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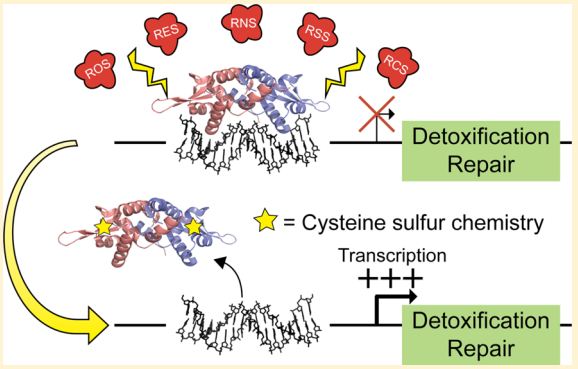
Cysteine Sulfur Chemistry in Transcriptional Regulators at the Host−Bacterial Pathogen Interface

Justin L. Luebke and David P. Giedroc*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405-7102, United States

ABSTRACT: Hosts employ myriad weapons to combat invading microorganisms as an integral feature of the host−bacterial pathogen interface. This interface is dominated by highly reactive small molecules that collectively induce oxidative stress. Successful pathogens employ transcriptional regulatory proteins that sense these small molecules directly or indirectly via a change in the ratio of reduced to oxidized low-molecular weight (LMW) thiols that collectively comprise the redox buffer in the cytoplasm. These transcriptional regulators employ either a prosthetic group or reactive cysteine residue(s) to effect changes in the transcription of genes that encode detoxification and repair systems that is driven by regulator conformational switching between high-affinity and low-affinity DNA-binding states. Cysteine harbors a highly polarizable sulfur atom that readily undergoes changes in oxidation state in response to oxidative stress to produce a range of regulatory post-translational modifications (PTMs), including sulfenylation (*S*-hydroxylation), mixed disulfide bond formation with LMW thiols (*S*-thiolation), di- and trisulfide bond formation, *S*-nitrosation, and *S*-alkylation. Here we discuss several examples of structurally characterized cysteine thiol-specific transcriptional regulators that sense changes in cellular redox balance, focusing on the nature of the cysteine PTM itself and the interplay of small molecule oxidative stressors in mediating a specific transcriptional response.



INTRODUCTION AND SCOPE

All microorganisms, irrespective of their specific niche(s), continuously monitor their immediate microenvironment and must be capable of responding to changes in cellular redox status. This is particularly true for bacterial pathogens that colonize the vertebrate host. The bacterial cytoplasm under unstressed conditions is strongly reducing, with a reduction potential ($E^{\circ\prime}$) of approximately −260 to −280 mV, determined for *Escherichia coli*.[1] This potential is maintained by the ratio of reduced to oxidized low-molecular weight (LMW) thiols, with far more reduced monothiol, RSH, than oxidized disulfide, RSSR (where R is a specific organic moiety), present in the cytoplasm (Figure 1A). Any small molecule that disrupts the LMW thiol pool or alters the ratio of reduced to oxidized LMW thiols makes the $E^{\circ\prime}$ more positive and therefore induces an oxidative stress response in the organism. Small molecules that comprise reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive electrophile species (RES), reactive chlorine species (RCS), and reactive sulfur species (RSS)[2,3] are fundamentally oxidative stressors because they target not only protein-based thiols but also the LMW thiol pool.

In mammals in particular, various ROS and RNS species as well as hydrogen sulfide (H_2S) are known to function as signaling molecules at low concentrations but become toxic at superphysiological concentrations. They disrupt the electron transport chain, disassemble Fe−S clusters and mononuclear transition metal complexes, induce DNA damage, and oxidize membrane lipids and proteins.[4−6] The host attempts to exploit this chemistry as an integral feature of the host innate immune response against bacterial pathogens.[7] Furthermore, it has long been known that the combination of multiple stressors such as ROS and RNS has synergistic effects on microbial killing.[8] This, in turn, necessitates a rapid sensing, transcriptional response, detoxification of the oxidative species, and damage repair to restore intracellular redox balance in the bacterium. The upregulation of detoxification enzymes used to combat changes in redox state is controlled by redox-sensing DNA-binding transcriptional regulatory proteins. Two broad types of stress-sensing mechanisms have been described for the vast majority of transcriptional regulatory proteins: those that utilize a prosthetic group and those that undergo oxidative post-translational modification (PTM^{ox}) of the Sγ atom in cysteine residues.

In this review, we summarize the chemistry of small molecule reactive species that comprise ROS, RNS, RES, RCS, and RSS and place it into the context of our understanding of the LMW thiol pool, thiol−disulfide homeostasis, and fundamental properties of cysteine. This is followed by a discussion of recently structurally characterized cysteine thiol-specific microbial transcriptional regulators that sense changes in cellular redox balance, with the goal of highlighting the structural and

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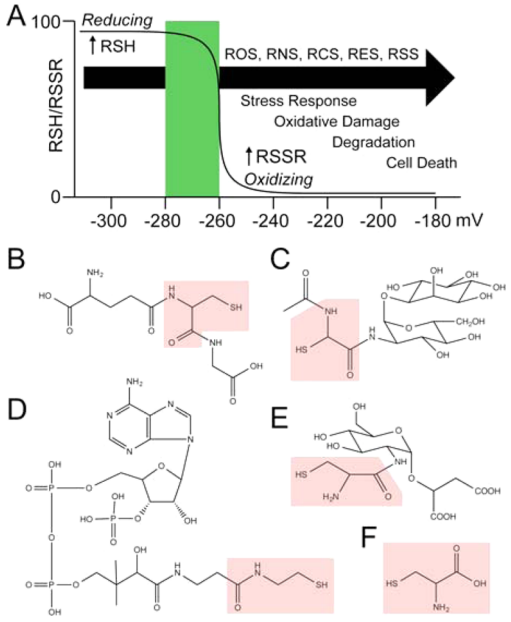

Figure 1. (A) Cellular redox potential where increasing stress leads to oxidative damage, degradation, and cell death if the stress surpasses the ability of the cell to adapt. (B–F) Major LMW thiols found in bacteria. Each possesses a thiol as part of the primary structure, highlighted in red: (B) glutathione (GSH), (C) mycothiol (MSH), (D) coenzyme A (CoA), (E) bacillithiol (BSH), and (F) cysteine (Cys).

chemical diversity of this stress response across the eubacterial kingdom. We will not specifically discuss other non-cysteine thiol-based modes of oxidative stress-induced regulation of transcription but instead refer the reader to recent work and reviews on these systems. These include heme-based redox sensors, exemplified by *Mycobacterium tuberculosis* DosS,[9,10] non-heme Fe-based sensors such as PerR from *Bacillus subtilis*,[11] Fe–S cluster-based sensors, including *E. coli* SoxR,[12,13] and *M. tuberculosis* WhiB proteins,[14] and regulators that directly survey the cellular NADH/NAD^+ ratio as observed with Rex from Gram-positive pathogens.[15−17] In addition, it is now established that cysteine is not the only amino acid that is a target of reversible oxidative chemistry in proteins, because the thioether Sδ atom of methionine has been characterized as the site of oxidation in hypochlorite sensor HypT from *E. coli*.[18,19] The evolution of a range of mechanisms for sensing and inducing an oxidative stress response beyond cysteine sulfur chemistry is discussed here and elsewhere[20] and serves to illustrate the essentiality of this cellular response. Distinct molecular mechanisms can also be used to fine-tune the specificity of a response, in part dictated by the micro-environmental niche.

