Redox-operated genetic switches: the SoxR and OxyR transcription factors

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Two redox-responsive transcription regulators have been well defined in *Escherichia coli* and serve as paradigms of redox-operated genetic switches. SoxR contains iron–sulfur centers that activate the protein when they are one-electron oxidized, or nitrosylated by nitric oxide. OxyR contains a pair of redox-active cysteine residues that activate the protein when they are oxidized to form a disulfide bond.

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Cells are under oxidative stress when the concentration of pro-oxidants (e.g. oxygen radicals) exceeds the cellular capacity to dispose of them[1]. Bacteria have evolved sophisticated molecular mechanisms to monitor oxidant levels and to activate antioxidant defense genes. In particular, the enteric bacterium *E. coli* has provided an excellent model to study gene regulation in response to oxidative stress[2,3]. *E. coli* (and other bacteria) can harness the deleterious effects of various oxidants, exploiting oxidative damage in proteins as physiological signals that trigger global antioxidant responses. This article is focused on the molecular mechanisms that mediate gene regulation in response to oxidative stress by the two best characterized redox-sensing proteins, SoxR and OxyR.

The SoxR and SoxS proteins: partners in redox-sensing and global transcription activation

The exposure of *E. coli* to sublethal doses of superoxide-generating agents, such as paraquat and menadione, renders the cells resistant to higher doses of these agents[4]. The inducible resistance to superoxide-generating agents depends on the integrity of the *soxRS* locus[5,6]. The *soxR* and *soxS* genes encode two separate transcription activators that participate in a two-step activation cascade that ultimately modulates more than 16 other genes (Fig. 1; see Ref. 2 for a more detailed review). The SoxR protein is produced constitutively at a low level of ~100 molecules per cell (Elena Hidalgo and B. Demple, unpublished), and activates expression of the *soxS* gene in response to superoxide-generating agents. The activity of SoxS is regulated only by its intracellular concentration and the protein binds promoter regions of the target genes to recruit RNA polymerase. These inducible genes[2,3,7] constitute the *soxRS* regulon, which includes *sodA* (manganese-containing superoxide dismutase), *zwf* (glucose-6-phosphate dehydrogenase), *fldA* and *fldB* (two distinct flavodoxins), *fpr* (NADPH-ferredoxin reductase), *fur* (another gene regulator that is mainly involved with iron metabolism), *nfo* (DNA repair endonuclease IV), *acrAB* (an efflux pump) and *micF* (an untranslated small RNA that downregulates the expression of the porin OmpF). Still other genes are being added to this list through analysis using genome-wide gene arrays (P.J. Pomposiello and B. Demple, unpublished).

The products of the induced *soxRS* regulon act collectively to avoid the eventual oxidative damage, or repair it using an assortment of mechanisms that include: scavenging of oxidants (superoxide dismutase), DNA repair (endonuclease IV), re-reduction of oxidized metals in prosthetic groups (flavodoxin and ferredoxin reductase), reconstitution of the NADPH pool (glucose-6-phosphate dehydrogenase), reduced permeability (*micF*) and excretion of toxicants (efflux pumps). The activation of the *soxRS* target genes renders the cell resistant not only to superoxide-generating agents, but also to organic solvents, macrophage-generated nitric oxide (NO), and antibiotics[5,8]. To date, this picture has emerged from studies of only *E. coli* and *Salmonella typhimurium* but SoxR homologs have so far been found in seven other species of bacteria.

SoxR: a transcription activator with iron–sulfur clusters

SoxR is a 17-kDa polypeptide that belongs to the MerR family of transcriptional activators[9,10]. The primary structure of SoxR predicts a helix-turn-helix motif in the amino end, and this region of proteins in the MerR family appears to confer sequence-specific DNA binding. SoxR forms a dimer in solution, with each monomer containing a [2Fe–2S] cluster[11,12] (Fig. 2). The ligands for metal binding in SoxR are the only four cysteine residues in the polypeptide, in a CX_2CXCX_5C sequence (in single-letter amino acid code and where x denotes any amino acid) near the carboxyl terminus[13]; this motif is unlike that of any other known iron–sulfur site. When SoxR is stripped of the metal centers, the protein remains dimeric and retains full affinity for its target DNA sequence in the *soxS* promoter[14]. Similarly, mutation of any of the SoxR cysteine residues yields a stable dimeric protein (apo-SoxR) that lacks detectable [2Fe–2S] clusters but still binds tightly to DNA (Ref. 13). Thus, the metal centers are not required either for the initial folding of SoxR or to maintain its structure and DNA-binding affinity.

