

THIOL-BASED REGULATORY SWITCHES

Mark S.B. Paget

*Department of Biochemistry, School of Life Sciences, University of Sussex,
Brighton BN1 9QG, United Kingdom; email: m.paget@sussex.ac.uk*

Mark J. Buttner

*Department of Molecular Microbiology, John Innes Centre, Colney, Norwich NR4 7UH,
United Kingdom; email: mark.buttner@bbsrc.ac.uk*

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■ **Abstract** Thiol-based regulatory switches play central roles in cellular responses to oxidative stress, nitrosative stress, and changes in the overall thiol-disulfide redox balance. Protein sulfhydryls offer a great deal of flexibility in the different types of modification they can undergo and the range of chemical signals they can perceive. For example, recent work on OhrR and OxyR has clearly established that disulfide bonds are not the only cysteine oxidation products that are likely to be relevant to redox sensing in vivo. Furthermore, different stresses can result in distinct modifications to the same protein; in OxyR it seems that distinct modifications can occur at the same cysteine, and in Yap1 a partner protein ensures that the disulfide bond induced by peroxide stress is different from the disulfide bond induced by other stresses. These kinds of discoveries have also led to the intriguing suggestion that different modifications to the same protein can create multiple activation states and thus deliver discrete regulatory outcomes. In this review, we highlight these issues, focusing on seven well-characterized microbial proteins controlled by thiol-based switches, each of which exhibits unique regulatory features.

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INTRODUCTION

The cytoplasm is a highly reducing environment in which protein cysteines are kept in their thiol (-SH) or thiolate ($-S^-$) state and in which stable disulfide bonds rarely form. This environment is maintained in part by millimolar concentrations of low-molecular-weight thiol buffers such as the cysteine-containing tripeptide glutathione (11) or the structurally unrelated mycothiol (74, 75). In addition to these thiol buffers, living cells also use a variety of reductive enzymatic pathways to remove disulfide bonds from the cytoplasm. These include the ubiquitous disulfide reductase, thioredoxin, which reduces oxidized protein substrates using electrons derived from NADPH via its reactivating enzyme, thioredoxin reductase, and the less widespread disulfide reductase, glutaredoxin, which is reduced by glutathione, which is in turn reduced by an NADPH-dependent glutathione reductase (11). However, in recent years it has become clear that unwanted thiol oxidation occurs in microbial cells in several conditions, including stasis and oxidative stress (4, 22).

Oxidative Stress

An inevitable consequence of aerobic metabolism is the production of reactive oxygen species (ROS) that are capable of modifying numerous cellular components, including proteins, nucleic acids, and lipids, leading to a deterioration of cellular function usually termed oxidative stress. Oxidative stress can also be imposed on cells by the extracellular environment. To counter oxidative stress, organisms activate numerous functions that detoxify ROS and repair cellular damage. To achieve this, transducing proteins (mostly transcription factors) function as “switches” that are either activated or inactivated in response to ROS. In many cases, these switches exploit the unique chemistry of sulfur contained in cysteine residues (sometimes as part of a metal center) to flip from one state to the other on exposure to ROS. Thus, a chemical signal is transduced into biological readout through an induced conformational change in a regulatory protein or enzyme.

Oxidation States of Cysteine

Exposure of cysteine to ROS such as hydrogen peroxide can give rise to a variety of different reaction products (Figure 1), and the stability of a given product depends on the local environment of the thiol involved. One such product is sulfenic acid (RSOH), which is normally unstable and can react further, either to yield more stable derivatives of a higher oxidation state such as sulfinic acid (RSOOH) or sulfonic acid (RSO₃H) (Figure 1a), or with another thiol to yield a disulfide

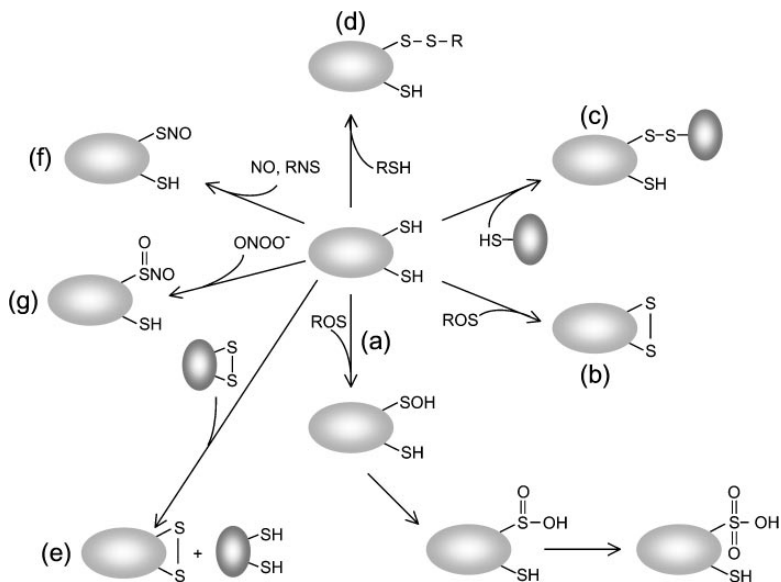


Figure 1 Chemical modifications to cysteine residues [adapted from (19)]. (a) Sequential oxidation of cysteine by ROS gives rise to sulfenic acid (R-SOH), sulfinic acid (R-SOOH), and sulfonic acid (R-SO₃H) derivatives. Alternatively, oxidation by ROS may promote (b) intramolecular cysteine disulfide bond formation within a protein, (c) intermolecular cysteine disulfide bond formation between proteins, or (d) a mixed disulfide between a cysteine-containing protein and a low-molecular-weight thiol such as glutathione or mycothiol. (e) Reduced cysteine residues can become oxidized to disulfides as a result of thiol-disulfide exchange reactions with an oxidized protein (or an oxidized low-molecular-weight thiol such as oxidized glutathione; G-S-S-G). Cysteine residues can also be modified by Reactive Nitrogen Species (RNS) such as nitric oxide (NO) to yield an S-nitrosothiol (R-SNO; f), and by peroxynitrite (NO₃⁻) to an S-nitrothiol (R-SNO₂; g).

bond (Figure 1b,c,d). Reduced cysteines can also become oxidized to disulfides through thiol-disulfide exchange reactions (Figure 1e), which are rapid and readily reversible. Through this route, a transducing regulatory protein might become oxidized because of a shift in the overall thiol-disulfide redox balance of the cell, which can potentially occur in the absence of ROS. Åslund & Beckwith (4) have coined the term “disulfide stress” to describe adverse changes in the overall thiol-disulfide redox balance. Cysteine residues can also be modified by Reactive Nitrogen Species (RNS) such as nitric oxide (NO) to yield an S-nitrosothiol (R-SNO), and by peroxynitrite (NO₃⁻) to yield an S-nitrothiol (R-SNO₂) (Figure 1f,g).

Despite intensive effort over the past decade, our understanding of the biological contribution of each of the different cysteine reaction products illustrated in

Figure 1 to cellular responses to oxidative and nitrosative stress is still limited. This point is exemplified by the recent controversy over the nature of the modification that controls OxyR activity (see below). As a further example, although sulfenic acids are known to form transiently at the active site of several enzymes as part of their catalytic cycle (7, 16, 24), it is only in the last year that the reversible formation of a stable cysteine sulfenic acid has been implicated in the activation of a transcription factor (see OhrR and OxyR, below). Here we review current knowledge of seven of the best-characterized thiol-based regulatory switches in microorganisms, covering three systems with no metal involvement (OxyR, OhrR, and CrtJ), three systems containing metals (RsrA, PerR, and Hsp33), and a thiol-based relay switch (Yap1).

SWITCHES WITH NO METAL INVOLVEMENT

OxyR

OxyR is frequently cited as an archetypal example of a redox regulatory protein, but the exact nature of the redox event that controls its activity has become the center of recent controversy (19, 30, 41). Seminal work in the laboratory of Gisela Storz (NIH, Bethesda) established that OxyR is a redox-sensing protein and that, in response to oxidation, it activates expression of a set of oxidative stress defense genes. Storz and colleagues also presented strong evidence, including crystal structures, that OxyR functions as an “on-off” switch, in which the active form of the protein contains a single intramolecular disulfide bond, and the inactive form contains reduced thiols. However, a recent paper from the group of Jonathan Stamler (Duke University, NC) challenges this view in two important respects. First, they find no evidence for intramolecular disulfide bond formation but instead demonstrate that OxyR can undergo a number of different stable chemical modifications at a single cysteine residue in response to oxidative or nitrosative stress. Second, they show that these different modifications have differential effects on OxyR DNA-binding activity and cooperativity *in vitro*. From this, they suggest that distinct chemical modifications may differentially affect OxyR activity, such that OxyR does not function as an “on-off” switch but is instead capable of mediating discrete responses to different effectors *in vivo*. To make the issues as clear as possible, we summarize the changing picture of OxyR regulation from a historical perspective and review the experimental evidence for and against the different models.

oxyR was originally identified genetically as a central regulator of the peroxide stress response in *Salmonella enterica* (then known as *Salmonella typhimurium*). In response to hydrogen peroxide, OxyR activates a regulon of >20 antioxidant genes, including loci encoding detoxifying enzymes such as hydroperoxidase I (*katG*), alkyl hydroperoxide reductase (*ahpCF*), components of disulfide reductase pathways such as glutathione reductase (*gorA*) and glutaredoxin I (*grxA*), and the regulatory locus *oxyS* (encoding a regulatory RNA) (93, 95, 104, 105).

THE THIOL-DISULFIDE SWITCH MODEL Storz et al. (92) showed that activation of OxyR by air oxidation was reversed by dithiothreitol (DTT), implicating one or more of the six cysteines in OxyR in redox regulation. In an attempt to determine which of these cysteines might be involved in the redox-sensing mechanism of OxyR, the Storz laboratory mutated each one individually to serine and assayed the mutant proteins for activity *in vivo* using a hydrogen peroxide/cumene hydroperoxide (CHP) zone-of-inhibition assay (61). From these experiments, they concluded that only one cysteine (C₁₉₉) was critical for OxyR activity and showed that a mutant form of OxyR, in which the other five cysteines were converted to serine, had wild-type activity *in vivo*, as judged by the same bioassay. As they were unable to detect any sign of intersubunit disulfide bond formation, they proposed that activation of OxyR occurred through oxidation of C₁₉₉ to a sulfenic acid. The following year, the Stamler laboratory showed that nitrosylating agents such as S-nitrosocysteine (Cys-SNO) could induce OxyR-dependent gene expression *in vivo* (although less effectively than hydrogen peroxide) and that this correlated with the ability to recover from cells OxyR protein carrying an S-nitrosothiol (-SNO) modification (although at a level of 0.1 to 0.2 SNO modifications per OxyR monomer) (39). Thus, the early observations of both groups appeared to be pointing in the same direction: that modification of a single cysteine (C₁₉₉), either to Cys-SNO or Cys-SOH, drives activation of OxyR-dependent gene expression. However, within Kullik et al. (61), there were several observations concerning C₂₀₈ that hinted that the OxyR story was likely to be less straightforward than it initially seemed. The first observation was that, in their bioassay, most of the strains gave two distinct zones of inhibition: the first zone corresponding to complete killing and the second to partial growth. The meaning of this double zone is not clear. Although the C₂₀₈S mutant appeared to have wild-type activity in this bioassay, it gave only a single zone of inhibition, not two, and this phenotype was also characteristic of the mutant form of OxyR in which five cysteines (all except C₁₉₉) were converted to serine. The second observation the Storz group made was that the C₂₀₈S mutant, although active *in vivo*, bound DNA in *in vitro* footprinting assays in a manner equivalent to the reduced rather than the oxidized protein, again suggesting that C₂₀₈ might somehow be involved in the redox switch.

