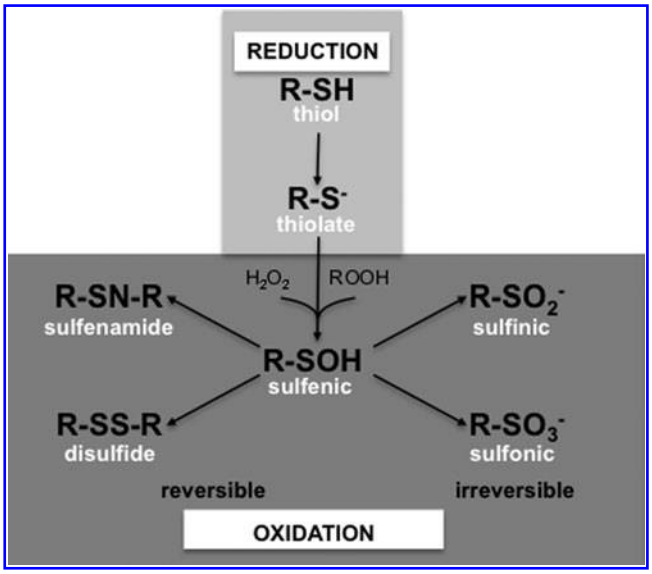

FIG. 6. Biological chemistry for the reaction of thiols with peroxides. Thiol groups of reactive cysteines are kept in the thiolated state through interactions with nearby charged residues. The thiolate reacts with H_2O_2 or organic hydroperoxides to form a −SOH. In *B. subtilis* OhrR, and *Sta. aureus* MgrA and SarZ, the sulfenylated cysteine can be quite stable. However, in most cases, the −SOH attacks nearby thiols, forming disulfide bonds that promote structural and functional changes in the protein. Disulfide bonds can form between cysteines within a protein (*e.g.*, *Escherichia coli* OxyR) or cysteines in the homodimer (*e.g.*, *X. campestris* OhrR). Mixed disulfides can also arise between the sulfenylated protein and LMWTs (*e.g.*, *Sta. aureus* SarZ). In addition, a −SOH can attack a nearby amide group, giving rise to a sulfenamide (*e.g.*, *B. subtilis* OhrR). All of these cysteine oxidation states are reversible, therefore providing flexibility as signaling switches. The −SOH intermediate can be oxidized further to sulfinic and sulfonic acids, which, for most part, are irreversible signatures of oxidative stress.

and, therefore, it is not endowed with the discriminatory power to serve as a signaling molecule. Superoxide dismutases detoxify $O_2^{\bullet -}$ with a rate constant of $2\times10^9\ M^{-1}\ s^{-1}$ (29), making the $O_2^{\bullet -}$-dependent oxidation of thiols of little physiological importance. On the other hand, H_2O_2 can react with fast kinetics with certain thiols. For example, the second-order rate constant for the reaction of OxyR Cys^{199} and H_2O_2 is about $10^5\ M^{-1}\ s^{-1}$ (2), making this regulator an excellent H_2O_2 sensor. As discussed elsewhere in this review, OxyR Cys^{199} reacts with RNS, but with apparently lower affinity than ROS. It is currently unknown why OxyR Cys^{199} in particular and redox active cysteines in general exhibit preferential affinity toward selected reactive species. To define the determinants that provide selectivity of redox-active thiolates for some reactive species but not others is an important goal for the future.

Oxidation of redox active thiols by H_2O_2

Although the reactivity of thiols with ROS is inversely related to their pKa (107), there are examples where this does not seem to be the case (106). In addition to a low pKa that ionizes the thiol to a thiolate, the environment of the protein is critical for the stability and reactivity of the thiolate. Reactive thiols in MarR, LysR, and zinc-finger family members just discussed have low pKa values and are generally located in hydrophobic pockets. As described in other systems (83), the two-electron oxidation of the redox-active thiolate in the regulators just presented by H_2O_2 or organic hydroperoxides gives rise to a sulfenic group. The oxidation of a thiol to a $-$SOH is not enough to transmit the signal in most regulatory proteins. The $-$SOH is frequently oxidized to a disulfide in order to drive the structural changes that modulate protein function. In the case of *E. coli* OxyR and *Str. coelicolor* RsrA, intramolecular disulfides are responsible for major structural changes, whereas mixed disulfides are necessary in the case of *B. subtilis* OhrR. In addition to mediating dramatic changes in secondary structures that transduce the signal, disulfide bonds prevent further oxidation of cysteine $-$SOHs to high-order oxidative species such as sulfinic and sulfonic groups. Disulfides are reversible modifications that on reduction turn off the signal elicited by H_2O_2, whereas the oxidation of thiols to sulfinic and sulfonic are, for the most part, irreversible and less amenable for signaling. Although the reaction of cysteines with peroxides is facilitated when the sulfur in the side chain is a thiolate, the modification of cysteines by other reactive species does not have to be limited to the ionic form of cysteines. For example, as will be discussed next, radicals can promote modifications of cysteines through a sulfenyl (*i.e.*, thiyl) radical intermediate.