LMW THIOL POOL

Organic thiol-containing small molecules contribute to the strong cellular reducing potential as a function of the relative concentrations of reduced (RSH) to oxidized (RSSR′) LMW thiols where R is a specific organic moiety with far more RSH that RSSR′.[1] The reducing potential typically includes a major cellular reductant such as glutathione (GSH), which is found in most Gram-negative bacteria, cysteine, or coenzyme A. However, glutathione is not synthesized by all bacteria but is replaced with other functionally analogous LMW thiols, including mycothiol (MSH), found in *M. tuberculosis* and other actinomycetes,[21] and bacillithiol (BSH) from many Gram-positive bacteria,[22] including *Bacillus* spp. and *Staphylococcus* spp. (Figure 1). Other bacteria, e.g., *Streptococcus pneumoniae*, are incapable of synthesizing GSH, MSH, or BSH and simply take up glutathione from their environment.[23]

The pK_a of the free thiol form is typically ≈8.5–9.0, and thus, the reduced form is defined by an equilibrium between protonated and deprotonated species. The intracellular [BSH] is in the range of 1 mM for mid-log *B. subtilis* cells,[24] while the intracellular [GSH] is roughly 10 mM in a typical Gram-negative bacterium; thus, these LMW thiols represent an enormous "sink" used to protect the cell from nonreparable oxidative damage. Although thiol–disulfide exchange is facile, LMW thiol–disulfide homeostasis is further kinetically facilitated by enzymes that catalyze the reduction of LMW disulfides to free thiols. These include the well-characterized thioredoxins and glutaredoxins and more recently described mycoredoxins[25,26] and bacilliredoxins[27] and, analogously, coenzyme A disulfide reductases.[28]

Finally, recent work reveals that the LMW thiol pool contributes to the transition metal buffering capacity of the cytoplasm, particularly for the more thiophilic metals Zn(II)[29] and Cu(I).[23,30] For example, in *B. subtilis*, BSH has been shown to function as a buffer for the labile or free Zn(II) in the cell.[31] As the concentration of the reduced bacillithiol decreases, the increased level of "free" zinc is efficiently sensed by the zinc efflux repressor, CzrA,[32] which leads to transcriptional derepression of a Zn(II) efflux transporter. The major point is that changes in the redox balance are likely always coincident with perturbations in metal speciation and can therefore potentially impact enzyme metalation.[33] This speaks to the interdependence of the oxidative stress response and metal homeostasis in cells.

CYSTEINE: A REDOX-SENSITIVE AMINO ACID

Cysteine is among the rarest and most functionally diverse of all the amino acids. It is often solvent-accessible, facilitating roles in redox chemistry, regulatory function, enzyme catalysis, and coordination of transition metals.[34,35] This diverse functionality is due to a polarizable sulfur atom that makes cysteine a thiol with highly tunable reactivity. Properties that control this reactivity include solvent accessibility, proximity to titratable groups, pK_a, and oxidation state.[36] The intrinsic pK_a of a cysteine residue in an unstructured peptide region is similar to that of the tripeptide glutathione at ≈8.5–9.0, but nearby basic residues such as histidine, lysine, and arginine stabilize the thiolate anion via electrostatic complementarity and/or hydrogen bonding to the peptide backbone or other side chains, thereby significantly lowering the pK_a to 5–7 in proteins;[37−39] indeed, several cysteines have been reported to possess pK_a values as low as 3.5.[36] Deprotonation of the cysteine thiol gives rise to the thiolate anion, RS^-, a potent nucleophile that is poised to react with electrophilic functional groups. On the other hand, physical proximity to deprotonated acidic residues, e.g., aspartate and glutamate, can increase the pK_a, thereby stabilizing the protonated thiol at neutral pH, which attenuates the reactivity of cysteine toward electrophiles. The critical importance of the cysteine microenvironment in proteins is highlighted by the observation that thiol reactivity with the simple oxidant hydrogen peroxide (H_2O_2) can vary over 7 orders of magnitude.[39]

Thiols with low pK_a values are far more susceptible to sulfenylation, a major post-translational modification induced

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by H_2O_2, which in turn enhances their reactivity toward cellular nucleophiles, e.g., LMW thiolate anion, because of the increased level of electrophilic character (Figure 2A, **7**).[38,40]

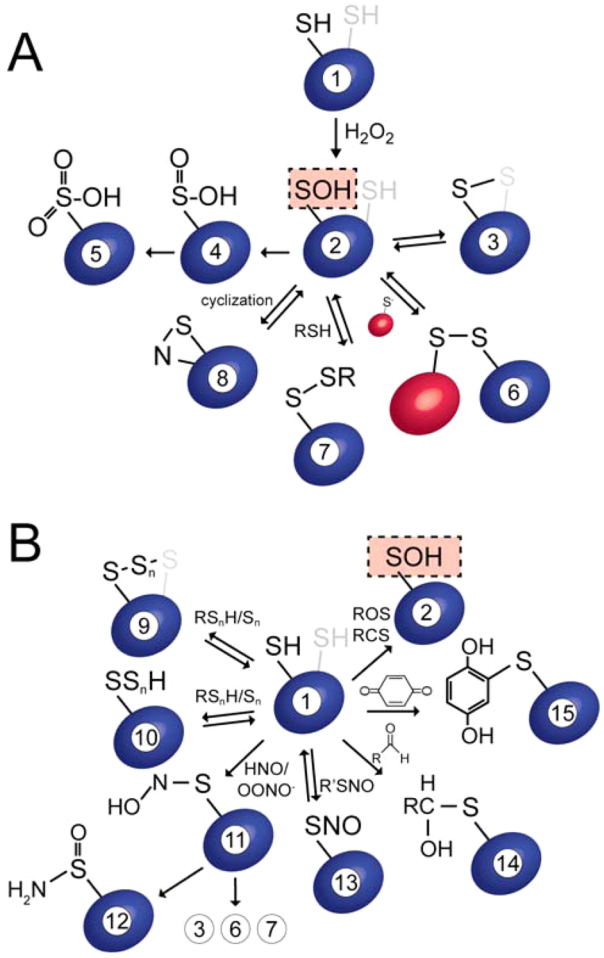

Figure 2. Schematic rendering of oxidative post-translational modifications (PTM^{ox}) of cysteine thiols. (A) Reactions that require cysteine sulfenylation (derivative **2**) prior to further reaction (**3**–**8**). (B) Reactions involving a reduced cysteine thiolate (**1**) (**2** and **9**–**15**). A resolving cysteine is required for some modifications and is colored gray: **1**, cysteine thiol; **2**, cysteinesulfenic acid; **3**, *intra*protomer disulfide; **4**, sulfinic acid; **5**, sulfonic acid; **6**, *inter*protomer disulfide; **7**, *S*-thiolation with a LMW thiol; **8**, cyclic sulfenamide derived from condensation of a sulfenate with a backbone amide group;[50] **9**, disulfide (n = 0), trisulfide (n = 1), and tetrasulfide (n = 2); **10**, *S*-sulfhydration (persulfide, n = 1; polysulfide, $n \geq 2$); **11**, *N*-hydroxysulfenamide; **12**, sulfinamide; **13**, *S*-nitrosation; **14**, *S*-hydroxymethylthiol; **15**, *S*-quinonization. This figure was adapted and significantly expanded from figures in refs 20 and 190.

Although protein sulfenylation was originally thought to be a transient PTM^{ox} en route to disulfide bond formation, the development of mass spectrometry-based proteomics methods[41] and *in situ* labeling[42] approaches has revealed that sulfenylated cysteines can be stable in certain proteins.[41] The crystal structure of sulfenylated SarZ, a global regulator from *Staphylococcus aureus*, provides molecular insights into this stabilization and is discussed in greater detail below.[43] A long-lived sulfenylated cysteine is particularly important for inducing a rapid response to changes in redox homeostasis in the cell. Many ROS, RES, and RCS stressors ultimately lead to cysteine sulfenylation as a primary PTM^{ox} (Figure 2B).