Subsequently, using a different assay (induction of the *oxyS* transcript, quantified by primer extension), the Storz group concluded that C₂₀₈ was indeed critical for OxyR activation *in vivo* (103), and they went on to discard the single-cysteine oxidation model for OxyR activation when they characterized oxidized OxyR biochemically. Working with a mutant protein (“4C→A”) carrying C₁₉₉ and C₂₀₈ but having alanine substitutions of the other four “noncritical” cysteine residues, they used MALDI-TOF mass spectrometry of tryptic digests of oxidized and reduced proteins to show that a C₁₉₉-C₂₀₈ intramolecular disulfide bond was present in their samples of the oxidized protein (103). Because genetic analysis showed that a C₂₀₈S mutant retained at least some activity, whereas C₁₉₉S was inactive, they proposed that C₁₉₉ is first oxidized to a sulfenic acid and that this (presumptively

highly reactive) intermediate reacts with C₂₀₈ to form the disulfide bond. The implication of this model was that the C₁₉₉ sulfenic acid intermediate was partially active, explaining why the C₂₀₈S mutant protein (but not the C₁₉₉S mutant) could acquire some activity without being able to undergo disulfide bond formation.

Disulfide bond formation seemed to be confirmed as the mechanism of OxyR activation when, in collaboration with the Storz group, the laboratory of Seong-Eon Ryu (Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea) solved the crystal structures of both reduced and oxidized OxyR (14). Intriguingly, in the reduced form of OxyR, C₁₉₉ and C₂₀₈ were some 17 Å apart but came together to form a disulfide bond in the oxidized protein, resulting in a significant structural rearrangement in the regulatory domain of the protein. Clearly, C₁₉₉ and C₂₀₈ are too far apart in the reduced protein for disulfide bond formation to occur without some prior conformational change. How could this happen? Choi et al. (14) suggested that the proposed sulfenic acid intermediate formed by oxidation of C₁₉₉ might not be stable in the pocket in which C₁₉₉ sits in the reduced protein, due to steric constraints and the hydrophobic nature of the surrounding residues. This might force the oxidized cysteine out of the pocket, driving the structural rearrangement that ultimately leads to disulfide bond formation, different oligomeric associations, and transcriptional activation.

THE MULTIPLE ACTIVATION STATE MODEL The Stamler group turned this apparently established picture upside down in 2002 (54). They proposed that C₁₉₉-C₂₀₈ disulfide bond formation plays no role in the regulation of OxyR. Instead, they presented evidence that its activity is controlled solely through a variety of modifications to C₁₉₉ in response to different stresses: by oxidative stress to C₁₉₉-SOH; by nitrosative stress to C₁₉₉-SNO; or through disulfide stress to form a mixed disulfide with glutathione (C₁₉₉-S-S-G). Further, they proposed that these modifications activate OxyR to varying extents, allowing different redox-related signals to initiate distinct transcriptional responses. Working *in vitro* with the full-length, wild-type protein, they found no evidence for C₁₉₉-C₂₀₈ disulfide bond formation in air-oxidized OxyR (the transcriptionally active form purified by standard methods). Instead, they identified a stable, single sulfenic acid at C₁₉₉ and showed that, by itself, this modification was sufficient to activate OxyR *in vitro*. Further, following hydrogen peroxide treatment of a reduced preparation of OxyR, they used chemical analysis to identify sulfenic acid formation (arsenite/DTT reversible product) as well as higher oxidation states such as sulfenic and sulfonic acids (arsenite/DTT irreversible products) but again found no evidence for disulfide bond formation (a DTT or borohydride reversible/arsenite irreversible product). Finally, they showed that C₁₉₉ could be converted stably to C₁₉₉-SNO by treatment of the purified protein with S-nitrosoglutathione (GSNO), or stably to C₁₉₉-S-S-G by treatment with oxidized glutathione. Not all protein chemical modifications that can be generated *in vitro* necessarily occur *in vivo*. However, the Stamler group had already isolated Cys-SNO OxyR from

S-nitrosocysteine-treated cells (39), and Kim et al. (54) recovered S-glutathionylated OxyR (and no other oxidized species) from hydrogen peroxide-treated cells.

CONCLUSIONS How is it possible to reconcile the conflicting data from the Storz and Stamler laboratories? The Stamler group point out that the biochemical data of Zheng et al. (103) and Åslund et al. (5) were generated using a mutant protein in which the four “noncritical” cysteine residues (C₂₅, C₁₄₃, C₁₈₀, and C₂₅₉) were substituted by alanine and that the crystal structure of OxyR (14) was solved using a truncated protein lacking the 79-residue N-terminal DNA-binding domain (including C₂₅) and that the remaining three “noncritical” cysteine residues (C₁₄₃, C₁₈₀, and C₂₅₉) were again substituted by alanine. They suggest that these changes (and the inevitably lengthy crystalization process) may favor nonphysiological disulfide bond formation (54). However, some of the data from the Storz group have been replicated using wild-type OxyR. Tao (96) showed that, on nonreducing SDS-polyacrylamide gels, a mobility shift consistent with intramolecular disulfide bond formation occurs in oxidized wild-type OxyR but not in C₁₉₉S or C₂₀₈S mutants. Further, he found that C₁₉₉S and C₂₀₈S mutants both failed to complement the hydrogen peroxide-sensitive phenotype of *oxyR* null mutants. It is also noteworthy that, unlike the other four cysteines present in *Escherichia coli* OxyR, the two cysteines proposed by Storz and coworkers to form the regulatory disulfide bond, C₁₉₉ and C₂₀₈, are absolutely conserved in all the OxyR homologues present in the databases. Furthermore, as pointed out by Georgiou (30), Kim et al. (54) did not take special precautions, such as the quenching of free cysteines, to preserve the oxidation state of the protein during isolation, and it is well established that disulfide bonds can rearrange or become reduced upon cell lysis in the absence of such precautions (56). On balance, the work of Stamler’s group clearly expands the range of cysteine modifications that are likely to be relevant to OxyR redox sensing and signal transduction in vivo. On the other hand, their inability to detect a C₁₉₉-C₂₀₈ disulfide bond in oxidized wild-type OxyR does not exclude this species as biologically relevant.

The title of this review is “Thiol-Based Regulatory Switches.” However, the most exciting hypothesis presented by the Stamler group in Kim et al. (54) is that, rather than acting as an “on-off” switch, OxyR may have multiple activation states [depending on whether C₁₉₉ is reduced (C₁₉₉-SH), or modified to C₁₉₉-SOH, C₁₉₉-SNO, or C₁₉₉-S-S-G], leading to a whole range of activation capacities in vivo. The Stamler group found in vitro that OxyR-S-S-G and OxyR-SH show noncooperative binding, whereas OxyR-SOH and OxyR-NO show cooperative binding (54). Further, they found that OxyR-SH shows weak DNA binding, whereas OxyR-S-S-G shows high-affinity binding. From these results, they propose that S-hydroxylation, S-nitrosylation and S-glutathionylation (and, presumably, by extension, C₁₉₉-C₂₀₈ disulfide bond formation) could produce discrete outcomes in gene expression in vivo. This suggestion provides a new paradigm in redox signaling, but it remains for the moment an unproven hypothesis. As pointed out

by Helmann (41), there is currently “no link between the distinct DNA-binding properties of the various OxyR forms and evidence of differential transcriptional responsiveness, either in vivo or in vitro. Indeed, as [Kim et al. (54)] report, DNA-binding affinity does not predict activator potency.”

The work of the Stamler group clearly shows that several stable chemical modifications can be generated at C₁₉₉ and that these modifications can activate OxyR in vitro. An important but technically demanding next step will be to determine the relative in vivo significance of these different chemical modifications and of C₁₉₉-C₂₀₈ disulfide bond formation. This will require the development of reliable methods to quantify the relative abundance of each OxyR species in vivo after exposure of cells to different stresses. It will then also be possible to determine if the different modifications can induce different patterns of gene expression within the totality of the OxyR regulon. One clear way forward is to compare the response of the *Escherichia coli* OxyR regulon to oxidative stress, nitrosative stress and disulfide stress using microarrays. A microarray analysis of the *E. coli* response to hydrogen peroxide has been published (105), and the response to GSNO has recently been examined (P. Mukhopadhyay, M. Zheng, R.A. LaRossa & G. Storz, personal communication). Based on this work, OxyR does not appear to mediate a major response to nitrosative stress, whereas it clearly does mediate a major response to peroxide stress (105). Although the response to the thiol-specific oxidizing agent diamide has been examined in other microorganisms, including *Streptomyces coelicolor* (81), *Bacillus subtilis* (63) and yeast (28), similar studies have not yet been reported for *E. coli*.

Finally, the OxyR-mediated response is attenuated in vivo through the reduction of oxidized OxyR by glutaredoxin 1, and this forms part of a homeostasis feedback loop because the glutaredoxin 1 structural gene, *grxA*, is a direct target for OxyR activation. If multiple OxyR oxidation states are important in vivo, then it will also be important to understand how each oxidized species is reduced and with what kinetics.

OhrR

Dissection of the redox-sensing mechanism of OxyR has been complicated by its multitude of cysteine residues. This section is concerned with OhrR, a newly identified redox-sensing repressor that contains just a single conserved cysteine residue. OhrR regulators control, and are usually genetically linked to, genes that encode a novel type of organic hydroperoxidase known as Ohr. Originally discovered in *Xanthomonas campestris* pv. *phaseoli* (70), Ohr homologues and their linked regulators have since been identified in several Gram-positive and Gram-negative bacteria (e.g., 1, 76, 89). OhrR mediates strong induction of *ohr* in response to organic peroxides such as *tert*-butyl hydroperoxide and CHP, but only weak induction in response to hydrogen peroxide (25, 94). Evidence for the direct involvement of OhrR in redox sensing has come from recent studies in both *X. campestris* pv. *phaseoli* and *B. subtilis*. In *B. subtilis*, OhrR binds to an operator

upstream from *ohrA*, one of two *ohr* genes (25, 26). Fuangthong & Helmann (26) demonstrated that the DNA-binding activity of OhrR was lost upon treatment with *tert*-butyl hydroperoxide, CHP, or hydrogen peroxide, but was regenerated upon treatment with DTT. The thiol-specific oxidant diamide also inhibited *B. subtilis* OhrR activity, further strengthening the idea that the single cysteine in OhrR (C₁₅) was the site of modification. Fuangthong & Helmann (26) found that mutation of this cysteine to serine or glycine did not affect the ability of OhrR to bind to its operator but rendered it redox insensitive. A similar result was noted by Panmanee et al. (82) for *X. campestris* OhrR; in this case, mutation of the equivalent residue (C₂₂) to alanine led to organic peroxide-insensitive repression of *ohr* in vivo. Because the oxidation of *B. subtilis* OhrR by organic peroxides did not lead to intermolecular disulfide bond formation, Fuangthong & Helmann (26) proposed that OhrR might sense oxidants through formation of a stable cysteine sulfenic acid (C₁₅-SOH). To test this, they exploited a useful reagent called 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) that reacts with sulfhydryls or sulfenic acids to form different products with distinct absorption characteristics. Sulfhydryls react with NBD-Cl to give a thioether ($\lambda_{\text{max}} = 420 \text{ nM}$), whereas sulfenic acids react to give a sulfenate ester ($\lambda_{\text{max}} = 347 \text{ nM}$) (24). This enabled the detection of a sulfenic acid in OhrR within 30 seconds of treatment with CHP. Extended treatment with CHP led to a decrease in the amount of sulfenic acid, presumably due to the further oxidation of the cysteine residue (26). Oxidation of the reactive cysteine in OhrR to sulfenic acid has not yet been demonstrated in vivo. Exciting questions for the future are how does cysteine sulfenic acid formation prevent DNA binding, and how is oxidized OhrR reactivated in vivo.