When extracted from *E. coli* using conventional techniques in the presence of atmospheric oxygen, SoxR is recovered with the [2Fe–2S] centers in the

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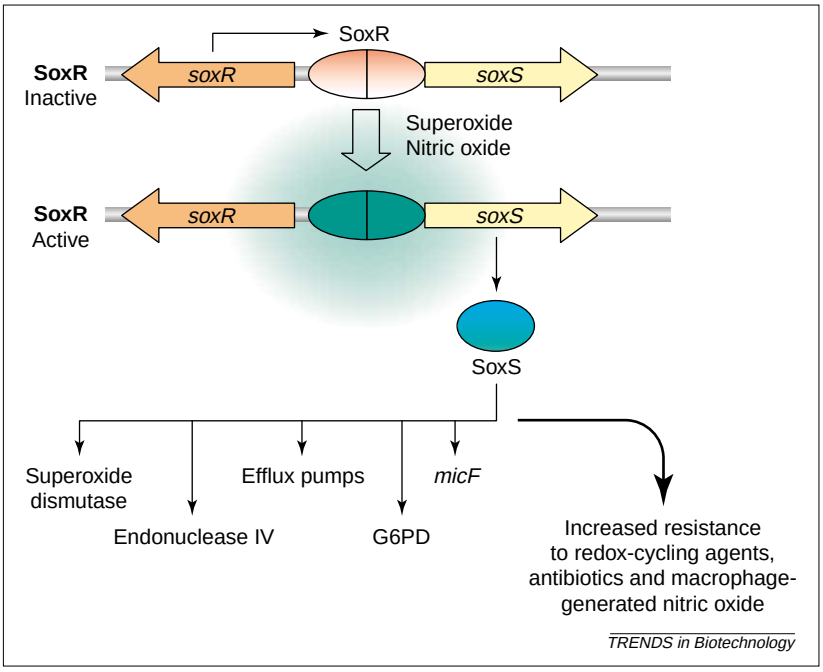

Fig. 1. The *soxRS* regulon. The *soxRS* locus is composed of the divergently transcribed *soxR* and *soxS* genes. The SoxR protein is produced constitutively and is activated upon exposure to superoxide-generating agents or nitric oxide (NO). The oxidized form of SoxR enhances the transcription of the *soxS* gene, the product of which is also a transcriptional activator. The SoxS protein activates transcription of genes that increase the resistance to oxidants. Additionally, activation of the SoxS-regulated genes increases the resistance to antibiotics and macrophage-generated NO. Abbreviation: G6PD, glucose-6-P-dehydrogenase.

oxidized (Fe^{3+}–Fe^{3+}) state (Fig. 2). This form of SoxR has powerful transcription-activating capacity (up to 100-fold) that is specific for the *soxS* promoter[14]. Adjusting the redox potential of *in vitro* transcription reactions to reduce the SoxR [2Fe–2S] clusters by one electron (to the Fe^{2+}–Fe^{3+} state) eliminates this transcriptional stimulation[15,16]. In general, Apo-SoxR is also inactive in transcription assays[14]. Similar to apo-SoxR, reduced SoxR retains high-affinity DNA-binding activity[16], which indicates that the transcriptional activation involves structural changes in the DNA–protein complex (Fig. 2).

Bound SoxR protein is localized to a site between the –10 and –35 elements of the *soxS* promoter, an unusual position for a transcriptional activator[14]. Also unusual, is the distance of 19 bp between the ends of the –10 and –35 hexamers of the *soxS* promoter, compared with 17±1 bp spacers for most *E. coli* promoters. Consistent with this unfavorable distance, transcription of *soxS* is very low in the absence of oxidized SoxR, even though the –10 and –35 elements themselves are good matches to consensus sequences and RNA polymerase binds the promoter with high affinity. A series of deletions engineered in the *soxS* promoter support the view that –10/–35 spacing is the critical regulatory feature of the *soxS* promoter[17]. Deleting 1 or 2 bp at various positions within the spacer dramatically increases SoxR-independent (basal) expression of *soxS*, and these 'normalized' *soxS* promoters are not further activated by SoxR (which still binds them tightly). Interestingly, truncation of the spacer region to 16 bp also increases basal *soxS* transcription, but SoxR represses this modified *soxS* promoter irrespective of its oxidation state.