Although the LMW thiol pool offers considerable protection against oxidative stress and nitric oxide toxicity,[44] cysteine residues on proteins are also susceptible to oxidative damage by hydrogen peroxide, for example, progressing from *S*-hydroxylation (sulfenylation), RSOH, to *S*-sulfinic acid to *S*-sulfonic acid formation (see Figure 2A, derivatives **2**, **4** ,and **5**). The latter two modifications, unlike sulfenylation and disulfide bond formation (Figure 2A, **3**), are irreversible and cannot be reduced by cellular reductants (note, however, that ATP-dependent sulfiredoxins can enzymatically reduce sulfinylated Cys[45]). When sulfenylation of a single, more reactive Cys occurs in the presence of a second Cys, the second Cys can act as a resolving cysteine either *intra*molecularly (Figure 2A, **3**) or *inter*molecularly (Figure 2A, **6**), exactly analogous to the Cys pair in the thioredoxins, peroxiredoxins, glutaredoxins, and related dithiol peroxidases that clear ROS and RNS.[45] Sulfenylated cysteines can also react with LMW thiols, to create mixed disulfides, known generically as *S*-thiolations (Figure 2A, **7**). The potent cellular oxidant diamide, for example, depletes the reduced LMW thiol pool (see Figure 1) and induces significant *S*-thiolation in *B. subtilis*, *S. aureus*, and *M. tuberculosis*[46,47] via thiol–disulfide exchange. There is emerging evidence that *S*-thiolation, e.g., *S*-glutathionation,[48] functions as a protective barrier to prevent the formation of nonrepairable higher oxidation state of cysteine, particularly in enzymes that require an active-site cysteine to function. It was recently shown that *S*-bacillithiolation occurs on the organohydroperoxide regulator, OhrR in *B. subtilis in vivo*,[49,50] and that de-*S*-bacillithiolation is mediated by bacilliredoxins BrxA and BrxB.[27] *S*-Bacillithiolation in Firmicutes[51] and *S*-mycothiolation in actinomycetes, including *Cornybacterium glutamicum*,[52] may well be a general defense strategy against other forms of oxidative stress, as these modifications are also protective against hypochlorite (HOCl) stress.

OXIDATIVE STRESS AT THE HOST–PATHOGEN INTERFACE

Although oxidative stress is commonly encountered by microorganisms in any given niche as a byproduct of aerobic cellular metabolism, it is most often discussed in the context of the host–microbial pathogen interface. A microbe encounters substantial oxidative stress upon being engulfed by a host immune cell, particularly in an intracellular phagosomal compartment or vacuole in macrophages[53] or neutrophils.[54] Here, microorganisms are bombarded with a myriad of small molecule oxidative stressors derived from NADPH oxidase (NOX), superoxide dismutase (SOD), nitric oxide synthase (NOS), myeloperoxidase (MPO), and other enzymes to form $O_2^{-\bullet}$, H_2O_2, NO, and HOCl, respectively (Figure 3).[55,56] Damage caused by these reactive species is widespread and includes disruption of metal centers and Fe-S clusters, lipid oxidation, amino acid oxidation, DNA damage, and general disruption of normal cellular physiology.[4−6,54] The specific stressors and quantities produced are dependent on the host immune cell type.

Many oxidative stressors are capable of reacting with one another to form additional toxic species either enzymatically or nonenzymatically (Figure 3).[6] For example, MPO is a major component of the phagocytic vacuole, comprising ≈25% of the protein content in these organelles,[57] and effectively catalyzes the dismutation of superoxide to H_2O_2, analogous to superoxide dismutases (Figure 3). In the presence of sufficient chloride anion, MPO converts H_2O_2 to hypochlorite (HOCl), a

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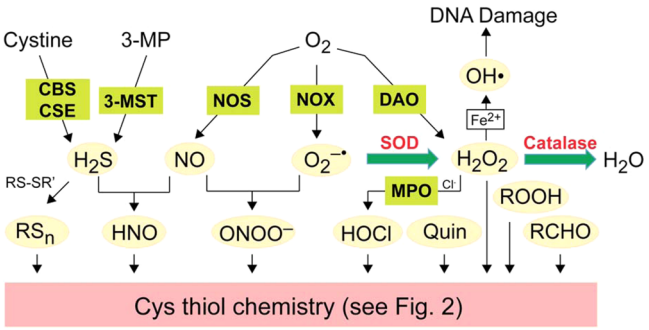

Figure 3. Interplay of ROS, RNS, RCS, RES, and RSS that potentially lead to a reaction with cellular thiols, upon which Figure 2 expands. Abbreviations: CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; 3-MP, 3-mercaptopyruvate; NOS, nitric oxide synthase; NOX, NADPH oxidase; DAO, D-amino acid oxidase; SOD, superoxide dismutase; MPO, myeloperoxidase; H_2S, hydrogen sulfide; RS_n, per/polysulfide; NO, nitric oxide; $O_2^{-\bullet}$, superoxide; $OH^\bullet$, hydroxyl radical; H_2O_2, hydrogen peroxide; HOCl, hypochlorite; HNO, nitroxyl; $ONOO^-$, peroxynitrite; ROOH, organic peroxide; RCHO, aldehyde/electrophile; Quin, quinone.

potent two-electron oxidant (Figure 3). MPO and other enzymes thus exploit ROS, RNS, and RCS as an integrated oxidative stress-inducing process designed to kill intracellular pathogens through the production of HOCl, chloramines, other RNS, hydroxyl radicals, and singlet oxygen species as recently reviewed (Figure 3).[56,58] An inducible NO synthase (NOS) in neutrophils is chiefly responsible for the production of NO by professional phagocytes (Figure 3), and more recently, it has been shown that RCS are generated by the enzyme dual oxidase (DUOX) in gut epithelial cells in *Drosophila* to control host–microbe interactions.[59]

Reactive Oxygen Species (ROS). ROS are the most widely recognized form of oxidative stress and are derived from the sequential one-electron reduction of molecular oxygen, from the superoxide anion ($O_2^{-\bullet}$), to hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), and ultimately H_2O (Figure 3). Other ROS include organic peroxides (ROOR′) and organic hydroperoxides (ROOH). ROS are capable of oxidizing cysteine, methionine, and tryptophan residues in proteins, as well as the LMW thiol pool. Superoxide itself is a relatively weak oxidant and a modest one-electron reductant; its toxicity may derive from subsequent reaction with the major RNS, nitric oxide, which generates peroxynitrite ($ONOO^-$) at diffusion-controlled rates (Figure 3).[6]

Both $O_2^{-\bullet}$ and H_2O_2 are capable of oxidizing iron–sulfur (Fe–S) clusters at sufficiently fast rates with the subsequent release of iron,[60,61] thereby increasing the bioavailable or "free" Fe in the cell. This reduced Fe(II) then reacts with H_2O_2 in a classical Fenton reaction to produce the highly oxidative hydroxyl radical ($OH^\bullet$) (Figure 3), which becomes catalytic under the reducing conditions of the cytoplasm. $OH^\bullet$, although short-lived (10^{-9} s) and generally considered a nonspecific oxidant,[62,63] induces DNA strand breaks,[64,65] and oxidation of DNA bases increases the number of mismatches and mutations.[66] ROS are capable of oxidizing cysteine, methionine, and tryptophan residues in proteins, as well as the LMW thiol pool. However, H_2O_2 also functions as a second messenger at lower concentrations in mammals.[67]

Superoxide has long been thought to be sensed directly in *E. coli* by SoxR,[68] a 2Fe–2S cluster-containing regulator from the MerR family of proteins.[13] However, recent work suggests that this may not be the case, and instead, SoxR is responsive to the intracellular NADPH/$NADP^+$ ratio.[69–71] Hydrogen peroxide, on the other hand, is directly sensed by OxyR, found largely in Gram-negative bacteria, e.g., *E. coli*, or PerR in Gram-positive bacteria, best characterized in *B. subtilis*.[72] Remarkably, each senses H_2O_2 through completely different structural mechanisms. OxyR employs reactive cysteine residues, discussed further below, while PerR utilizes an iron atom capable of binding peroxide through an open coordination site, which ultimately leads to the oxidation of two Fe-coordinating histidine residues to 2-oxo-His *in vivo*.[11] PerR ligand oxidation results in release and dissociation of Fe from the DNA operator–promoter region, leading to transcriptional derepression of the PerR regulon.[73] In contrast, organic hydroperoxides are sensed by the regulator OhrR in *B. subtilis* through a reactive cysteine residue,[74,75] also discussed below.