PpsR/CrtJ

The purple non-sulfur bacterium *Rhodobacter sphaeroides* is metabolically highly versatile. It can derive energy from aerobic respiration, anaerobic respiration, and fermentation, but it can also use light as an energy source in the process of anoxygenic photosynthesis. The decision to grow photosynthetically is triggered primarily by oxygen levels, such that the photosynthetic (PS) apparatus is only produced when the oxygen tension falls below $\sim 2.5\%$ (53). The PS apparatus consists of three multimeric transmembrane complexes: the light harvesting complexes (LHI and LHII); the reaction center (RC); and the cytochrome bc₁ complex. The genes that encode the structural and assembly proteins of the PS complex are regulated primarily at the transcriptional level by a complex network of regulators and signals, as described in detail elsewhere (102). In this section we focus on one of these regulators, PpsR, which is a transcriptional repressor whose activity is modulated, in part, by reversible disulfide bond formation. PpsR functions in the oxygen-dependent regulation of many bacteriochlorophyll (*bch*) and carotenoid (*crt*) genes, as well as the *puc* operon that encodes the proteins of the LHII complex (32, 83). We also discuss the regulation of CrtJ, a PpsR orthologue present in

Rhodobacter capsulatus (85), which behaves in a manner very similar to that of PpsR but with interesting differences.

PpsR is a homotetrameric repressor that contains two PAS domains in its central region (31, 97, 106). Under aerobic conditions, PpsR binds to operators that either overlap or lie downstream of target gene promoters. Initial clues that PpsR and CrtJ might function as redox sensors came from studies with CrtJ. Ponnampalam & Bauer (84) found that the DNA-binding activity of CrtJ increased fivefold when buffers were saturated with oxygen and decreased in the presence of the reducing agent sodium dithionite, suggesting that CrtJ might contain a redox switch, although no redox metals, heme groups, or flavin groups were present in the purified protein. More detailed investigations by Bauer's group using thiol-trapping approaches showed that the oxidized form of CrtJ contained an intramolecular disulfide bond and that this formed in an oxygen-dependent manner both in vitro and in vivo (67). A disulfide bond also forms in PpsR under aerobic conditions, although this influences the DNA-binding ability of PpsR to a lesser degree (66). Two cysteines are conserved between CrtJ and PpsR and were therefore considered the likely redox-sensing residues: C₂₅₁ and C₄₂₄ in PpsR, and C₂₄₉ and C₄₂₀ in CrtJ. Mutation to alanine of either C₄₂₀ in CrtJ or C₄₂₄ in PpsR significantly decreased the DNA-binding activity of the repressors in vitro (66, 67). Significantly, a CrtJ C₂₄₉A mutant retained a greater level of activity than a C₄₂₀A mutant. This is reminiscent of OxyR mutagenesis experiments in which mutation of C₁₉₉ gave a more severe in vivo phenotype than mutation of C₂₀₈, its proposed disulfide partner in the oxidized active protein (61, 103). As with OxyR (54), this raises the possibility that CrtJ activity might be modulated by oxidation of C₄₂₀ alone.

CrtJ AND PpsR RESPOND SPECIFICALLY TO MOLECULAR OXYGEN Importantly, the disulfide bond in CrtJ and PpsR appears to occur specifically in response to molecular oxygen rather than to other oxidants. For example, exposure of reduced CrtJ to oxygen led to disulfide formation within 30 min, whereas the addition of as much as 1 mM hydrogen peroxide did not stimulate the oxidation of CrtJ (67). This is entirely consistent with the biological role of CrtJ and PpsR, but it raises interesting questions as to how this specificity is achieved. Further, the midpoint potential for the oxidation of CrtJ is -180 mV, and yet a stable disulfide bond appears to form in the reducing environment of the cytoplasm (-222 to -224 mV). These observations suggest that CrtJ is not in redox equilibrium with the rest of the cytoplasm and that it responds specifically to oxygen levels. The mechanism of CrtJ and PpsR oxidation is not clear, but it is tempting to speculate that the two PAS domains are somehow involved, given that PAS domains are frequently involved in redox sensing (97, 106). Oh & Kaplan (77) suggest that the quinone pool might play a key role in modulating the redox status of CrtJ/PpsR. This is attractive because the redox status of the quinone pool is closely linked to oxygen consumption.

AppA-A REGULATOR OF PpsR ACTIVITY In *R. sphaeroides*, PpsR activity is modulated by another regulator called AppA, although an AppA homologue is not

present in *R. capsulatus*. *appA* was identified in a screen for multicopy suppressors of mutations in the two-component *prpB-prpA* regulatory system (33) and was subsequently shown to be essential for the induction of PS expression in response to anoxia. A genetic link between *appA* and *ppsR* was established when it was found that *appA* was dispensable for PS expression in a *ppsR* null mutant and that all photosynthesizing suppressor mutations of *appA* mapped to *ppsR* (34). Gomelsky & Kaplan (34) extended this study by expressing *R. sphaeroides* genes in a heterologous host, *Paracoccus denitrificans*, demonstrating that *ppsR* could repress expression of a *bchF::lacZ* fusion and that *appA* antagonized this repression.

PpsR SENSES OXYGEN AND LIGHT THROUGH TWO DISTINCT MECHANISMS AppA modulates the DNA binding activity of PpsR in response to either oxygen levels or light using two distinct mechanisms. The regulation of PpsR activity in response to oxygen levels appears to be controlled by AppA through its disulfide reductase activity. Masuda & Bauer (66) demonstrated that either dark-adapted or light-excited reduced AppA could break the disulfide bond in oxidized PpsR. However, the reverse reaction, the oxidation of reduced PpsR by oxidized AppA, was not detected.

Light-dependent regulation of PpsR activity is based on the behavior of AppA as an antirepressor. Masuda & Bauer (66) demonstrated that AppA binds to PpsR to form a 1:2 AppA:PpsR complex that is unable to bind PpsR operator sites. Blue light, however, prevents the formation of this complex by exciting a flavin cofactor in AppA, presumably leading to a conformational change. This fits well with the observed repression of PS expression in response to blue light (90). Furthermore, blue-light repression under semiaerobic conditions was seen in *R. sphaeroides*, which contains AppA, but not in *R. capsulatus*, which does not (8). Therefore, AppA appears to decrease the DNA-binding activity of PpsR in two ways, by binding to it to form an inactive complex, or by reducing a disulfide bond that is essential for its full activity.

SWITCHES CONTAINING METALS

Hsp33—A Chaperone with a Redox Switch

Although Hsp33 is not a regulator of gene expression, it is included in this review because it offers important insights into how metal-coordinating cysteine residues can play key roles in thiol-based regulatory switches. Chuang & Blattner (15) identified *hsp33* (originally named *hslO*) as one of 26 new genes that were induced by the heat-shock-specific sigma factor, σ^{32} , in *E. coli*. Because the products of heat-shock genes so often play roles in protein refolding, Bardwell and colleagues investigated whether Hsp33 possessed chaperone activity and found that it was indeed able to prevent the thermal aggregation of model unfolded substrates (50). Further, an *hsp33* null mutation caused a 0.5°C drop in maximal growth temperature,

and the maximal growth temperature dropped an additional degree when combined with a *trxB* (thioredoxin reductase) mutation, which caused increased thioredoxin-dependent oxidation of cytoplasmic proteins. The *trxB hsp33* double mutant was also extremely sensitive to hydrogen peroxide. Together, these results suggest that Hsp33 plays a key role in protecting proteins from the adverse effects of oxidative stress and heat stress (50).

Although Hsp33 behaved like a typical chaperone, its activity was regulated in a novel way. The Bardwell group noticed that the chaperone activity of Hsp33 was dramatically decreased by reducing agents such as DTT (50), which was surprising given that Hsp33 resides in the reducing environment of the cytoplasm. This inactivation was fully reversed by the addition of oxidized glutathione or hydrogen peroxide, raising the possibility that Hsp33 activity was regulated by a redox switch. In its active, oxidized form, Hsp33 was found to contain two disulfide bonds, involving cysteine residues that are conserved in Hsp33 homologues from a wide range of bacteria (50). These cysteines are present in the C-terminal domain of Hsp33 in a C₂₃₂XC₂₃₄ and a C₂₆₅XXC₂₆₈ cluster. In their reduced thiolate form, these four cysteines coordinate a tightly associated zinc ion (49, 50) in a novel zinc-binding motif. Upon exposure to oxidants such as hydrogen peroxide, two disulfide bonds are formed, C₂₃₂-C₂₃₄ and C₂₆₅-C₂₆₈, and the zinc ion is released (6). Zinc appears to play dual roles in the function of Hsp33. Zinc enhances the proteolytic stability of Hsp33, as seen from comparative studies of zinc-loaded, reduced Hsp33 and zinc-free, reduced Hsp33 (49). Zinc also plays an important role in the activation process itself. Zinc-loaded, reduced Hsp33 can be activated by hydrogen peroxide at a much faster rate than zinc-free, reduced Hsp33. There are several possible explanations for these results: Zinc may help keep the reactive cysteines in a suitable alignment for correct disulfide bond formation; zinc may play a role in the catalytic activation of Hsp33 itself; and/or zinc might alter the reactivity of the conserved cysteines by reducing their pK_a, keeping them in their reactive thiolate form.

OXIDATION TRIGGERS Hsp33 DIMERIZATION Although disulfide bond formation is necessary for Hsp33 activation, it is not sufficient. Careful comparison of the kinetics of Hsp33 oxidation and the kinetics of Hsp33 activation revealed that an additional step was required, which turned out to be the dimerization of oxidized monomers (36). Fluorescence studies, which allowed the simultaneous monitoring of dimerization and disulfide bond formation, indicated that dimerization is the rate-limiting step in the activation of Hsp33 by hydrogen peroxide. Graumann et al. (36) proposed that the zinc-loaded, reduced cysteine domain blocks the dimerization domain, and that disulfide bond formation and zinc release cause a structural rearrangement that allows the dimerization domains to interact. The overall purpose of the thiol-based redox switch in Hsp33 might therefore be to precipitate a major structural change in the protein, which somehow allows a higher-order complex to be formed. Interestingly, dimerization was also stimulated by heat, which may be significant for the faster activation of Hsp33 under heat

stress (36). This again underlines the close link between oxidative stress and the heat-shock response.

σ^R -RsrA

Unusually among redox regulatory proteins, σ^R , from the mycelial, antibiotic-producing bacterium *S. coelicolor*, was first characterized biochemically rather than genetically, and orthologues have since been characterized in related, pathogenic actinomycetes such as *Mycobacterium tuberculosis* (52a, 64, 86). σ^R was identified in preparations of *S. coelicolor* RNA polymerase holoenzyme, and the *sigR* gene was cloned using a degenerate oligonucleotide designed from the N-terminal sequence of the purified protein (51, 80). The role of σ^R as a key regulator of the oxidative stress response was revealed by phenotypic analysis of a constructed *sigR* null mutant, which was somewhat sensitive to the superoxide-generating, redox-cycling compounds menadione and plumbagin, and was particularly sensitive to the thiol-specific oxidant diamide (80). The diamide-sensitive phenotype suggested that *sigR* mutants might be unable to respond to adverse changes in the cytoplasmic thiol–disulfide redox balance, the condition termed disulfide stress. This hypothesis was borne out by the demonstration of lowered levels of cytoplasmic disulfide reductase activity in *sigR* mutants (80). Leading on from this, Paget et al. (80) made a simple but important observation. They showed that, in response to disulfide stress (imposed using diamide), disulfide reductase activity rises sharply in wild-type *S. coelicolor*, but there is no such response in a *sigR* mutant. In *Streptomyces*, all of the measurable disulfide reductase activity is accounted for by the thioredoxin system (2, 17). Paget et al. (80) made the connection between the *sigR* mutant phenotype and disulfide reductase activity when they showed that *trxBp1*, one of the two promoters of the thioredoxin reductase thioredoxin (*trxBA*) operon, is a direct biochemical target for σ^R -containing RNA polymerase (Figure 2). Further, they showed that *trxBp1* promoter activity was rapidly and massively induced by the addition of diamide to wild-type mycelium but remained uninduced in the *sigR* null mutant, suggesting that the induction of the *trxBp1* promoter accounted for all of the previously observed induction of enzymatic activity.