Selective reaction of thiols with peroxides

Not all redox active cysteines react equally with ROS. Cys^{15} of *B. subtilis* OhrR is an excellent case in point. Antioxidant defenses and intrinsic allosteric determinants explain the selective reaction of *B. subtilis* OhrR with organic hydroperoxides. H_2O_2 can oxidize the thiol of OhrR; however, this reactive species does not appear to be a relevant signal *in vivo*. The activity of catalases in *B. subtilis* diminishes the effective concentration of H_2O_2 to levels that are insufficient to initiate the oxidation of the critical cysteine residue in OhrR (31). The reaction of OhrR with organic hydroperoxides is fast. A patch of hydrophobic residues in the vicinity of the redox active cysteine increases the affinity of OhrR for organic hydroperoxides (42). Most eukaryotic organic hydroperoxides are derived from membrane lipids. A radical abstracts an allylic hydrogen in the polyunsaturated hydrocarbon, giving rise to a conjugated diene that is susceptible to the O_2 attack for the generation of lipid hydroperoxides (41). The lack of polyunsaturated hydrocarbons in prokaryotic lipids greatly limits the formation of lipid hydroperoxides in their membranes. Although extremely active when supplied in pure form, it remains to be seen whether organic hydroperoxides are the actual signal for members of the OhrR family of regulators *in vivo*. The thiol oxidizer diamide also derepresses the OhrR regulon (31), raising the possibility that the cysteines triggered in OhrR may respond to thiol/disulfide exchange reactions. OhrR also appears to be a sensor of hypochlorite stress (17).

Reaction of thiols with RNS

Thiol groups in regulators such as OxyR and SsrB can be modified by RNS. S-nitrosothiols can be generated by several independent mechanisms (Fig. 7). Transnitrosation and radical reactions may account for the nitrosylation of redox-active thiols in the regulators discussed in this review. In the case of OxyR, RNS modify the Cys^{199} thiolate to form an S-nitrosothiol. The nitrosonium cation is the most likely candidate that covalently modifies the nucleophilic thiolate of OxyR Cys^{199}. However, nitrosonium does not exist free in solution. It has consistently been reported that OxyR is more efficiently activated by nitrogen oxides in GSH-deficient *E. coli* strains, suggesting that transnitrosation reactions from species other than S-nitrosoglutathione are the most likely nitrosating species. Lancaster has proposed that dinitrosyl iron complexes (DNICs) are the most important nitrating agents in the cell (8). DNIC arising from the complexation of NO, iron, and cysteine, or other LMWTs, could be a source of the nitrosonium-like species that S-nitrosylates the OxyR Cys^{199} thiolate group.

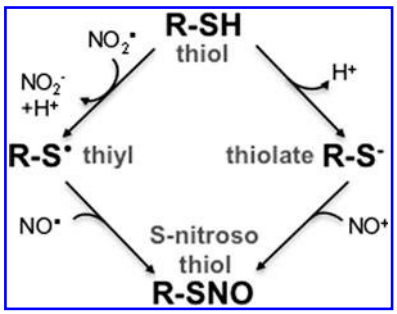

FIG. 7. Mechanisms for the formation of S-nitrosothiols in biological systems. The formation of S-nitrosothiols in biological systems involves two dominant biochemistries. Thiolates in redox active cysteines are strong nucleophiles that react with the nitrosonium cation (NO^+) to form an S-nitrosothiol. Transnitrosation reactions from low-molecular-weight S-nitrosothiols, dinitrosyl iron complexes, and dinitrogen trioxide (N_2O_3) are likely donors of NO^+. In addition, S-nitrosothiols can be generated through the reaction of thiyl and NO radicals. Nitrogen dioxide ($NO_2^{\bullet}$) generated in the autoxidation of NO likely mediates the abstraction of an electron from the thiol to form the thiyl radical ($S^{\bullet}$).