Reactive Nitrogen Species (RNS). Reactive nitrogen species are generally discussed in the context of the nitric oxide radical ($NO^\bullet$, or simply NO), a gasotransmitter that has long been known for its role in smooth muscle relaxation and neurotransmission in mammals.[76] However, NO stress can lead to the formation of other nitrogen-containing oxidants, including nitrogen dioxide ($NO_2^\bullet$), peroxynitrite ($ONOO^-$), and dinitrogen trioxide (N_2O_3) (Figure 3). At elevated concentrations, RNS disrupt Fe–S clusters and Fe metabolism, induce *S*-nitrosation of cysteine residues, form metal–nitrosyl complexes, and catalyze protein nitration, typically with tyrosine to form 3-nitrotyrosine. Many of these concepts were recently and comprehensively reviewed.[6] It should be noted, however, that the direct reaction of NO with thiols does not occur at a physiologically relevant rate, in contrast to the transnitrosation reaction (Figure 2B, **13**).[77,78]

More recently, nitroxyl (HNO), the one-electron reduced and protonated form of NO, and designated reactive nitrogen oxide species (RNOS) has emerged as a candidate gasotransmitter. HNO is strongly thiophilic and is characterized by a signaling pathway that is distinct from that of nitric oxide.[79–81] In mammalian systems, HNO has been suggested to mediate vasodilation through HNO-mediated disulfide bond formation in the transient receptor protein channel A1 (TRPA1), resulting in calcium influx.[82] In contrast to mammalian systems, the microbial stress response to nitroxyl is completely uncharacterized. HNO reacts with cysteine thiolates to form an N-hydroxysulfenamide intermediate (Figure 2B, **11**) that is either resolved by a second thiolate (derived from a second cysteine or a LMW thiol) to form a disulfide bond with the liberation of hydroxylamine (Figure 2B, **6** and **7**), or in the absence of a thiol, a sulfinamide (Figure 2B, **12**).[82,83]

HNO can be produced from a two-step reaction in which H_2S and NO or *S*-nitrosothiols (RSNO) react to form thionitrous acid (HSNO). HSNO subsequently reacts with a second equivalent of HS^- to form H_2S_2 (hydrodisulfide) and HNO.[82,84] HNO can also react with molecular oxygen to generate $ONOO^-$ at diffusion-controlled rates,[85] again suggesting significant interplay among multiple RNS, ROS, RCS, and RSS species (Figure 4). HNO exemplifies the complexity of interacting oxidative small molecule stressors at the host–pathogen interface and further connects this oxidative chemistry with hydrogen sulfide homeostasis and cysteine catabolism (Figure 3).

All NO sensors that have been described to date harbor heme or 4Fe–4S cluster prosthetic groups[86,87] in which NO

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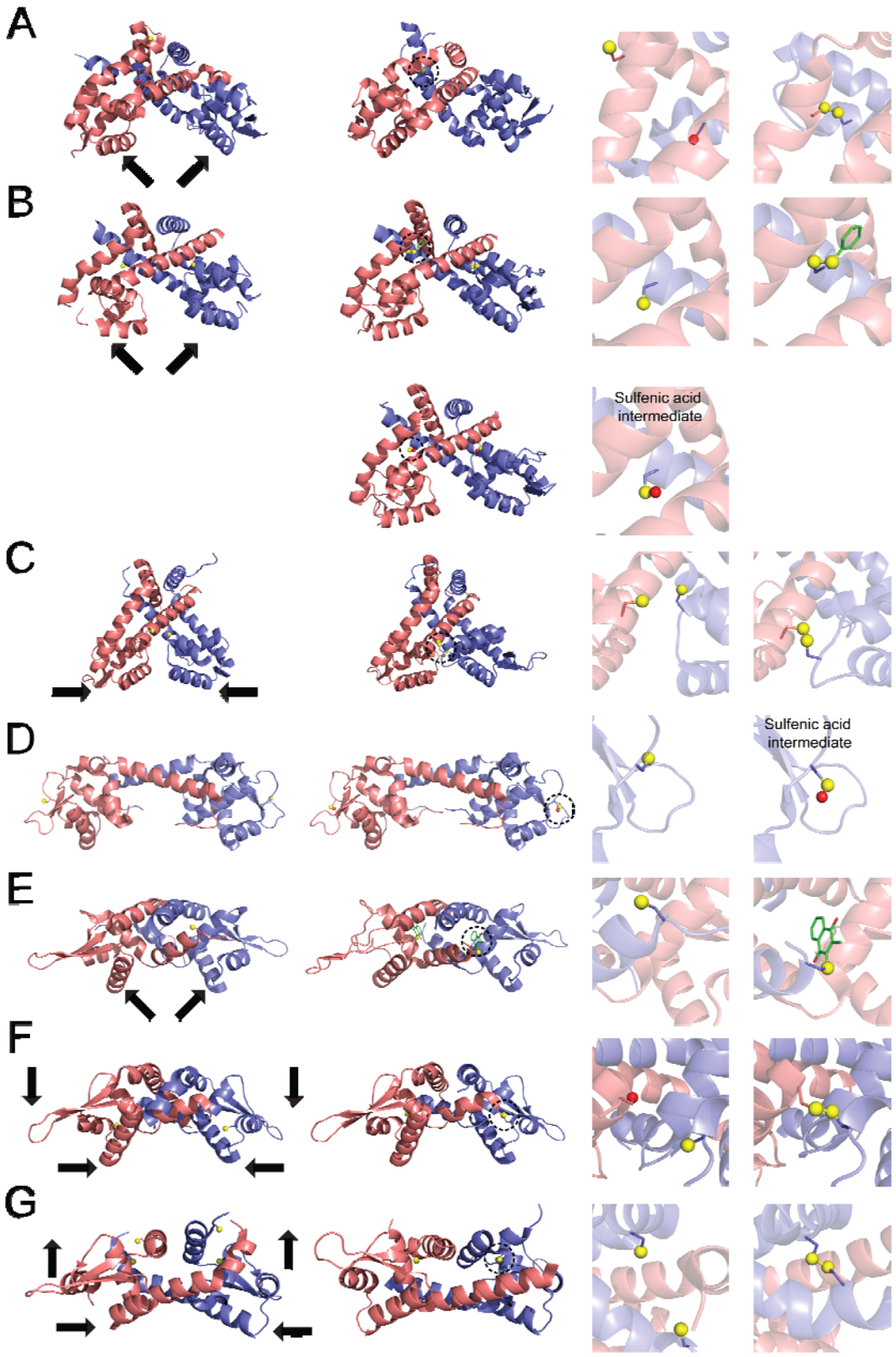

Figure 4. Ribbon representations of selected crystal structures of oxidative stress-sensing transcriptional repressors from the (A−C) MarR, (D) Rrf2, and (E−G) ArsR/SmtB families in the reduced (first and third columns) and oxidized (second and fourth columns) states. Global structures are shown (first and second columns), with the region highlighted by the black dashed circle expanded to the immediate right (third and fourth columns). All proteins shown are homodimers (protomers colored salmon and slate) and use cysteine as a regulatory switch to induce a quaternary structural change that results in dissociation from the DNA operator in the oxidized state. Arrows (first column) schematize secondary structure movement(s) upon transit from the reduced to the oxidized states, with thiol chemistry highlighted on the right (sulfurs, colored yellow; oxygens, colored red; *S*-thiolation and *S*-quinonizations, colored green). (A) *Xanthomonas campestris* OhrR [Protein Data Bank (PDB) entry 2PEX, reduced; PDB entry 2PFB, oxidized][138] with the reduced form (third column) modeled by a Cys-to-Ser substitution. (B) *S. aureus* SarZ (PDB entry 3HSE,