REGULATION OF σ^R ACTIVITY The second σ^R target promoter to be identified, *sigRp2*, lay upstream of its own structural gene, *sigR*, thereby establishing a positive feedback loop for its own synthesis (80) (Figure 2). It thus became clear that, in order to prevent an upward spiral of σ^R synthesis, there must be a negative regulator in place to ensure that σ^R is switched on only when necessary and to ensure that its activity is effectively switched off when the disulfide stress has been dealt with. This key negative regulator was identified as RsrA (regulator of Sigma R), a zinc-containing, σ^R -specific anti- σ factor that is encoded by the gene lying immediately downstream of *sigR* (52). Anti- σ factors are proteins that inhibit σ factor activity either by binding to it and preventing its interaction with core RNA polymerase, or by binding to the σ factor when it is part of the

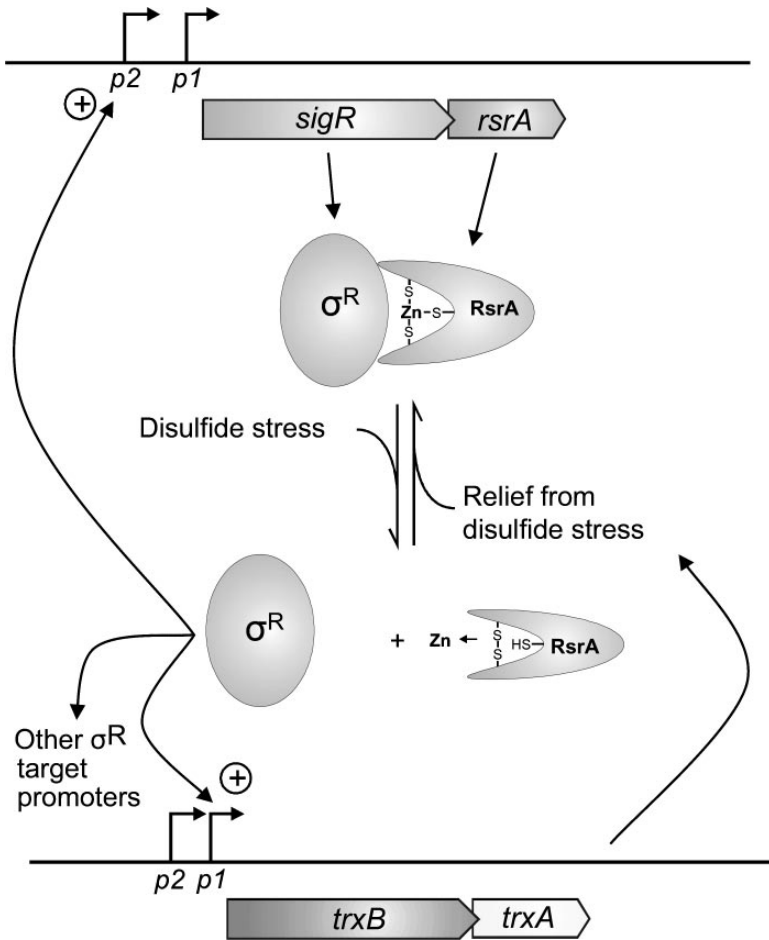


Figure 2 A model for the regulation of σ^R activity in response to disulfide stress [adapted from (79)]. The thiol-disulfide status of *S. coelicolor* is controlled by a novel regulatory system consisting of a σ factor, σ^R , and RsrA, a zinc-containing, redox-sensitive, σ^R -specific, anti- σ factor. Under reducing conditions, RsrA binds to σ^R and prevents it from activating transcription. Exposure to disulfide stress induces the formation of an intramolecular disulfide bond in RsrA, which causes it to lose its affinity for σ^R , releasing σ^R to activate transcription of >30 genes and operons, including *trxBA*. Increased *trxBA* expression in turn leads to the thioredoxin-dependent reduction of oxidized RsrA back to its σ^R -binding conformation, thereby shutting off σ^R -dependent transcription. In addition, σ^R positively autoregulates expression of the *sigR-rsrA* operon. Consequently, disulfide stress not only activates σ^R posttranslationally but also induces its de novo synthesis.

holoenzyme form, thereby preventing promoter binding (40, 45). Purified RsrA can bind tightly to σ^R and inhibit σ^R -directed transcription in vitro. However, RsrA can only perform this function under reducing conditions (Figure 2). Kang et al. (52) showed that, in the absence of thiol reductants such as DTT, RsrA can neither bind to σ^R nor inhibit σ^R -directed transcription. Moreover, if *rsrA* is deleted from the *S. coelicolor* chromosome, σ^R target promoters are constitutively expressed at the fully induced level (78). In other words, the regulation of σ^R activity by disulfide stress appears to be mediated solely by RsrA, with RsrA itself acting as the direct sensor of the thiol-disulfide redox status of the cell. Indeed, unlike σ^R , which contains no cysteines, RsrA, a protein of only 105 residues, contains seven cysteines and forms intramolecular disulfide bonds in the absence of thiol reducing compounds (52). Figure 2 illustrates the current model for the σ^R -RsrA regulatory switch. σ^R protein is present in the hyphae all of the time but under normal reducing conditions is held inactive in an RsrA: σ^R complex. σ^R is released during disulfide stress as a direct consequence of the inactivation of RsrA through intramolecular disulfide bond formation. σ^R is then free to associate with core RNA polymerase and activate transcription of its target genes, including *trxBA* and other thiol-disulfide oxidoreductase genes [for a detailed description of the genes of the σ^R regulon, see (79, 81)]. At least in vitro, oxidized RsrA is a direct biochemical substrate for purified thioredoxin, the product of the *trxA* gene (52). If the thioredoxin system also reduces (reactivates) RsrA in vivo, this would allow it to rebind σ^R and shut down the response, thereby creating a simple homeostasis feedback loop in which the σ^R regulon is regulated in response to changes in the thiol-disulfide redox status of the hyphae.

In *E. coli*, OxyR mediates responses to both disulfide and peroxide stress (5). In contrast, it is noteworthy that *Streptomyces* appears to separate these two responses. None of the >30 σ^R targets identified is associated with peroxide stress (81). Indeed, the alkylhydroperoxidases and catalases identified in *Streptomyces* have instead been shown to be regulated by OxyR, Fur, OhrR, and σ^B homologues (13a, 13b, 37, 37a, 37b). Thus, the σ^R -RsrA switch appears to specifically mediate responses to disulfide stress, implying that peroxide stress and disulfide stress are not always encountered together.

THE ROLE OF ZINC Metals analysis showed that overexpressed RsrA, purified from *E. coli*, contains one Zn(II) ion per RsrA monomer (78; W. Li & C. Kleanthous, personal communication). Zn(II) appears to have a relatively small role in sigma factor binding and in anti-sigma factor structure but plays a critical role in determining the kinetics of RsrA oxidation.

Removal of zinc from RsrA does not prevent binding to σ^R (W. Li & C. Kleanthous, personal communication). RsrA that cannot bind zinc can be generated by alkylating all seven cysteines with iodoacetamide (RsrA-7CAM). Like native (zinc-containing) RsrA, RsrA-7CAM can form a complex with σ^R on nondenaturing gels but, unlike native RsrA, RsrA-7CAM cannot inhibit σ^R activity in an

in vitro transcription assay (i.e., in the presence of core RNA polymerase). Presumably, fully alkylated RsrA cannot function as an effective anti-sigma factor because it cannot compete effectively with core RNA polymerase for σ^R , whereas native RsrA can. Moreover, preliminary experiments suggest that the CD spectra of reduced, native RsrA, and RsrA-7CAM are very similar, showing primarily β -sheet structure. In contrast, the CD spectrum of RsrA changes substantially upon formation of a single disulfide bond, with a shift to α helical character, and the formation of this single disulfide alone [trapped by alkylation of the other five reduced cysteines (RsrA-5CAM)] is sufficient to prevent RsrA from binding to σ^R (W. Li & C. Kleanthous, personal communication).

Whereas zinc has comparatively subtle effects on RsrA binding to σ^R , it has dramatic effects on the kinetics of RsrA oxidation (W. Li & C. Kleanthous, personal communication). Reduced apo-RsrA, separated from DTT by FPLC gel filtration, is substantially air-oxidized on elution from the column and completely oxidized within 5 h, whereas no oxidation is detected after 5 h when native RsrA is treated in the same way. Like the redox-sensing chaperone Hsp33 (6) and eukaryotic metallothionein (65), RsrA releases zinc upon thiol oxidation (W. Li & C. Kleanthous, personal communication). Thus, formation of a single intramolecular disulfide bond appears to occur concomitantly with zinc release, a dramatic change in RsrA structure, and loss of σ^R -binding activity (Figure 3).

IDENTIFICATION OF ESSENTIAL CYSTEINES Mutational analysis showed that three cysteine residues (C₁₁, C₄₁, C₄₄) and one histidine residue (H₃₇) are individually essential for anti-sigma factor function in vivo and in vitro (78; M.S.B. Paget, unpublished data). Three of these residues (H₃₇, C₄₁, C₄₄) correspond to an invariant HisXXXCysXXCys motif present in all known RsrA-related proteins (29, 78, 79). In contrast, the other four cysteines (C₃, C₃₁, C₆₁, C₆₂) are collectively dispensable both for redox sensing and anti-sigma factor function (78).

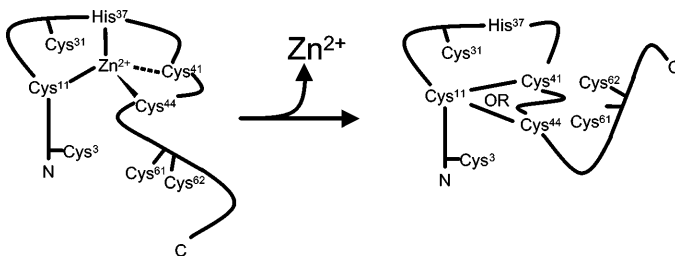


Figure 3 A speculative model for the redox inactivation of RsrA. In the reduced state, C₁₁, H₃₇, C₄₁, and C₄₄ coordinate zinc. In response to oxidative stress, an intramolecular disulfide forms between C₁₁-C₄₁ or C₁₁-C₄₄, and this oxidation event occurs concomitantly with zinc release, a dramatic change in RsrA structure, and loss of σ^R -binding activity (W. Li & C. Kleanthous, personal communication).

MAPPING THE FIRST DISULFIDE BOND Using a combination of iodoacetamide modification, proteolysis, and Q-TOF mass spectrometry to study the oxidation of apo-RsrA, Li and Kleanthous (personal communication) identified the first disulfide bond as a mixture of C₁₁-C₄₁ and C₁₁-C₄₄ (Figure 3). The three cysteines involved correspond to the three cysteines that are individually essential for anti-sigma factor function. Whether the RsrA redox switch is genuinely degenerate, or whether this mixed product arises by partial isomerization of the first disulfide bond remains to be determined.