Given that transcriptional activation by SoxR does not appear to rely on recruitment of RNA polymerase[14], we hypothesize that activated SoxR effects some form of 'remodeling' of the *soxS* promoter that compensates for its over-long –10/–35 spacer (Fig. 2). In the absence of stress, reduced SoxR binds to the *soxS* promoter without enhancing transcription. RNA polymerase can bind to the SoxR–DNA complex, but can only form a 'closed' complex. Upon exposure to superoxide-generating agents, the [2Fe–2S] clusters are oxidized, and activated SoxR stimulates RNA polymerase to form the open complex. Results from probing the SoxR–*soxS* promoter complexes with structure-sensitive reagents is consistent with this hypothesis[11], although an exact definition of the structural changes in the *soxS* promoter is lacking. A parallel hypothesis was proposed earlier for the homologous MerR protein, which is activated by binding mercury ions (Hg^{2+}) rather than a redox reaction, and has been extended recently to another *E. coli* protein, ZntR (Ref. 18).

Sensing free radical stress: distinct activation pathways for SoxR by superoxide stress or NO

The [2Fe–2S] clusters in SoxR can undergo reversible one-electron oxidation and reduction. Because the reduced [2Fe–2S] clusters are paramagnetic, these changes can be followed quantitatively by electron paramagnetic resonance (EPR) spectroscopy[11,12]. Although the normal *in vivo* level of SoxR (50–100 molecules per cell) is too low to detect by EPR, the signal for reduced SoxR can be detected in intact *E. coli* when SoxR is expressed at ~1% of total cellular protein, under which conditions its activity appears to be regulated normally. The observed EPR signal shows that the SoxR [2Fe–2S] clusters are >95% reduced during aerobic growth[15,16]. Upon exposure of the cells to paraquat or other redox-cycling compounds, the reduced signal disappears rapidly (<2 min), and reappears within a few minutes when the oxidative stress is withdrawn[19]. The kinetics of *soxS* transcription *in vivo* exactly parallel the oxidation and re-reduction of SoxR measured by EPR in intact cells[19].

The foregoing observations are consistent with oxidation of the SoxR [2Fe–2S] clusters as the essential regulatory signal in cells, but the EPR studies alone did not rule out alternative mechanisms, such as the destruction and resynthesis of [2Fe–2S] clusters in the protein. This possibility was eliminated by showing that the full EPR signal could be restored in paraquat-treated cells by permeabilization and treatment with the chemical reductant dithionite[19]. A parallel line of evidence arises from studies of mutant forms of SoxR that are constitutively active (i.e. switched on even in the absence of oxidative stress agents)[20,21]. These mutant forms become oxidized during normal aerobic growth, and one was shown to be more susceptible to oxidation *in vitro*[21].

The question of how SoxR is maintained in the reduced form is still unanswered. Recently, a SoxR-reducing activity dependent on NADPH was

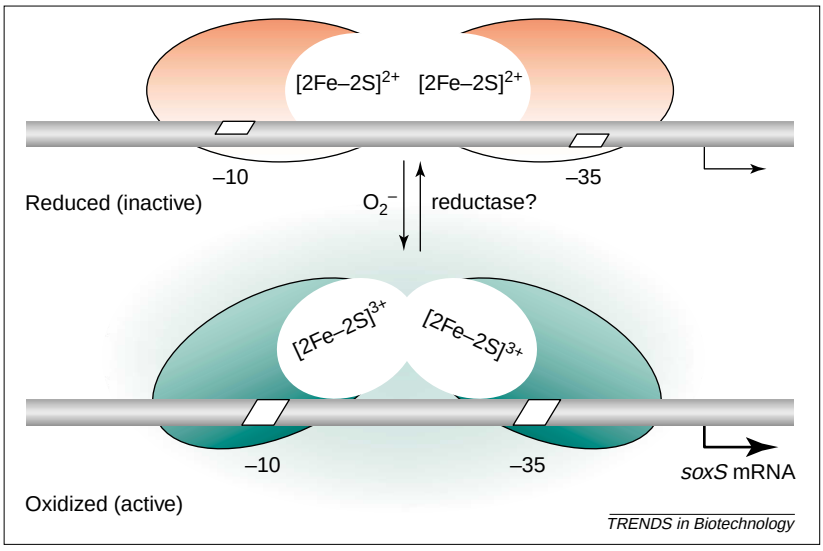

Fig. 2. Mechanism of SoxR activation by superoxide. The SoxR dimer can bind DNA in either the reduced or the oxidized form. However, only oxidized SoxR activates transcription of *soxS*. After exposure to superoxide-generating agents, the iron in the Fe–S clusters is oxidized. The model for the activation of *soxS* proposes a conformational change in SoxR that modifies the local DNA topology at the promoter and compensates for a dysfunctional spacing between promoter elements. The oxidation of SoxR is rapid and transient: after cessation of the superoxide stress, SoxR is completely re-reduced in a few minutes. The mechanism of SoxR re-reduction remains elusive.