The Cys^{203} of the response regulator SsrB has also been shown to react with nitrogen oxides (43). The DNA binding activity of SsrB is inhibited by high fluxes of NO in the presence of O_2. This indicates that products of the autooxidation of NO mediate the inhibition of binding of SsrB with cognate promoters. Together with DNIC and S-nitrosothiols, dinitrogen trioxide (N_2O_3) is a potent nitrosating agent that serves as a source of nitrosonium groups (54). The production of N_2O_3 is second order for NO, and some investigators have argued that the generation of N_2O_3 is not favored under physiological conditions. Alternatively, nitrogen dioxide produced in the autooxidation of NO could abstract an electron from the thiol group of SsrB Cys^{203}, generating a thiyl radical that could condensate with the NO radical to form S-nitroso SsrB. As is the case for most biomolecules, the chemical species that mediates S-nitrosylation of SsrB is currently unknown.

Concluding Remarks

Cysteine and methionine have a sulfur atom in their structures. Sulfur in methionine is frequently oxidized to a sulfoxide group. This oxidative modification can be repaired through the enzymatic activity of the methionine sulfoxide reductases. The reactivity of methionines with ROS, the reversibility of the oxidation, and the enrichment of oxidizable methionines in protein surface areas and enzyme active sites have led to the proposal that the sulfur in methionine acts as an endogenous antioxidant which protects biomolecules from the attack of reactive species (65, 68). Despite being redox active, methionine has not yet been found to be a redox-sensing moiety of bacterial transcriptional regulators. Several reasons may explain the predominance of cysteines rather than methionines as common signaling redox determinants. Cysteines and methionines can be found in 10 and 3 different oxidation states in the cell, respectively (46). Thus, cysteines provide a larger repertoire in oxidation states, making it possible to sense diverse ROS and RNS. However, of all possible oxidation states, thiol-dependent bacterial sensors rely quite frequently on thiolates and disulfide bonds as effective ways to transduce a signal. Why would disulfide bonds be a preferred mode of signal transduction? Disulfides are reversible modifications that can be in balance with thiolates by the action of Trx, GSH, and Grx thiol/disulfide exchange systems. Equally important, the dramatic structural rearrangements associated with disulfide bond formation are accompanied by changes in function. A case in point is the fact that the newly formed disulfide between Cys^{199} and Cys^{208} in oxidized OxyR brings together regions separated by 17 Å in the reduced protein (18). This massive structural reorganization allows binding of OxyR to the RNA polymerase with the consequent initiation of transcription.

The regulators discussed in this review reveal variability in the chemistry by which disulfide bonds are generated, and the diverse structural means by which they modulate molecular function (Fig. 8). In addition to the aforementioned intramolecular disulfide bond that drives OxyR transcriptional activity, intermolecular and mixed disulfide bonds affect the function of bacterial regulatory proteins. Intermolecular disulfide bonds in *X. campestris* OhrR and mixed disulfides in redox active thiolates in lone cysteine OhrR-type regulators are additional ways by which redox-active cysteines transduce signals in response to oxidative and nitrosative stress.