A MODEL FOR THE RsrA REDOX SWITCH Taken together, the cysteine mutagenesis, zinc studies, and disulfide bond mapping suggest the following speculative model. In the reduced state, C₁₁, H₃₇, C₄₁, and C₄₄ coordinate zinc (Figure 3). In response to oxidative stress, an intramolecular disulfide forms between C₁₁-C₄₁ or C₁₁-C₄₄, and this oxidation event occurs concomitantly with zinc release, a dramatic change in RsrA structure, and loss of σ^R -binding activity (W. Li & C. Kleanthous, personal communication). The zinc content of individual RsrA cysteine and histidine mutants has not been examined, and so the assignment of C₁₁, H₃₇, C₄₁, and C₄₄ as the ligands to zinc is based on the fact that these residues are individually essential for anti-sigma factor function, and that H₃₇, C₄₁, and C₄₄ correspond to the invariant HisXXXCysXXCys motif present in all known RsrA-related proteins.

PerR

Bacillus subtilis PerR is the best-characterized example of a family of peroxide-sensing repressors that control the expression of peroxide resistance genes. The PerR family forms an evolutionary offshoot of the Fur superfamily of metalloregulators. Each member of this superfamily consists of an N-terminal DNA-binding domain that contains a helix-turn-helix motif, and a C-terminal domain that usually contains two metal ions coordinated by cysteines. One of the metal-binding sites coordinates a structural zinc ion (3, 48), whereas the other site tends to be more promiscuous, probably reflecting its regulatory role. In the case of PerR, there is evidence that the regulatory site can be occupied either by Fe(II) or Mn(II) and that, crucially, the metal content dictates the ability of PerR to sense peroxides. Although the focus of this section is the seminal work on the *B. subtilis* PerR repressor, other members of the PerR family, some with distinct properties, have recently been identified in a wide variety of organisms, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *S. coelicolor* (37, 44, 55).

The first clue that a repressor was involved in the peroxide stress response in *B. subtilis* was the isolation, in 1994, of a hydrogen peroxide-resistant mutant (MA991) in which several peroxide resistance proteins were overproduced, including the major catalase (KatA), a DNA protection protein (MrgA), and an alkyl hydroperoxidase (AhpCF) (38). In John Helmann's laboratory, it had earlier been demonstrated that the gene encoding one of these proteins, *mrgA*, was subject

to metal regulation—*mrgA* was induced in postexponential phase, but only when levels of manganese and iron were low (12). In 1995, Helmann's group further strengthened the possible link between metal regulation and the peroxide stress response when they identified a conserved repressor-binding site (the *per* box) that was necessary for both peroxide and metal regulation, and isolated new *trans*-acting mutants with a similar phenotype to MA991 (13). The identity of PerR was finally established a few years later when the same group showed that both the MA991 and their own *trans*-acting mutants contained causative lesions in a *fur*-like gene (9).

PerR CAN BIND Fe(II) OR Mn(II) The Helmann group has presented evidence that the PerR metalloprotein repressor can exist in two forms, PerR-Fe and PerR-Mn, differing as to whether the regulatory metal site is occupied by Fe(II) or Mn(II). Further, they have evidence that changing the metal content of the growth medium can change the intracellular form of the PerR, which in turn completely changes its hydrogen peroxide sensitivity (42). Hydrogen peroxide can induce PerR target genes in exponentially growing cells, but the metal ion content of the medium affects this induction. In the presence of added iron, induction is maximal, comparable to the level seen in a *perR* null mutant. In contrast, added manganese causes PerR to repress its target genes, irrespective of peroxide stress. These *in vivo* data are paralleled by *in vitro* results. PerR purified from overexpressing *E. coli* cells contains stoichiometric amounts of Fe(II), and PerR-Fe binds to *per* boxes in DNaseI footprinting assays and is inhibited by 10 mM hydrogen peroxide, consistent with a role as a direct peroxide sensor. However, the coordinated Fe(II) can be efficiently displaced by Mn(II) and, although PerR-Mn also binds tightly to *per* sites, it is insensitive to added hydrogen peroxide, even at levels up to 75 mM (42). Thus, it seems likely that PerR can exist in two forms *in vivo*, one that is responsive to peroxide stress (PerR-Fe) and one that is insensitive to peroxides (PerR-Mn).

CAN THE DNA-BINDING SELECTIVITY OF THE MANGANESE AND IRON FORMS OF PerR DETERMINE THE PEROXIDE RESPONSIVENESS OF INDIVIDUAL PerR TARGET GENES? A further exciting twist to this story emerged when Fuangthong et al. (27) showed that, although both the *perR* and *fur* genes are members of the PerR regulon, they are not induced by hydrogen peroxide. Moreover, they are only repressed by PerR when manganese, not iron, is added to the growth medium. This led the Helmann group to propose that PerR-Fe and PerR-Mn might differ in their DNA-binding specificities, allowing genes to be differentially regulated depending on which form of PerR they are targeted by (27, 42). This exciting hypothesis has clear parallels with the proposal of Stamler's group that OxyR might be able to elicit different genetic outputs depending on the nature of the cysteine modification that activates it (54).

How PerR actually senses peroxides and why PerR-Fe and PerR-Mn appear to differ in their sensitivity are questions that remain to be answered, but Helmann's

group have put forward several speculative models (42). Peroxide regulation is likely to involve the regulatory metal-binding site, including the metal itself and the two conserved cysteine ligands, which are arranged in a CysXXCys motif. In the first model, Fe(II) is oxidized to Fe(III), leading to its dissociation. In the second, Fe(II) promotes hydroxyl radical formation that in turn oxidizes and inactivates PerR. This kind of metal-catalyzed oxidation of the protein could take many forms (even including cleavage of the peptide backbone), some of which would not be reversible *in vivo*. In the third, preferred model, cysteine thiolate ligands coordinated to the regulatory metal ion react directly with hydrogen peroxide. This leads to disulfide bond formation and release of the associated metal ion, as described for Hsp33 (49, 50). Evidence in support of thiol oxidation comes from unpublished mutagenesis studies cited by Mongkolsuk & Helmann (69). They find that at least one of the cysteine residues can be replaced with a non-thiol metal ligand such as histidine, leaving a protein that is active in DNA binding but insensitive to hydrogen peroxide. A thiol-based mechanism has also been proposed for the regulation of a PerR homologue—CatR from *S. coelicolor*. CatR regulates the expression of the major vegetative catalase (CatA) in *S. coelicolor* in response to peroxide stress, and oxidized CatR loses its ability to bind to the *catA* operator (37). To investigate this oxidation event, Hahn et al. (37) made use of a reagent called 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonate (AMS), a reagent of ~500-Da mass that covalently modifies reduced cysteines in proteins to give a visible mobility shift on SDS-polyacrylamide gels. *In vitro* studies using pure protein showed that, in contrast to reduced CatR, hydrogen peroxide-treated CatR did not exhibit any retardation upon AMS treatment, implying that all four cysteine residues in oxidized CatR are involved in disulfide bond formation. In summary, it appears that the PerR family has evolved from a superfamily of metal-binding proteins to a family of regulators that use their metal-coordinating cysteine ligands for a different purpose—to sense peroxides.

A THIOL-BASED RELAY SWITCH

Yap1

Yap1 plays a major role in activating gene expression in response to oxidative stress in the budding yeast, *Saccharomyces cerevisiae*. Considerable progress has been made in unravelling the mechanism of Yap1 activation, leading to several important conceptual breakthroughs. In particular, it is now clear that Yap1 senses peroxide stress and disulfide stress through two separate mechanisms and that Yap1 senses H₂O₂ indirectly, receiving a redox signal relayed by a partner protein.

Yap1 is a bZIP DNA-binding protein of the AP-1 family that was originally identified by its ability to recognize the mammalian AP-1 binding site (72). The gene was also identified independently in multicopy screens for increased resistance to a variety of unrelated toxic drugs and to cadmium (43, 87, 100). *YAP1* mutants are hypersensitive to hydrogen peroxide as well as to other ROS-producing

compounds such as menadione and methyl viologen (87, 88), and Kuge & Jones (58) showed that Yap1 mediated induction of the *TRX2* thioredoxin gene in response to hydroperoxides and diamide and that *TRX2* was essential for Yap1-dependent resistance to oxidative stress. Following the identification of this key target, the size of the known Yap1 regulon has been greatly expanded through proteomic analysis, DNA microarray-based transcriptional profiling, and the construction of large-scale transcriptional fusion libraries (10, 23, 28, 62). The regulon includes genes encoding antioxidant enzymes and components of the cellular thiol-reducing pathways, including, for example, the thioredoxin system [*TRX2* (58); *TRR1* (71)], the glutaredoxin system [*GSH1* (99); *GLR1* (35)], superoxide dismutases [*SOD1* and *SOD2* (62)], glutathione peroxidase [*GPX2* (46)], and thiol-specific peroxidases [*TSA1* and *AHP1* (62)]. The Yap1 regulon partially overlaps that of another important oxidative stress regulator in yeast called Skn7, which is a member of the response-regulator family of proteins (71).

Yap1 IS REGULATED BY NUCLEAR LOCALIZATION In response to oxidative stress, Yap1 binds to Yap1-responsive elements [consensus T(T/G)ACTAA] located upstream of target genes (58, 62, 99). Yap1-dependent transcription peaks about 30 min after treatment with 0.3 mM H₂O₂, before falling back to uninduced levels within one hour (20). However, the DNA-binding activity of Yap1 barely increases upon oxidation (58). Instead, activation of Yap1 is controlled by nuclear localization (59). The use of Yap1-GFP translational fusions revealed that Yap1 is actively transported into and out of the nucleus and that both processes occur continuously (20, 47, 59). The outcome is a relatively low nuclear level of Yap1 in unstressed cells. However, the addition of hydrogen peroxide or diamide inhibits export from the nucleus by blocking the interaction between Yap1 and the export receptor Crm1/Xpo1 (60, 101). This leads to the rapid accumulation of Yap1 in the nucleus, with kinetics that mirror the activation of Yap1-dependent transcription (20, 59).