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Figure 4. continued

reduced; PDB entry 3HRM, sulfenylated; PDB entry 3HSR, *S*-thiolated with benzenethiol).[43] (C) *Pseudomonas aeruginosa* MexR (LNW,[153] reduced; PDB entry 3MEX,[191] oxidized). (D) *S. aureus* CymR (PDB entry 3T8R, reduced; PDB entry 3T8T, oxidized)[168] illustrating little observable structural change upon sulfenylation with *S*-thiolation known to negatively regulate DNA binding. (E) *S. aureus* QsrR (PDB entry 4HQE, reduced; PDB entry 4HQM, *S*-quinonized).[107] (F) *B. subtilis* HypR (PDB entry 4A5N, reduced; PDB entry 4A5M, oxidized),[109] with the reduced form (third column) modeled by a Cys-to-Ser substitution. (G) *Xylella fastidiosa* BigR (PDB entry 3PQJ, reduced; PDB entry 3PQK, oxidized)[128]forms an intramolecular disulfide bond *in vitro* and is regulated by sulfide stress *in vivo*.[128]

reacts rapidly with redox-sensitive transition metal complexes, e.g., Fe(III)−porphyrin complexes.[6] A bona fide cysteine thiol-dependent NO-sensing transcriptional regulator has not yet been identified, likely because of the low reactivity of NO toward cysteine thiolates. NmlR (*Neisseria* MerR-like regulator) from *Neisseria gonorrheae* and *Streptococcus pneumoniae* was originally proposed as one such thiol-based NO sensor, given that NmlR activates the expression of what was hypothesized to be an *S*-nitrosoglutathione reductase.[88,89] Indeed, it has been established that *S. pneumoniae* NmlR and *B. subtilis* AdhR can be *S*-nitrosated with a LMW *S*-nitrosothiol, GSNO, or cysteine nitrosothiol, in a transnitrosation reaction (Figure 2B, **13**).[90] The most recent work suggests that NmlR-type regulators may function in formaldehyde sensing or in other types of carbonyl or electrophile stress (RES).[91−93] Far more prevalent are NO sensors that harbor heme or 4Fe−4S cluster prosthetic groups.[86,87]

Reactive Electrophile Species (RES). RES are electron-poor small molecules that are generated during normal cellular metabolism and from lipid peroxidation and nitration of nucleic acids and lipids. They are capable of reacting with cysteine, histidine, and lysine residues in addition to DNA bases. Proteins containing a cysteine thiol with a low pK_a are particularly susceptible to electrophile stress. Although RES are not considered specific to the host−pathogen interface, some forms of oxidative stress can lead to their generation as collateral damage. For example, hypochlorite (HOCl) stress leads to an increase in the level of production of methylglyoxal (MG),[94] and thus, bacteria must be capable of clearing toxic carbonyl compounds that include the electrophiles formaldehyde and related short chain aldehydes, as well as MG. A number of recent reviews discuss RES in detail,[95−97] including mechanisms of toxicity[98] and RES-based cellular signaling.[99] AdhR[91] (aldehyde dehydrogenase regulator) from *B. subtilis* and NmlR[91−93] from streptococci and *Neisseria* ssp. are two candidate examples of RES-sensing transcriptional regulators.

Quinones make up another class of carbon-based electrophiles, are involved in electron transport and quorum sensing, and are the major redox component of soils.[100] Ubiquinone and menaquinone are produced endogenously and function in electron transport[95,101] and sulfide oxidation and detoxification.[102] Quinone derivatives are also found in a number of cytotoxic molecules, including anthracycline and cercosporin. Electrophilic quinones can undergo Michael addition with thiols, termed *S*-alkylation (Figure 2B, **15**).[103,104] Several RES-sensing transcriptional repressors have recently been characterized, including YodB, MhqR, and QsrR (Figure 4).[105−107] The regulation and homeostasis of quinones are generally poorly understood.

Reactive Chlorine Species (RCS). Reactive chlorine species (RCS), notably HOCl, are potent killers of intracellular pathogens sequestered within phagosomes.[5,108] HOCl is also the active ingredient in many disinfectants used ubiquitously in households and hospitals. The effectiveness of RCS lies in its broad spectrum chemical reactivity with virtually every cellular component, including metal centers, lipids, DNA, amino acids (including cysteines) and small molecule metabolites. Reaction with proteins leads to unfolding followed by degradation or aggregation, leading to cell death. These concepts were recently and comprehensively reviewed.[5] Several microbial RCS-sensing regulators have been identified to date and include HypR[109] from *B. subtilis*, discussed further below, and NemR[94] and RclR,[110] each found in *E. coli*.

Hydrogen Sulfide (H_2S) and Reactive Sulfur Species (RSS). Hydrogen sulfide (H_2S) is a recently classified "gasotransmitter" or signaling molecule that plays important roles in many (patho)physiological processes, including vasorelaxation, cardioprotection, and neurotransmission in mammals.[111−113] H_2S is freely membrane permeable and once inside the cell is readily deprotonated, and the more nucleophilic HS^- anion predominates. HS^- is then either assimilated or, in some organisms, effluxed via active transport[114] and has been the subject of numerous recent reviews.[112,113,115,116] At increased concentrations, H_2S poisons cytochrome *c* oxidase of the electron transport chain. H_2S is produced endogenously via the transsulfuration pathway involving cysteine or cystine[2] and 3-mercaptopyruvate (3-MP) and the action of cystathionine β-synthase (CBS),[117] cystathionine γ-lyase (CSE),[111] and 3-mercaptopyruvate sulfurtransferase (3-MST).[118] However, the relationship between mammalian-derived H_2S and bacterial pathogens is completely unknown but is of interest because in several prominent human microbial pathogens, including *S. aureus*, *E. coli*, *P. aeruginosa*, and *Bacillus anthracis*, H_2S in concert with NO, plays a protective role in resistance to generalized antibiotic-derived oxidative stress via an as yet unknown mechanism.[119]

Reactive sulfur species (RSS) is a general term given to sulfite and bisulfite, as well as sulfane sulfur-containing species, including hydrogen sulfide, persulfides (RSSH), polysulfides [$RS(S_n)SR$, where $n \geq 1$], and hydrogen polysulfides [$RS(S_n)H$, where $n > 1$]. Hydrogen polysulfides are estimated to be present in mammalian cells at micromolar concentrations, a finding consistent with an emerging picture of sulfane sulfur species as primary RSS derived from H_2S, rather than H_2S itself.[2] Cysteine *S*-sulfhydration is becoming an increasingly recognized cysteine PTM^{ox} (Figure 2B, **10**)[2,120−123] despite the lack of direct reactivity between H_2S and cysteine thiols.[121,124] Rather, *S*-sulfhydration must be catalyzed by oxidized metal ion complexes, e.g., Fe(III)−porphyrin complexes, that catalyze the one-electron oxidation of H_2S to the $HS^{-\bullet}$ radical or via reaction of the more electrophilic cysteine species, most notably a cysteinesulfenic acid or other more oxidized sulfur species,[125] including organic and inorganic per- and polysulfides.[113,125] A number of specific *S*-sulfydration targets have been identified in mammalian cells, including protein tyrosine phosphatases and nuclear factor κB (NF-kB).[126,127] In bacteria to date, two sulfide stress-sensing transcriptional regulators

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have been characterized and include *Agrobacterium tumefaciens* BigR[128] and *S. aureus* CstR,[124] which are discussed further below.

REGULATORY MECHANISMS OF CYSTEINE THIOL OXIDATION-SENSING TRANSCRIPTIONAL REPRESSORS

The ability of the bacterial cell to counteract attempts of the host to use oxidative stress to attenuate viability requires that the cell "sense" or detect a specific stress and turn on a transcription profile that leads to an adaptive response. Here we discuss structural features of a number of oxidation-sensing transcriptional regulators that collectively highlight the chemical and mechanistic diversity of this adaptive response to specific chemical stimuli (Figure 4). A common feature is negative allosteric regulation (inhibition) of DNA operator binding by the regulator upon specific cysteine thiol modification, leading to dissociation from the DNA and transcriptional derepression of downstream genes, the products of which mount the cellular response to a specific oxidative insult.