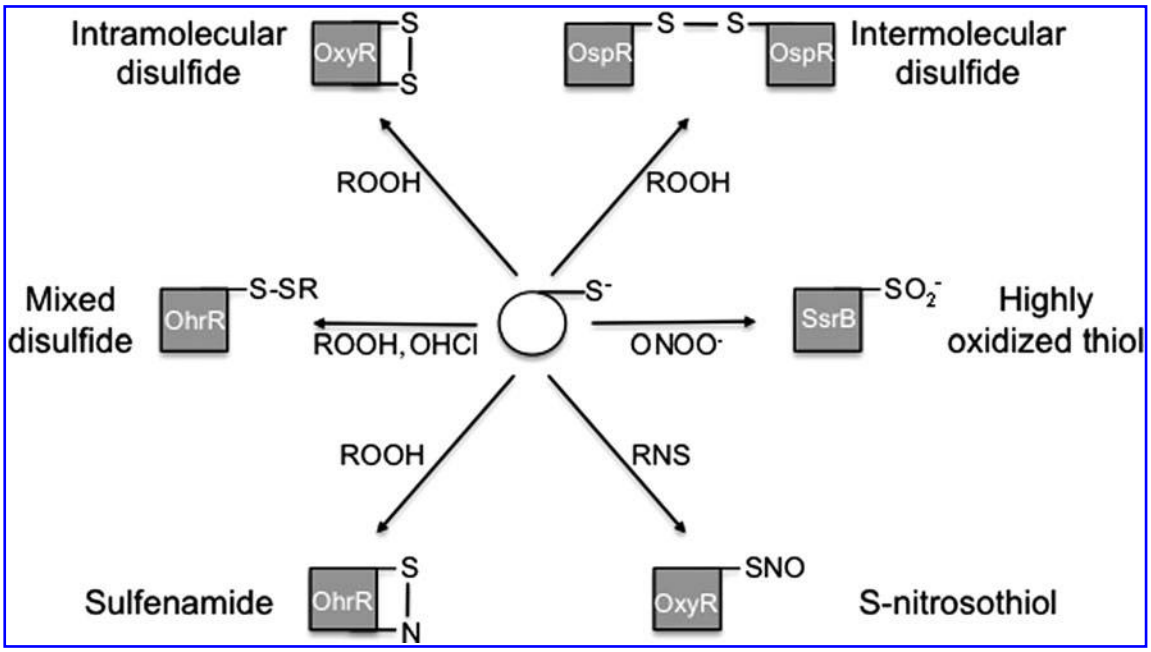

FIG. 8. Changes in protein structure after thiol oxidation. Reduced thiolates in regulatory proteins are susceptible to oxidation by a variety of inorganic or organic hydroperoxides and hypochlorate. Further oxidation of −SOH intermediate to intramolecular, intermolecular, and mixed disulfides induces changes in structure that are followed by switches in molecular function. The −SOH intermediate can also react with adjacent amines to form sulfenamide. In addition, redox active thiols can be modified by peroxynitrite ($ONOO^-$) and other RNS for the generation of highly oxidized thiols and S-nitrosothiols. Examples of such regulators are shown in *white*. Redox-active thiols in regulators such as OxyR can be modified by several reactive species.

The mechanisms underlying the S-thiolation of redox-active cysteines are equally varied in nature. Mixed disulfides in the OhrR-family members have been shown to occur through a –SOH oxidative intermediate. S-thiolation can independently arise through an S-nitrosylated intermediate as described for OxyR (55). In either case, mixed disulfides make possible the structural and functional changes of the oxidized or S-nitrosylated regulatory protein.

Thiol-dependent redox sensing coordinates bacterial responses to changes in physiology and pathogenesis. OxyR, OhrR, RshA, and SsrB add to the antioxidant and anti-nitrosative defenses of bacteria. In addition, the oxidized thiols in some sensors such as OxyR and RsrA have been shown to be substrates of the antioxidant defenses activated in their regulons. This feedback regulation ensures that redox homeostasis is re-established after the burst of reactive species has subsided. This is an important consideration, as bacterial cells experience fluctuations in oxidative stress during different growth phases and during preferential utilization of certain metabolic pathways (57). In addition, pathogenic microorganisms are exposed to a burst of intense oxidative stress (102). The redox activity of thiol-based sensors increases fitness of saprophytic, commensal, and pathogenic bacteria to fluctuations in oxidative and nitrosative stress in their ever-changing environment.

Since investigations with *E. coli* and *Salmonella* discovered the thiol-based H_2O_2 sensor OxyR (20), an increasing number of members within the LysR, MarR, RsrA, and NarL families of transcriptional regulators have been shown as using thiol-based redox sensors that coordinate responses to endogenous and exogenous ROS and RNS. As the range of thiol-based prokaryotic redox sensors broadens, it will be interesting to see the extent that the concepts discussed in this review apply.

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Abbreviations Used

DNIC = dinitrosyl iron complexes
Grx1 = glutaredoxin 1
GSH = glutathione
GSSG = oxidized glutathione
H_2O_2 = hydrogen peroxide
iNOS = inducible nitric oxide synthase
LMWTs = low molecular weight thiols
NO = nitric oxide
NO^+ = nitrosonium cation
N_2O_3 = dinitrogen trioxide
NO_2^- = nitrite
$NO_2^\bullet$ = nitrogen dioxide
O_2 = oxygen
$O_2^{\bullet-}$ = superoxide
$ONOO^-$ = peroxynitrite
RNS = reactive nitrogen species
ROS = reactive oxygen species
$S^\bullet$ = thiyl radical
$-S^-$ = thiolate group
–SOH = sulfenic acid
SPI2 = *Salmonella* pathogenicity island 2
TGN = trans-Golgi network
Trx = thioredoxin
ZAS = zinc-containing antisigma

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