Yap1 SENSES PEROXIDE STRESS AND DISULFIDE STRESS THROUGH TWO SEPARATE MECHANISMS The C-terminal domain of Yap1 is a key determinant in the regulated localization of Yap1 and includes a nuclear export signal embedded in a cysteine-rich domain (c-CRD), which contains three cysteines, C₅₉₈, C₆₂₀, and C₆₂₉ (Figure 4). Deletion of the nuclear export signal leads to ~tenfold higher basal levels of Yap1-dependent transcription and the accumulation of a GFP-Yap1 fusion protein in the nucleus (59). The spatial localization of Yap1 in response to diamide is determined by the oxidation state of the c-CRD. A hybrid protein consisting of the DNA-binding domain of Gal4 fused to GFP and the c-CRD was found to be dispersed throughout the cell in unstressed cells, but it concentrated in the nucleus during thiol-specific oxidative stress (59). Rather than the c-CRD carrying a unique thiol-disulfide redox switch, it appears from mutational studies that several disulfide combinations can occur among the three c-CRD cysteine residues, each able to obstruct the nuclear export signal within the c-CRD and

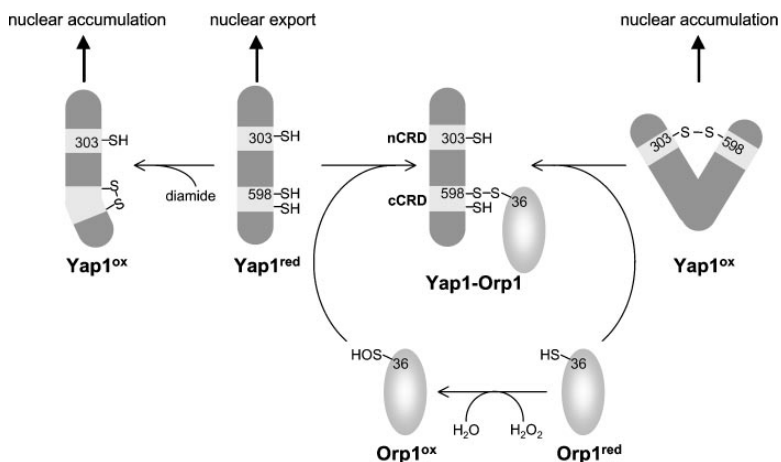


Figure 4 A model for the regulation of Yap1 in response to peroxide stress and disulfide stress (adapted from 21). The activity of the Yap1 transcription factor in *S. cerevisiae* is controlled primarily at the level of nuclear localization. Exposure to the disulfide stress-inducing oxidant diamide leads to the formation of one of several possible disulfide bonds in the C-terminal cysteine-rich domain (c-CRD), which inhibits nuclear export, promoting the transcriptional activation of Yap1 target genes. In response to hydrogen peroxide, a thiol-disulfide relay switch involving Orp1 leads to the formation of a disulfide bond between Yap1 C₃₀₃ and C₅₉₈, again leading to nuclear accumulation and activation of Yap1 target genes. Orp1 reacts with hydrogen peroxide to yield H₂O and a sulfenic acid, C₃₆-SOH. C₃₆-SOH then reacts with Yap1 C₅₉₈ in the c-CRD to form an Orp1-Yap1 mixed disulfide. Thiol-disulfide exchange results in the generation of a C₃₀₃-C₅₉₈ intramolecular disulfide bond in Yap1 and the regeneration of reduced Orp1.

thus prevent the interaction between Yap1 and the Crm1 export receptor in vivo (57).

Remarkably, although the c-CRD alone is sufficient for the regulated localization of Yap1 in response to certain oxidants, such as diamide, it is not sufficient to respond to others, such as hydrogen peroxide. Peroxide sensing requires both the c-CRD and an additional region of Yap1 called the N-terminal cysteine-rich domain (n-CRD) (Figure 4). A Yap1 mutant protein carrying a deletion of amino acids 225–335, which removes the n-CRD, remains inducible by diamide but is not inducible by hydrogen peroxide (98). Further, expression of this deletion mutant in a $\Delta yap1$ background leads to a higher level of diamide resistance than that of the wild type, but fails to restore resistance to hydrogen peroxide. The n-CRD contains another cluster of three cysteines: C₃₀₃, C₃₁₀, and C₃₁₅. Strikingly, mutation of just C₃₀₃ to alanine prevents the induction of Yap1 by hydrogen peroxide but does not prevent induction by diamide (18). These data strongly suggest that Yap1

senses peroxide stress (i.e., hydrogen peroxide) and disulfide stress (i.e., diamide) using different mechanisms, a conclusion supported by *in vivo* acid-trapping experiments performed by Toledano and coworkers (20). Treatment of yeast cells with hydrogen peroxide leads to the formation of a disulfide bond in Yap1 that causes a conformational change in the protein, as indicated by a shift in its mobility on SDS-polyacrylamide gels. Although diamide induces Yap1 activity, it does not cause this mobility shift, which suggests that the disulfide bond(s) introduced by diamide do not cause a major structural change in the protein. Toledano and coworkers (20) pinpointed C₃₀₃ in the n-CRD and C₅₉₈ in the c-CRD (Figure 4) as key cysteine residues required for the hydrogen peroxide-dependent oxidation of Yap1 since a mutant that lacked the other four cysteines in the n-CRD and the c-CRD retained the ability to undergo the redox-dependent shift in mobility in response to hydrogen peroxide. Furthermore, using a two-hybrid assay, they showed that, whereas the interaction between wild-type Yap1 and the nuclear export receptor Crm1 was inhibited by hydrogen peroxide, mutation of either C₃₀₃ or C₅₉₈ rendered this interaction insensitive to hydrogen peroxide (20).

A PARTNER PROTEIN MEDIATES OXIDATION OF Yap1 IN RESPONSE TO PEROXIDE STRESS Until recently, all of the available data pointed toward Yap1 being a direct sensor of peroxides, responding directly with the formation of an intramolecular disulfide bond between C₃₀₃ and C₅₉₈. However, in an unexpected twist, it has become apparent that Yap1 does not sense hydrogen peroxide directly and that treatment with hydrogen peroxide *in vitro* does not even result in C₃₀₃-C₅₉₈ disulfide bond formation. During their acid-trapping experiments, Delaunay et al. (21) noticed a Yap1 species that was ~20 kDa larger than Yap1 itself, which appeared and disappeared with the same kinetics as the peroxide-dependent activation of Yap1. This suggested that Yap1 might form a transient, covalent interaction with another protein. The Yap1 partner protein was identified as Gpx3, one of three yeast proteins thought to function as glutathione peroxidases. However, biochemical analysis revealed that Gpx3 uses reducing equivalents derived from thioredoxin to reduce peroxides, and therefore Gpx3 was renamed Orp1 (Oxidant receptor peroxidase 1). Importantly, in a $\Delta orp1$ mutant in which the Yap1-Orp1 complex cannot assemble, the C₃₀₃-C₅₉₈ disulfide in Yap1 does not form after hydrogen peroxide treatment and Yap1 is not activated. In contrast, Orp1 is not required for the disulfide stress response. Consequently, in an $\Delta orp1$ mutant, Yap1 is activated normally by diamide through oxidation of the c-CRD. Toledano and colleagues (21) have proposed the following model for the role of Orp1 in the activation of Yap1 by peroxides (Figure 4). Treatment of yeast cells with hydrogen peroxide leads initially to the oxidation of the active site cysteine of Orp1 (C₃₆) to a sulfenic acid, as is the usual case for peroxidases (16). Orp1 C₃₆ then reacts with Yap1 C₅₉₈ to form a mixed disulfide. Rearrangement of this disulfide by thiol-disulfide exchange leads to formation of the Yap1 C₃₀₃-C₅₉₈ intramolecular disulfide bond, thus recycling the now-reduced Orp1. The C₃₀₃-C₅₉₈ disulfide bond obstructs the nuclear export signal, preventing export of Yap1, leading to its accumulation in the

nucleus and induction of the Yap1 regulon. The purification of the Yap1-Orp1 covalent complex that allowed Orp1 to be identified was greatly facilitated by using a strain carrying a *YAP1* C₃₀₃A mutation, which caused the complex to accumulate because the thiol-disulfide exchange reaction that forms the Yap1 C₃₀₃-C₅₉₈ intramolecular disulfide bond, thereby liberating Orp1 from the complex, was blocked (21).

After its initial oxidation, an alternative pathway for Orp1 might be intramolecular disulfide bond formation as part of its peroxidase catalytic cycle, which would not lead to the activation of Yap1. However, there is evidence to suggest that Yap1 and Orp1 associate under all conditions irrespective of oxidative stress, thereby forming a complex that is primed to form the mixed disulfide in response to peroxide-specific oxidation (21).

PERSPECTIVES

This review has focused on a selection of well-studied microbial proteins, mostly transcriptional regulators, to illustrate how thiol-based regulatory switches can transduce a redox signal into a regulatory output. In conclusion, we outline themes that have emerged from research in this field and highlight future challenges. One emerging theme is that protein sulfhydryls offer a great deal of flexibility in the different types of modification they can undergo and the range of signals they can perceive. Work on OhrR and OxyR has clearly established that disulfide bonds are not the only cysteine oxidation products that are likely to be relevant to redox sensing *in vivo*. Furthermore, the concept that different stresses can result in different modifications to the same protein has gained support not only from work on OxyR but also from the analysis of Yap1 in yeast, in which a partner protein ensures that the Yap1 oxidation event induced by hydrogen peroxide is different from the oxidation event induced by other stresses. In addition, as pointed out by Helmann (41), there are strong hints that other regulators may also be able to initiate distinct programs of gene expression in response to different stresses. Although the definitive experiments have yet to be done, it seems that differences in the DNA-binding selectivity of the manganese and iron forms of the *B. subtilis* PerR regulator may determine the peroxide responsiveness of individual PerR target genes (27, 42).

A general issue for the field is the relationship between *in vitro* and *in vivo* observation. Part of this problem resides in the difficulty in generating proteins in a homogenous state *in vitro* and the current absence of reliable techniques for quantifying the extent and homogeneity of cysteine modifications *in vivo*. Further, not all protein chemical modifications that can be generated *in vitro* necessarily occur *in vivo*, and, unlike certain other regulatory modifications, such as enzymatic phosphorylation, we cannot safely assume that all modifications of proteins by ROS and RNS, even if detected *in vivo*, necessarily serve a regulatory function (19).

Another important emerging theme, highlighted by Yap1, is that thiol-based regulatory switches are not necessarily the primary sensors of the redox signal. Yap1

is regulated by disulfide bond formation in response to peroxide stress. However, the primary sensor of peroxides is Orp1, a thiol-specific peroxidase that relays the redox signal to Yap1. We suspect that this pattern will prove to be more widespread than is currently anticipated and that many more such relay switches will be identified in the future. Another currently underexplored possibility is the indirect modulation of thiol-based redox switches through thiol-disulfide exchange. Storz and colleagues showed that when *E. coli* is exposed to 200 μM hydrogen peroxide, thioredoxin becomes completely oxidized with very rapid kinetics [cited in (5)]. Further, Beckwith and colleagues (91) showed that, in a reversal of its normal in vivo role, thioredoxin, when oxidized, can actively introduce disulfides into reduced proteins. Thus, thioredoxin could potentially act as both the in vivo oxidant and reductant of regulatory proteins such as RsrA, allowing the regulator to respond indirectly to the overall thiol-disulfide redox poise of the cell.

For each regulatory switch, identification of the reductive pathway that resets the switch is of direct importance, and, if multiple activation states are important in vivo, as proposed for OxyR (54), then it will also be important to understand how each oxidized species is reduced and with what kinetics. The low-molecular-weight thiol buffers (e.g., glutathione, mycothiol) or disulfide reductase enzymes (e.g., thioredoxin, glutaredoxin) may be involved. However, genetic and biochemical investigations into the contribution of these different pathways can be complicated by overlapping substrate specificity and by compensatory changes in the expression of alternative pathways when one pathway is blocked (e.g., 68, 73). For several of the regulators (e.g., OxyR, Yap1, RsrA), the induced regulon includes components of the reductive pathway, thereby providing a homeostatic feedback loop for regulation. Dedicated reductases may also be involved, as appears to be the case for AppA, the disulfide reductase of PpsR. The further development of in vivo thiol-trapping techniques in combination with sensitive mass spectrometric methods, as exemplified in the discovery of Orp1 (21), seems the most promising approach to identify in vivo thiol-disulfide redox partners in the future.

From the three examples of metal-containing switches discussed in this review (Hsp33, RsrA, and PerR), it is clear that metal-coordinating cysteines can be involved in redox switches, undergoing redox reactions that lead to changes in protein conformation and activity. There is poor understanding of the role of these metals ions, but the limited information currently available suggests that they may serve diverse roles. In Hsp33, zinc enhances the reactivity of the cysteine ligands, whereas in RsrA zinc appears to protect the cysteine ligands from oxidation. Moreover, PerR can bind either iron or manganese, and the nature of the bound metal appears to modulate the redox-sensing capacity of the regulator.