ROS-Inducible MarR Family Regulators. The MarR (multiple-antibiotic resistance regulator) family of transcriptional regulators is named for *E. coli* MarR[129] and contains a number of well-characterized oxidative stress sensors that employ cysteine oxidative chemistry to allosterically inhibit DNA operator binding. MarR proteins are homodimeric, roughly triangularly shaped, and all α-helical repressors characterized by a C-terminal dimerization domain and an N-terminal winged helix−turn−helix (wHTH) DNA-binding domain (Figure 4A−C). MarR proteins involved in oxidative stress sensing contain either one or two regulatory cysteines but are characterized by a broad range of thiol perturbations and regulatory structural mechanisms in response to ROS. These include intra- and interprotomer disulfide bond formation, *S*-thiolation, cysteine phosphorylation, and *S*-quinonization, the latter of which occurs in response to RES.

OhrR. OhrR (organic hydrogen peroxide resistance regulator) is the most extensively characterized oxidative stress-sensing transcriptional regulator of the MarR family. OhrR regulates the expression of the ROOH detoxification system that includes OhrA, a peroxiredoxin that employs thiol chemistry to catalyze the reduction of ROOH to the less toxic alcohol.[130] OhrRs have been described in a number of organisms, including *B. subtilis*,[131] *Xanthomonas campestris*,[132] *Enterococcus faecalis*,[133] *S. aureus*,[134] *Deinococcus radiodurans*,[135] *P. aeruginosa*,[136] and *A. tumefaciens*.[137] The sensing mechanism typically involves one or more cysteines that engage in disulfide bond formation, *S*-thiolation, or sulfenamide formation, resulting in an ≈28° rigid body rotation of the wHTH DNA-binding domain relative to the dimerization domain, and dissociation from the DNA operator (Figure 4A).[43,138] It is hypothesized that all OhrRs and related transcriptional regulators undergo a similar conformational change.

B. subtilis OhrR harbors a single cysteine (Cys15) that mediates a response to cumene hydrogen peroxide (CHP) and hypochlorite stress.[49,50,139] Although it was originally proposed that the PTM^{ox} responsible for negative regulation of DNA binding and transcriptional regulation was *S*-sulfenylation of this single cysteine,[139] subsequent studies revealed that sulfenylation was necessary but not sufficient to trigger derepression and required *S*-thiolation by cysteine, coenzyme A, or a previously unknown thiol,[50] later identified as bacillithiol (Figure 1E).[22,27] In OhrR reacted with CHP in the absence of LMW thiols, or from cells depleted of LMW thiols by diamide, significant cyclic sulfenamide was detected, which results when Cys15-sulfenate condenses with a neighboring backbone amide group (Figure 2A, **8**); this modification can also be reversed by reduction by thiol−disulfide exchange, although the kinetics are slow.[50] In any case, it appears that OhrRs must first form a cysteine sulfenic acid that is free to react with the nearest free thiol, either LMW or within the protein, or condense with the peptide backbone. Promiscuity here may provide the cell with a more rapid response rather than requiring a specific *S*-thiolation.

SarZ. These findings with OhrR were further extended by crystallographic studies in the single-cysteine OhrR homologue SarZ (staphylococcal accessory protein Z) from *S. aureus*.[43] Here, SarZ was crystallized in three different states, including the reduced, sulfenylated, and *S*-thiolated forms. Comparison of these structures revealed that the reduced and sulfenylated adducts of SarZ adopted similar structures, mirroring other MarRs in an active DNA-binding conformation (Figure 4B). Consistent with this, sulfenylation of SarZ fails to negatively regulate DNA operator binding *in vitro*. However, formation of a mixed disulfide with benzene thiol to create *S*-thiolated SarZ induces a large conformational change projected to reduce DNA binding affinity (Figure 4B). Oxidation in the presence of coenzyme A also leads to dissociation from the DNA.[43] Although benzene thiol is not likely to be biologically relevant, these studies are consistent with an emerging picture that shows that sulfenylation in and of itself is not sufficient to induce a regulatory conformational switch, requiring subsequent cysteine *S*-thiolation to drive transcriptional derepression.[43] The high intracellular concentrations of various LMW thiols, coupled with the high reactivity of sulfenylated cysteine, will facilitate this in cells. SarZ is one of three global stress regulators in *S. aureus*, in addition to MgrA and SarA, that mount a comprehensive oxidative stress response.[140−142]

MgrA. MgrA (multiple-gene regulator A) controls the expression of ≈350 genes, with little overlap between SarA/Z and MgrA regulons,[140−142] and is required for virulence and disease progression.[134,140,143,144] This regulator contains a single cysteine residue near the dimer interface, similar to *B. subtilis* OhrR, which upon sulfenylation was shown initially to negatively regulate DNA binding to the MgrA-*sarV* promoter region;[134] however, subsequent studies revealed that the formation of an interprotomer disulfide bond was the major form of MgrA isolated from cell extracts.[145] Interestingly, MgrA is subjected to other types of post-translational modifications that significantly expand the regulatory repertoire of this global regulator. For example, two serine phosphorylation sites have been identified in MgrA, each of which appears to regulate DNA binding affinity at different promoters.[146] In addition, MgrA is also subject to cysteine phosphorylation, a PTM that is generally considered rare, which also negatively regulates DNA binding.[147] This study identified a cysteine kinase/phosphatase pair, Stk1/Stp1, that controls the phosphorylation state of not only MgrA but also the oxidation-sensing cysteines in both SarA and SarZ in *S. aureus*.[147] The extent to which differential PTMs of this oxidation-sensing Cys in these global regulators control virulence and antibiotic and oxidative stress resistance in *S. aureus* has not yet been established but is of interest given that other pathogens encode one or a small number of kinase/phosphatase pairs as documented virulence factors.[148]

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MexR. MexR (multidrug efflux operon repressor) from *P. aeruginosa* provides another example of how formation of an *inter*protomer disulfide bond drives negative regulation of the *mexAB-oprM* operon. This operon encodes a multidrug efflux system, capable of effluxing a diverse range of antibiotics, including tetracycline, chloramphenicol, β-lactams, trimethoprim, and others.[149−151] MexR was initially identified by homology to MarR from *E. coli*,[152] and the structure is highly similar to those of other MarR repressors (Figure 4C).[153] Each protomer of MexR contains two cysteine residues, and biochemical studies reveal that the formation of one or two interprotomer disulfide bonds effects regulation of DNA operator binding by MexR. The cysteine pair possesses a redox potential of −155 mV,[141] similar to that of the master oxidative stress sensor OxyR from *E. coli*, −185 mV (see Figure 1A).[154] This is consistent with the idea that many antibiotics induce cellular redox stress through the formation of reactive oxygen species[155] and thus protect the cell against a range of structurally diverse compounds. *E. coli* MarR itself was recently shown to be subjected to Cu(II)-dependent disulfide bond formation across a tetramer interface in the presence of clinically important antibiotics, thereby sequestering the DNA-binding helices and abrogating DNA binding.[156]

MosR. In addition to those regulators that employ *inter*protomer disulfide bonds to effect allosteric switching, a MarR family regulator from *M. tuberculosis* that forms a regulatory *intra*protomer disulfide bond was recently characterized. MosR (*M. tuberculosis* oxidative stress regulator) regulates transcription of an uncharacterized putative oxidoreductase, Rv1050. MosR contains four cysteine residues per protomer and forms two intraprotomer disulfide bonds, one between Cys10 and Cys12 and the other between Cys96 and Cys147. The Cys10−Cys12 intraprotomer disulfide is the primary regulatory modification that occurs upon exposure to H_2O_2 stress *in vivo* and negatively regulates DNA binding.[157,158] Companion crystallographic and computational studies of the reduced DNA-bound and oxidized forms of MosR revealed an ≈25° rotation of the α4 DNA-binding helices in each protomor,[158] a structural rearrangement similar to the one that occurs in *B. subtilis* OhrR[138] and *S. aureus* SarZ (Figure 4A,B).[43] Interestingly, mutation of Cys12 to serine maintains the responsiveness of MosR to H_2O_2 stress *in vivo*, suggesting the possibility of an alternative mechanism of regulation involving only the remaining cysteine, Cys10, which is conserved in other MarRs, including MgrA and OhrR.[158] This suggests that oxidative perturbation of Cys10 through *S*-thiolation may indeed be relevant, but further studies are required to confirm this.