Finally, CrtJ/PpsR shows that thiol-based regulatory switches are not confined to oxidative or nitrosative stress response pathways. These proteins regulate transcription of a variety of photosynthetic genes in *Rhodobacter* and have evolved to respond specifically to molecular oxygen, such that formation of the regulatory disulfide bond is triggered by O_2 but not by reactive oxygen species such as hydrogen peroxide. It is also noteworthy that, despite the highly reducing

cytoplasmic environment, the regulatory disulfide bond in CrtJ is stable under aerobic conditions, suggesting that CrtJ is not in redox equilibrium with the rest of the cytoplasm.

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LITERATURE CITED

1. Atichartpongkul S, Loprasert S, Vattanaviboon P, Whangsuk W, Helmann JD, Mongkolsuk S. 2001. Bacterial Ohr and OsmC paralogues define two protein families with distinct functions and patterns of expression. *Microbiology* 147:1775–82
2. Aharonowitz Y, Av-Gay Y, Schreiber R, Cohen G. 1993. Characterization of a broad-range disulfide reductase from *Streptomyces clavuligerus* and its possible role in β -lactam antibiotic biosynthesis. *J. Bacteriol.* 175:623–29
3. Althaus EW, Outten CE, Olson KE, Cao H, O'Halloran TV. 1999. The ferric uptake regulation (Fur) repressor is a zinc metalloprotein. *Biochemistry* 38:6559–69
4. Åslund F, Beckwith J. 1999. Bridge over troubled waters: sensing stress by disulfide bond formation. *Cell* 96: 751–53
5. Åslund F, Zheng M, Beckwith J, Storz G. 1999. Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. *Proc. Natl. Acad. Sci. USA* 96:6161–65
6. Barbirz S, Jakob U, Glocker MO. 2000. Mass spectrometry unravels disulfide bond formation as the mechanism that activates a molecular chaperone. *J. Biol. Chem.* 275:18759–66
7. Boschi-Muller S, Azza S, Sanglier-Cianferani S, Talfournier F, Van Dorsselaar A, Branlant G. 2000. A sulfenic acid enzyme intermediate is involved in the catalytic mechanism of peptide methionine sulfoxide reductase from *Escherichia coli*. *J. Biol. Chem.* 275:35908–13
8. Braatsch S, Gomelsky M, Kuphal S, Klug G. 2002. A single flavoprotein, AppA, integrates both redox and light signals in *Rhodobacter sphaeroides*. *Mol. Microbiol.* 45:827–36
9. Bsat N, Herbig A, Casillas-Martinez L, Setlow P, Helmann JD. 1998. *Bacillus subtilis* contains multiple Fur homologues: identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. *Mol. Microbiol.* 29:189–98
10. Carmel-Harel O, Stearman R, Gasch AP, Botstein D, Brown PO, Storz G. 2001. Role of thioredoxin reductase in the Yap1p-dependent response to oxidative stress in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 39:595–605
11. Carmel-Harel O, Storz G. 2000. Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress. *Annu. Rev. Microbiol.* 54:439–61
12. Chen L, James LP, Helmann JD. 1993. Metalloregulation in *Bacillus subtilis*: isolation and characterization of two genes differentially repressed by metal ions. *J. Bacteriol.* 175:5428–37
13. Chen L, Keramati L, Helmann JD. 1995. Coordinate regulation of *Bacillus subtilis*

- peroxide stress genes by hydrogen peroxide and metal ions. *Proc. Natl. Acad. Sci. USA* 92:8190–94
- 13a. Cho Y-H, Lee E-J, Ahn B-E, Roe J-H. 2001. SigB, an RNA polymerase sigma factor required for osmoprotection and proper differentiation of *Streptomyces coelicolor*. *Mol. Microbiol.* 42:205–14
 - 13b. Cho Y-H, Lee E-J, Roe J-H. 2000. A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor*. *Mol. Microbiol.* 35:150–60
 14. Choi H, Kim S, Mukhopadhyay P, Cho S, Woo J, Storz G, Ryu S. 2001. Structural basis of the redox switch in the OxyR transcription factor. *Cell* 105:103–13
 15. Chuang SE, Blattner FR. 1993. Characterization of twenty-six new heat shock genes of *Escherichia coli*. *J. Bacteriol.* 175:5242–52
 16. Claiborne A, Yeh JI, Mallett TC, Luba J, Crane EJ, Charrier V, Parsonage D. 1999. Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. *Biochemistry* 38:15407–16
 17. Cohen G, Yanko M, Mislovati M, Argaman A, Schreiber R, et al. 1993. Thioredoxin-thioredoxin reductase system of *Streptomyces clavuligerus*: sequences, expression and organization of the genes. *J. Bacteriol.* 175:5159–67
 18. Coleman ST, Epping EA, Steggerda SM, Moye-Rowley WS. 1999. Yap1p activates gene transcription in an oxidant-specific fashion. *Mol. Cell. Biol.* 19:8302–13
 19. Cooper CE, Patel RP, Brookes PS, Darley-Usmar VM. 2002. Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem. Sci.* 27:489–92
 20. Delaunay A, Isnard AD, Toledano MB. 2000. H₂O₂ sensing through oxidation of the Yap1 transcription factor. *EMBO J.* 19: 5157–66
 21. Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB. 2002. A thiol peroxidase is an H₂O₂ receptor and redox-transducer in gene activation. *Cell* 111:471–81
 22. Dukan S, Nyström T. 1998. Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. *Genes Dev.* 12:3431–41
 23. Dumond H, Danielou N, Pinto M, Bolotin-Fukuhara M. 2000. A large-scale study of Yap1p-dependent genes in normal aerobic and H₂O₂-stress conditions: the role of Yap1p in cell proliferation control in yeast. *Mol. Microbiol.* 36:830–45
 24. Ellis HR, Poole LB. 1997. Novel application of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydroperoxide reductase. *Biochemistry* 36:15013–18
 25. Fuangthong M, Atichartpongkul S, Mongkolsuk S, Helmann JD. 2001. OhrR is a repressor of *ohrA*, a key organic hydroperoxide resistance determinant in *Bacillus subtilis*. *J. Bacteriol.* 183:4134–41
 26. Fuangthong M, Helmann JD. 2002. The OhrR repressor senses organic hydroperoxides by reversible formation of a cysteine-sulfenic acid derivative. *Proc. Natl. Acad. Sci. USA* 99:6690–95
 27. Fuangthong M, Herbig AF, Bsath N, Helmann JD. 2002. Regulation of the *Bacillus subtilis fur* and *perR* genes by PerR: not all members of the PerR regulon are peroxide inducible. *J. Bacteriol.* 184:3276–86
 28. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, et al. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell.* 11:4241–57
 29. Gehring AM, Yoo NJ, Losick R. 2001. An RNA polymerase sigma factor that blocks morphological differentiation by *Streptomyces coelicolor* A3(2). *J. Bacteriol.* 183:5991–96
 30. Georgiou G. 2002. How to flip the (redox) switch. *Cell* 111:607–10

31. Gomelsky M, Horne IM, Lee HJ, Pemberton JM, McEwan AG, Kaplan S. 2000. Domain structure, oligomeric state, and mutational analysis of PpsR, the *Rhodobacter sphaeroides* repressor of photosystem gene expression. *J. Bacteriol.* 182:2253–61
32. Gomelsky M, Kaplan S. 1995. Genetic evidence that PpsR from *Rhodobacter sphaeroides* 2.4.1 functions as a repressor of *pu*c and *bchF* expression. *J. Bacteriol.* 177:1634–37
33. Gomelsky M, Kaplan S. 1995. *appA*, a novel gene encoding a trans-acting factor involved in the regulation of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. *J. Bacteriol.* 177:4609–18
34. Gomelsky M, Kaplan S. 1997. Molecular genetic analysis suggesting interactions between AppA and PpsR in regulation of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. *J. Bacteriol.* 179:128–34
35. Grant CM, Collinson LP, Roe JH, Dawes IW. 1996. Yeast glutathione reductase is required for protection against oxidative stress and is a target gene for yAP-1 transcriptional regulation. *Mol. Microbiol.* 21:171–79
36. Graumann J, Lilie H, Tang X, Tucker KA, Hoffmann JH, et al. 2001. Activation of the redox-regulated molecular chaperone Hsp33 - a two-step mechanism. *Structure* 9:377–87
37. Hahn J-S, Oh S-Y, Chater KF, Cho Y-H, Roe J-H. 2000. H₂O₂-sensitive Fur-like repressor CatR regulating the major catalase gene in *Streptomyces coelicolor*. *J. Biol. Chem.* 275:38254–60
- 37a. Hahn J-S, Oh S-Y, Roe J-H. 2000. Regulation of the *furA* and *catC* operon, encoding a ferric uptake regulator homologue and catalase-peroxidase, respectively, in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* 182:3767–74
- 37b. Hahn J-S, Oh S-Y, Roe J-H. 2002. Role of OxyR as a peroxide-sensing positive regulator in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* 184:5214–22
38. Hartford OM, Dowds BC. 1994. Isolation and characterization of a hydrogen peroxide resistant mutant of *Bacillus subtilis*. *Microbiology* 140:297–304
39. Hausladen A, Privalle CT, Keng T, DeAngelo J, Stamler JS. 1996. Nitrosative stress: activation of the transcription factor OxyR. *Cell* 86:719–29
40. Helmann JD. 1999. Anti-sigma factors. *Curr. Opin. Microbiol.* 2:135–41
41. Helmann JD. 2002. OxyR: a molecular code for redox sensing? *Sci STKE*. 2002(157):PE46
42. Herbig AF, Helmann JD. 2001. Roles of metal ions and hydrogen peroxide in modulating the interaction of the *Bacillus subtilis* PerR peroxide regulon repressor with operator DNA. *Mol. Microbiol.* 41:849–59
43. Hertle K, Haase E, Brendel M. 1991. The *SNQ3* gene of *Saccharomyces cerevisiae* confers hyper-resistance to several functionally unrelated chemicals. *Curr. Genet.* 19:429–33
44. Horsburgh MJ, Clements MO, Crossley H, Ingham E, Foster SJ. 2001. PerR controls oxidative stress resistance and iron storage proteins and is required for virulence in *Staphylococcus aureus*. *Infect. Immun.* 69:3744–54
45. Hughes KT, Mathee K. 1998. The anti-sigma factors. *Annu. Rev. Microbiol.* 52:231–86
46. Inoue Y, Matsuda T, Sugiyama K, Izawa S, Kimura A. 1999. Genetic analysis of glutathione peroxidase in oxidative stress response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 274:27002–9
47. Isoyama T, Murayama A, Nomoto A, Kuge S. 2001. Nuclear import of the yeast AP-1-like transcription factor Yap1p is mediated by transport receptor Pse1p, and this import step is not affected by oxidative stress. *J. Biol. Chem.* 276:21863–69
48. Jacquamet L, Aberdam D, Adrait A,

- Hazemann JL, Latour JM, Michaud-Soret I. 1998. X-ray absorption spectroscopy of a new zinc site in the Fur protein from *Escherichia coli*. *Biochemistry* 37:2564–71
49. Jakob U, Eser M, Bardwell JC. 2000. Redox switch of Hsp33 has a novel zinc-binding motif. *J. Biol. Chem.* 275:38302–10
50. Jakob U, Muse W, Eser M, Bardwell JC. 1999. Chaperone activity with a redox switch. *Cell* 96:341–52
51. Kang J-G, Hahn M-Y, Ishihama A, Roe J-H. 1997. Identification of sigma factors for growth phase-related promoter selectivity of RNA polymerases from *Streptomyces coelicolor* A3(2). *Nucleic Acids Res.* 25:2566–73
52. Kang J-G, Paget MSB, Seok Y-J, Hahn M-Y, Bae J-B, et al. 1999. RsrA, an anti-sigma factor regulated by redox change. *EMBO J.* 18:4292–98
- 52a. Kaushal D, Schroeder BG, Tyagi S, Yoshimatsu T, Scott C, et al. 2002. Reduced immunopathology and mortality despite tissue persistence in a *Mycobacterium tuberculosis* mutant lacking alternative σ factor, SigH. *Proc. Natl. Acad. Sci. USA* 99:8330–35
53. Kiley PJ, Kaplan S. 1988. Molecular genetics of photosynthetic membrane biosynthesis in *Rhodobacter sphaeroides*. *Microbiol. Rev.* 52:50–69
54. Kim SO, Merchant K, Nudelman R, Beyer WF Jr, Keng T, et al. 2002. OxyR: a molecular code for redox-related signaling. *Cell* 109:383–96
55. King KY, Horenstein JA, Caparon MG. 2000. Aerotolerance and peroxide resistance in peroxidase and PerR mutants of *Streptococcus pyogenes*. *J. Bacteriol.* 182:5290–99
56. Kishigami S, Akiyama Y, Ito K. 2001. Redox states of DsbA in the periplasm of *Escherichia coli*. *FEBS Lett.* 364:55–58
57. Kuge S, Arita M, Murayama A, Maeta K, Izawa S, et al. 2001. Regulation of the yeast Yap1p nuclear export signal is mediated by redox signal-induced reversible disulfide bond formation. *Mol. Cell Biol.* 21:6139–50
58. Kuge S, Jones N. 1994. YAP1-dependent activation of *TRX2* is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides. *EMBO J.* 13:655–64
59. Kuge S, Jones N, Nomoto A. 1997. Regulation of yAP-1 nuclear localization in response to oxidative stress. *EMBO J.* 16:1710–20
60. Kuge S, Toda T, Iizuka N, Nomoto A. 1998. Crm1 (Xpo1) dependent nuclear export of the budding yeast transcription factor yAP-1 is sensitive to oxidative stress. *Genes Cells* 3:521–32
61. Kullik I, Toledano MB, Tartaglia LA, Storz G. 1995. Mutational analysis of the redox-sensitive transcriptional regulator OxyR: regions important for oxidation and transcriptional activation. *J. Bacteriol.* 177:1275–84
62. Lee J, Godon C, Lagniel G, Spector D, Garin J, et al. 1999. Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J. Biol. Chem.* 274:16040–46
63. Leichert LI, Scharf C, Hecker M. Global characterization of disulfide stress in *Bacillus subtilis*. *J. Bacteriol.* 185:1967–75
64. Manganelli R, Voskuil MI, Schoolnik GK, Dubnau E, Gomez M, Smith I. 2002. Role of the extracytoplasmic-function sigma factor σ^H in *Mycobacterium tuberculosis* global gene expression. *Mol. Microbiol.* 45:365–74
65. Maret W. 1994. Oxidative metal release from metallothionein via zinc-thiol/disulfide interchange. *Proc. Natl. Acad. Sci. USA* 91:237–41
66. Masuda S, Bauer CE. 2002. AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in *Rhodobacter sphaeroides*. *Cell* 110:613–23

67. Masuda S, Dong C, Swem D, Setterdahl AT, Knaff DB, Bauer CE. 2002. Repression of photosynthesis gene expression by formation of a disulfide bond in CrtJ. *Proc. Natl. Acad. Sci. USA* 99:7078–83
68. Miranda-Vizueté A, Rodríguez-Ariza A, Toribio F, Holmgren A, López-Barea J, Pueyo C. 1996. The levels of ribonucleotide reductase, thioredoxin, glutaredoxin 1, and GSH are balanced in *Escherichia coli* K12. *J. Biol. Chem.* 271:19099–103
69. Mongkolsuk S, Helmann JD. 2002. Regulation of inducible peroxide stress responses. *Mol. Microbiol.* 45:9–15
70. Mongkolsuk S, Praituan W, Loprasert S, Fuangthong M, Chamnongpol S. 1998. Identification and characterization of a new organic hydroperoxide resistance (*ohr*) gene with a novel pattern of oxidative stress regulation from *Xanthomonas campestris* pv. *phaseoli*. *J. Bacteriol.* 180:2636–43
71. Morgan BA, Banks GR, Toone WM, Raitt D, Kuge S, Johnston LH. 1997. The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* 16:1035–44
72. Moye-Rowley WS, Harshman KD, Parker CS. 1989. Yeast *YAP1* encodes a novel form of the jun family of transcriptional activator proteins. *Genes Dev.* 3:283–92
73. Muller EG. 1996. A glutathione reductase mutant of yeast accumulates high levels of oxidized glutathione and requires thioredoxin for growth. *Mol. Biol. Cell.* 7:1805–13
74. Newton GL, Arnold K, Price MS, Sherill C, Delcardayre SB, et al. 1996. Distribution of thiols in microorganisms: mycothiol is a major thiol in most actinomycetes. *J. Bacteriol.* 178:1990–95
75. Newton GL, Fahey RC. 2002. Mycothiol biochemistry. *Arch. Microbiol.* 178:388–94
76. Ochsner UA, Hassett DJ, Vasil ML. 2001. Genetic and physiological characterization of *ohr*, encoding a protein involved in organic hydroperoxide resistance in *Pseudomonas aeruginosa*. *J. Bacteriol.* 183:773–78
77. Oh JI, Kaplan S. 2001. Generalized approach to the regulation and integration of gene expression. *Mol. Microbiol.* 39:1116–23
78. Paget MSB, Bae J-B, Hahn M-Y, Li W, Kleanthous C, et al. 2001. Mutational analysis of RsrA, a zinc-binding anti-sigma factor with a thiol-disulfide redox switch. *Mol. Microbiol.* 39:1036–47
79. Paget MSB, Hong H-J, Bibb MJ, Buttner MJ. 2002. The ECF sigma factors of *Streptomyces coelicolor* A3(2). In *Signals, Switches, Regulons and Cascades: Control of Bacterial Gene Expression*, ed. DA Hodgson, CM Thomas, pp. 105–25. Cambridge: Cambridge Univ. Press
80. Paget MSB, Kang J-G, Roe J-H, Buttner MJ. 1998. σ^R , an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). *EMBO J.* 17:5776–82
81. Paget MSB, Molle V, Cohen G, Aharonowitz Y, Buttner MJ. 2001. Defining the disulfide stress response in *Streptomyces coelicolor* A3(2): identification of the σ^R regulon. *Mol. Microbiol.* 42:1007–20
82. Panmanee W, Vattanaviboon P, Eiamphungporn W, Whangsuk W, Sallabhan R, Mongkolsuk S. 2002. OhrR, a transcription repressor that senses and responds to changes in organic peroxide levels in *Xanthomonas campestris* pv. *phaseoli*. *Mol. Microbiol.* 45:1647–54
83. Penfold RJ, Pemberton JM. 1994. Sequencing, chromosomal inactivation, and functional expression in *Escherichia coli* of *ppsR*, a gene which represses carotenoid and bacteriochlorophyll synthesis in *Rhodobacter sphaeroides*. *J. Bacteriol.* 176:2869–76
84. Ponnampalam SN, Bauer CE. 1997. DNA

- binding characteristics of CrtJ. A redox-responding repressor of bacteriochlorophyll, carotenoid, and light harvesting-II gene expression in *Rhodobacter capsulatus*. *J. Biol. Chem.* 272:18391–96
85. Ponnampalam SN, Buggy JJ, Bauer CE. 1995. Characterization of an aerobic repressor that coordinately regulates bacteriochlorophyll, carotenoid, and light harvesting-II expression in *Rhodobacter capsulatus*. *J. Bacteriol.* 177:2990–97
86. Raman S, Song T, Puyang X, Bardarov S, Jacobs WR, Husson RN. 2001. The alternative sigma factor σ^H regulates major components of oxidative and heat stress responses in *Mycobacterium tuberculosis*. *J. Bacteriol.* 183:6119–25
87. Schnell N, Entian KD. 1991. Identification and characterization of a *Saccharomyces cerevisiae* gene (*PARI*) conferring resistance to iron chelators. *Eur. J. Biochem.* 200:487–93
88. Schnell N, Krems B, Entian KD. 1992. The *PARI* (*YAP1/SNQ3*) gene of *Saccharomyces cerevisiae*, a *c-jun* homologue, is involved in oxygen metabolism. *Curr. Genet.* 21:269–73
89. Shea RJ, Mulks MH. 2002. *ohr*, encoding an organic hydroperoxide reductase, is an *in vivo*-induced gene in *Actinobacillus pleuropneumoniae*. *Infect. Immun.* 70:794–802
90. Shimada H, Iba K, Takamiya K. 1992. Blue-light irradiation reduces the expression of *puf* and *puc* operons of *Rhodobacter sphaeroides* under semi-aerobic conditions. *Plant Cell Physiol.* 33:471–75
91. Stewart EJ, Åslund F, Beckwith J. 1998. Disulfide bond formation in the *Escherichia coli* cytoplasm: an *in vivo* role reversal for the thioredoxins. *EMBO J.* 17:5543–50
92. Storz G, Tartaglia LA, Ames BN. 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. *Science* 248:189–94
93. Storz G, Zheng M. 2000. Oxidative stress. In *Bacterial Stress Responses*, ed. G Storz, R Hengge-Aronis, pp. 47–59. Washington DC: ASM Press
94. Sukchawalit R, Loprasert S, Atichartpongkul S, Mongkolsuk S. 2001. Complex regulation of the organic hydroperoxide resistance gene (*ohr*) from *Xanthomonas* involves OhrR, a novel organic peroxide-inducible negative regulator, and posttranscriptional modifications. *J. Bacteriol.* 183:4405–12
95. Tao K. 1997. *oxyR*-dependent induction of *Escherichia coli* *grx* gene expression by peroxide stress. *J. Bacteriol.* 179:5967–70
96. Tao K. 1999. *In vivo* oxidation-reduction kinetics of OxyR, the transcriptional activator for an oxidative stress-inducible regulon in *Escherichia coli*. *FEBS Lett.* 457:90–92
97. Taylor BL, Zhulin IB. 1999. PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol. Mol. Biol. Rev.* 63:479–506
98. Wemmie JA, Steggerda SM, Moyer-Rowley WS. 1997. The *Saccharomyces cerevisiae* AP-1 protein discriminates between oxidative stress elicited by the oxidants H_2O_2 and diamide. *J. Biol. Chem.* 272:7908–14
99. Wu AL, Moyer-Rowley WS. 1994. *GSH1*, which encodes γ -glutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation. *Mol. Cell. Biol.* 14:5832–39
100. Wu A, Wemmie JA, Edgington NP, Goebel M, Guevara JL, Moyer-Rowley WS. 1993. Yeast bZip proteins mediate pleiotropic drug and metal resistance. *J. Biol. Chem.* 268:18850–58
101. Yan C, Lee LH, Davis LI. 1998. Crm1p mediates regulated nuclear export of a yeast AP-1-like transcription factor. *EMBO J.* 17:7416–29
102. Zeilstra-Ryalls J, Gomelsky M, Eraso JM, Yeliseev A, O’Gara J, Kaplan S. 1998. Control of photosystem formation in *Rhodobacter sphaeroides*. *J. Bacteriol.* 180:2801–9
103. Zheng M, Åslund F, Storz G. 1998.

- Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* 279:1718–21
104. Zheng M, Wang X, Doan B, Lewis KA, Schneider TD, Storz G. 2001. Computation-directed identification of OxyR DNA binding sites in *Escherichia coli*. *J. Bacteriol.* 183:4571–79
105. Zheng M, Wang X, Templeton LJ, Smulski DR, LaRossa RA, Storz G. 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* 183:4562–70
106. Zhulin IB, Taylor BL, Dixon R. 1997. PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox. *Trends Biochem. Sci.* 22:331–33