ROS-Sensing Non-MarR Family Regulators. Although the canonical MarR dimer fold appears to be prevalent among one- and multiple-cysteine ROS sensors, other protein scaffolds are also capable of incorporating the same chemistry or a similar chemistry to elicit a response to ROS. It is also the case that not all MarR family repressors are ROS sensors. For example, the Zn(II) uptake regulator, AdcR (adhesin-competent repressor), from Streptococci binds Zn(II) and activates DNA binding to repress the expression of a zinc uptake transporter that brings Zn(II) into the cell.[159,160]

OxyR. OxyR is a well-characterized global ROS regulator found exclusively in Gram-negative bacteria and a member of the LysR family of transcriptional activators.[154] OxyR activates expression of catalase, alkylhydroperoxide reductase (AhpCF), and proteins involved in thiol−disulfide homeostasis. This occurs in response to peroxide, superoxide, and nitrosative stress through reversible disulfide bond formation,[154,161,162] *S*-sulfenylation, *S*-nitrosation,[163,164] or *S*-thiolation.[165] Although OxyR is widely accepted as a paradigm peroxide sensor required when cells are grown under oxygenated conditions, when *E. coli* is grown anaerobically using nitrate as a terminal electron acceptor, OxyR is reported to function as an NO sensor, regulating the expression of a distinct subset of genes.[163] It has also been shown that modification of only one of the two cysteines (the more N-terminal one in *E. coli*, Cys199) is both necessary and sufficient to regulate DNA binding.[164] Consistent with this finding, other OxyR proteins harbor a single cysteine residue that is necessary and sufficient to regulate DNA binding, a notable example of which is *D. radiodurans* OxyR.[166] Although the high-resolution structure of intact OxyR is still not available, the structures of the reduced and disulfide-oxidized regulatory domains are, which provides strong support for a structural switching model concomitant with Cys198−Cys208 disulfide bond formation.[162] How sulfenylation versus disulfide bond formation versus *S*-nitrosation converts OxyR from a repressor into a transcriptional activator at specific promoters is not yet known but clearly involves a change in the DNA-binding footprint and recruitment of RNA polymerase to OxyR-regulated promoters via a physical interaction with the RNAP α-subunit.[167]

CymR. CymR is the master regulator of cysteine sulfur metabolism and regulates the expression of more than 300 genes in *S. aureus*. CymR has been structurally characterized in both *S. aureus*[168] and *B. subtilis*[169] and adopts a canonical Rrf2 family fold, first characterized in the global regulator of Fe−S status in *E. coli*, IscR, which reversibly binds a single 2Fe−2S cluster that drives regulation of DNA operator binding.[170] CymR harbors no such cofactors, and studies of *B. subtilis* reveal that CymR senses cellular cysteine status by forming a protein−protein complex with CysK, an *O*-acetylserine (OAS)-thiol-lyase.[171] OAS is the substrate for the CysK-CysE cysteine synthase complex, and when OAS levels are low, CysK stabilizes the CymR−DNA complex, which represses the transcription of cysteine catabolism genes. When OAS levels rise, CysK binds OAS, leading to disassembly of the CymR−DNA complex. The available structure of *B. subtilis* CymR provides limited insight into how CysK activates DNA binding.[169] However, recent studies of *S. aureus* CymR suggest a thiol-specific oxidation mechanism in which a single Cys (Cys25) in the N-terminal wHTH DNA-binding domain can be sulfenylated *in vitro* by H_2O_2 (Figure 4D), which like OhrR, is not sufficient to negatively regulate DNA binding; subsequent *S*-thiolation by coenzyme A enhances DNA dissociation and transcription derepression via a poorly understood mechanism.[168] We point out that Cys25 is not conserved in other closely related CymRs from *B. subtilis* and *Listeria innocua*, suggesting that this oxidation-sensing mechanism may not be a general property of all CymRs.

RES-Sensing Regulators. *AdhR and NmlR.* RES such as formaldehyde, methylglyoxal, and quinones react directly with cysteine thiols in an *S*-alkylation reaction through Michael addition (Figure 2B, **15**). Genes that encode formaldehyde dehydrogenases and short chain aldehyde oxidoreductases are often inducible, necessitating the presence of a specific regulator that directly senses these electrophiles. This process is arguably best understood in *B. subtilis*, where it has been established that AdhR (aldehyde dehydrogenase regulator) controls the expression of AdhA, a formaldehyde dehydrogen-

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ase, and YraA, a cysteine protease, which are proposed to repair and degrade, respectively, electrophile adduction products.[91] *B. subtilis* AdhR is homologous to NmlRs discussed above and harbors a single cysteine residue that is essential for transcriptional activation of the *adh* operon. It has been suggested that this Cys reacts directly with formaldehyde in a Michael addition reaction to form an *S*-hydroxymethylthiol (Figure 2B, **14**), which in the presence of an exogenous amine or the primary α-amine could form a thiazolidine-like adduct.[172] Interestingly, *E. coli* harbors a formaldehyde sensor FmrR, a single cysteine-containing repressor from the tetrameric helical bundle CsoR family[172] that, like in *B. subtilis*, regulates the expression of a putative NAD-dependent formaldehyde dehydrogenase.[173] The nature of the cysteine thiol adduct formed in cells under carbon electrophile stress in AdhR and FmrR is not yet known, nor are their structures available.

YodB, MhqR, and QsrR. Reactive aromatic electrophiles from the quinone family can react directly with cysteine thiols or via general disulfide stress, thus eliciting a regulatory response to RES. Several homologous single cysteine-containing ArsR family[a] transcriptional regulators, including *B. subtilis* YodB,[105,174] *B. subtilis* MhqR,[106] and *S. aureus* QsrR,[107] have been characterized, and each undergoes a specific thiol modification. YodB forms an interprotomer disulfide bond,[174] while QsrR undergoes *S*-quinonization (Figure 4E).[107] MhqR was identified in a proteomic study that examined the effects of catechol or 2-methylquinone stress in *B. subtilis* and harbors a single cysteine residue, like YodB and QsrR. The DNA binding activity of the MhqR is likely regulated by *S*-quinonization or formation of an interprotomer disulfide bond. Examination of the structures of the reduced and *S*-quinonizated forms of QsrR reveals a 10° rigid body rotation of one protomer relative to the other, coupled with a 9 Å increase in the separation of the DNA-binding helices of the wHTH motif in the alkylated and underivatized reduced forms (Figure 4E), thus providing a plausible mechanism for the regulation of DNA binding by RES.[107] This global structural transition in QsrR upon *S*-quinonization is strongly reminiscent of that which occurs upon Zn(II) binding for the zinc-sensing metalloregulatory protein from *S. aureus*, CzrA, which leads to dissociation of the apo-CzrA−DNA operator complex.[32]

RCS-Sensing Regulators. *HypR.* *B. subtilis* HypR (hypochlorous acid regulator) is a member of the ArsR/SmtB[a] family[175,176] and regulates transcription of the putative nitroreductase YfkO through the formation of an interprotomer disulfide bond. HypR contains two cysteines, Cys14 and Cys49, that upon oxidation from HOCl stress form an interprotomer disulfide bond between Cys14 and Cys49′. The pK_a values of Cys14 and Cys49 are 6.4 and 8.5, respectively, suggesting that Cys14 is initially sulfenylated and then subsequently attacked by Cys49′ as the resolving cysteine. Disulfide bond formation results in a conformational change in HypR that pulls the α4 and α4′ helices ≈4 Å closer, moving them out of register relative to consecutive major grooves on the same face of the pseudosymmetric DNA operator (Figure 4F). Cys14 is stabilized by a hydrogen bonding network that is lost upon oxidation.[109]

RclR and NemR. *E. coli* RclR[110] is a member of the AraC family of transcriptional activators that regulates genes required for survival under RCS. Activation is thought to be achieved by the formation of an intramolecular disulfide bond following cysteine oxidation in RclR.[94] NemR, first characterized from *E. coli*, is a member of the tetracycline repressor (TetR) family,[177] contains six cysteines, and responds to HOCl and *N*-chlorotaurine, but not ROS or RNS. NemR controls the regulation of *N*-ethylmaleimide reductase and glyoxalase 1, enzymes responsible for the detoxification of methylglyoxal and other reactive electrophiles, via a currently uncharacterized PTM[ox] of a single cysteine residue.[94] It is possible that NemR forms either an intra- or intermolecular disulfide bond with another cysteine, becomes *S*-thiolated, and/or condenses with the peptide backbone to form a cyclic sulfenamide (Figure 2B, **8**), similar to the process that occurs in *B. subtilis* OhrR.[50]

RSS-Sensing Regulators. The sensing of reactive sulfur species[3] and hydrogen sulfide stress by transcriptional regulatory proteins further expands the repertoire of cysteine thiol chemistry in transcriptional regulatory proteins. Reversible *S*-sulfhydration[121,178] is a cysteine PTM[ox] that has been observed in a number of proteins, including cysteine desulfurases, sulfide:quinone oxidoreductases, rhodaneses, sulfurtransferases,[2] and coenzyme A disulfide reductases,[179] and proteomic approaches that allow profiling of *S*-sulfhydration in cells have just begun to appear.[2,121] However, only recently has this PTM[ox] been described as a regulatory modification for bacterial transcriptional regulators *in vivo*.[124]

To date, two transcriptional repressors that sense RSS have been described. These are the ArsR/SmtB family[175,176] transcriptional regulator BigR (biofilm-associated growth repressor) found in the plant pathogens *Xylella fastidiosa* and *A. tumefaciens*[128,180] and CstR (CsoR-like sulfurtransferase repressor) from *S. aureus*.[124,181] In *X. fastidiosa*, BigR controls expression of an operon consisting of three putative membrane proteins (XF0766−XF0764) and a candidate sulfur dioxygenase (XF0768) in response to hydrogen sulfide stress under anaerobic, biofilm-inducing conditions. DNA binding is negatively regulated by formation of an intraprotomer disulfide bond between two critical cysteine residues *in vitro* (Figure 4G), leading to derepression of the BigR regulon.

The second RSS-sensing repressor, CstR, is a per- and polysulfide sensor from *S. aureus* that regulates the transcription of the divergently transcribed *cst* operon, which is induced by sulfide or inorganic polysulfide stress.[124] Three genes encode a nearly complete sulfide oxidation system[122] and include a three-domain sulfurtransferase (CstA[182]), sulfur dioxygenase-rhodanese fusion protein (CstB), and sulfide:quinone oxidoreductase.[124,181] CstR is a member of the CsoR/RcnR family of transcriptional regulators, characterized by an unusual disc-shaped homotetrameric, helical bundle architecture that lacks a canonical DNA-binding domain[172,183] and is the first CsoR family member to be characterized that is not regulated by transition metals.[124,181] The DNA binding activity of CstR is negatively regulated by the formation of interprotomer di-, tri-, and tetrasulfide bonds (RS-S_n-SR′, where n = 0−2) across the dimer interface following reaction with inorganic polysulfides (Na_2S_4) or organic persulfides [glutathione persulfide (GSSH)] *in vitro*. Although derivatization of the more N-terminal cysteine (Cys31) is sufficient for negative regulation of DNA binding *in vitro*,[184] *in vivo* studies reveal that both reactive (Cys31) and resolving (Cys60) cysteines are essential for inducing the operon under sulfide stress.[124] The regulatory modification in cells is unknown for CstR but is predicted to involve a Cys31−Cys60′ interprotomer disulfide bond.

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SUMMARY AND PERSPECTIVES

There is much to be learned regarding our understanding of cysteine thiol chemistry and PTM^{ox} and the interplay among myriad reactive small molecules that define the oxidative stress component of the host−microbial pathogen interface. The ongoing development of mass spectrometry (MS)-based thiol proteomic approaches[185] that can be used to identify and quantify, on a proteome-wide scale, a specific PTM^{ox} in response to a specific stressor promises new insights into the cellular response to a specific oxidative insult. These methods, in principal, will also allow the direct determination of the specific thiol modifications of transcriptional regulatory proteins in bacterial cells. More importantly, they will allow the elucidation of the degree of global, proteome-wide overlap among a number of distinct PTM^{ox} events, e.g., sulfenylation[186] versus *S*-sulfhydration[2,121] versus *S*-nitrosation,[163,187] in response to a particular stressor. Although operon inducer specificity can be readily probed using conventional transcriptomics[124] and related proteomics approaches, structural methods and direct determination of PTM^{ox} in regulatory proteins isolated from cells[11,188] are required to unravel sensing mechanisms.

The precise factors that control the specificity of a particular thiol for a given oxidant remain incompletely understood. For example, the RSS sensor CstR from *S. aureus* detects sulfide stress but is not activated by other common oxidative stressors, including NO, selenite, HOCl, H_2O_2, and paraquat.[124] This inducer specificity may well be a common characteristic of specific cysteine-containing oxidative stress sensors that can not be explained by simple tuning of the intrinsic reactivity of a cysteine residue, e.g., specifically by the pK_a. Limiting cysteine reactivity to a particular compound or collection of chemically similar compounds potentially provides a more direct or tailored response to oxidative stress. To illustrate, a cysteine sequestered in a hydrophobic binding pocket may well be more reactive toward an organic hydroperoxide than a more hydrophilic oxidant such as hydrogen peroxide. Alternatively, a more solvent-exposed cysteine may be more promiscuous and ideal for sensing generalized oxidative stress. How the microenvironment surrounding a specific regulatory cysteine(s) influences the chemical reactivity and selectivity in a transcriptional regulatory protein is generally not well understood, which limits our understanding of stressor selectivity.

We also highlight the myriad response of small molecule oxidative stressors employed by host immune cells and reactions that lead to additional and often more reactive toxic compounds (Figure 3), resulting in a synergistic microbial killing effect.[8] Thus, a universal strategy used by bacteria might be to prevent broad spectrum oxidative damage through the detoxification or clearance of less reactive oxidative "parent" species, e.g., H_2O_2 and NO, to minimize subsequent reactions with metals or other oxidants that lead to the generation of highly reactive, less specific oxidative species such as peroxynitrite, superoxide, and other radicals. In many cases, it is unclear if this parent stressor reacts directly with a transcriptional regulator *in vivo*. The intersection of microbiology and transcriptomics, mass spectrometry-based proteomics and metabolomics approaches, and structural biology mentioned here promises new insights into the understanding of cysteine thiol-based redox biology that operates at the host−microbial pathogen interface.[2,116,124,128,189]

AUTHOR INFORMATION

Corresponding Author

*E-mail: giedroc@indiana.edu. Telephone: (812) 856-3178. Fax: (812) 856-5710.

Author Contributions

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[a]Both *S. aureus* QsrR[107] and *B. subtilis* HypR[109] have been previously characterized as MarR family repressors. We argue these should be reclassified as ArsR/SmtB family repressors because they lack the more extensive all α-helical C-terminal dimerization domain of MarRs that give them their characteristic triangular structure and instead employ a single C-terminal α-helix (compare panels C and E of Figure 4, for example).[176] Although their winged helix−turn−helix domains are structurally homologous, ArsR family repressors can typically be distinguished from MarRs by a smaller number of residues per protomer (≈100−130 vs ≈140−160 residues).

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