isolated from *E. coli* but the responsible protein(s) were not characterized genetically[22]. The positive regulation of flavodoxins (products of the genes *fldA and fldB*) and ferredoxin-NADPH oxido-reductase (product of the gene *fpr*) by SoxR suggests that these proteins could be SoxR reductases. However, mutations in these genes do not alter basal or paraquat-activated *soxS* expression[7]. Moreover, incubation of oxidized SoxR *in vitro* with ferredoxin oxido-reductase, ferredoxin, and NADPH does not reduce or inactivate SoxR (Ref. 16). To address this question more generally, defects in the pentose–phosphate shunt that generates NADPH were also tested for effects on SoxR regulation. In a *zwf*-null mutant (lacking glucose-6-phosphate dehydrogenase, another SoxR-regulated function), the paraquat-inducible expression of *soxS* was only partially affected[23], but *zwf* mutants grow poorly in any case. Thus, the physiological pathway(s) that hold SoxR in the resting state remain to be established, although it is probable that an enzymatic mechanism operates to maintain reduced [2Fe–2S] clusters in intact cells.

SoxR exhibits an additional mechanism of activation by NO. Exposure of cells to pure NO gas or solutions of NO, or co-cultivation with NO-generating murine macrophages, results in the SoxR-dependent activation of *soxS* expression[24,25]. The activation of SoxR by NO is independent of the presence of oxygen; in fact, NO is a more efficient activator of SoxR in anaerobic than in aerobic conditions. Thus, oxygen-dependent NO products, such as peroxynitrite, are not involved in signaling to SoxR.

EPR spectroscopy has also been useful in dissecting the mechanism of NO-dependent activation of SoxR. The reaction of NO with iron–sulfur centers produces mixed dinitrosyl–iron–dithiol complexes with a clear EPR signature[26]. EPR analyses have shown that, both *in vivo* and *in vitro*, SoxR reacts with NO to form such dinitrosyl–iron clusters. The nitrosylated form of SoxR is nearly as active in *soxS* transcription *in vitro* as oxidized SoxR. *In vivo*, exposure of cells to a bolus of NO results in the rapid nitrosylation of SoxR (within 2 min), and when the NO is withdrawn, the EPR signal for the dinitrosyl–iron complexes disappears rapidly and is replaced by the signal for unmodified, reduced SoxR. The rise and fall of the EPR signal for nitrosylated SoxR is paralleled exactly by the increase and decrease of *soxS* transcription *in vivo*[26]. These results indicate that, *in vivo*, nitrosylated SoxR has activity comparable with that of oxidized SoxR. In summary, NO activation of SoxR appears to proceed by a chemically distinct pathway from the response to paraquat-induced oxidative stress.

The *in vitro* experiments show that nitrosylated SoxR is relatively stable and can be purified. In contrast, SoxR nitrosylation *in vivo* seems to disappear rapidly, with a half-life of just a few minutes[26]. Evidently, physiological factor(s) reverse or remove nitrosylated SoxR effectively, which accounts for the relatively inefficient activation of the *soxRS* regulon by a bolus treatment with pure NO. We tested whether some important cellular thiols [glutathione (GSH), glutaredoxin and thioredoxin] might contribute to the turnover of nitrosylated SoxR, but none of these species seemed to affect the stability of the modified protein. Nevertheless, because the level of SoxR protein remains approximately constant in NO-treated *E. coli*, it seems probable that the dinitrosyl–iron–dithiol complexes are actively removed *in vivo* and replaced by normal [2Fe–2S] clusters.

The observation that distinct forms of SoxR can activate transcription accentuates the question of how this protein transduces free radical signals into gene activation. The oxidized [2Fe–2S] centers contrast with the nitrosylated form(s) generated by NO – the former remain intact, the latter are partially destroyed. Determining the structural basis of transcription activation by these distinct forms of SoxR constitutes an important goal.

OxyR: hydrogen peroxide sensor and transcription activator

In parallel with the effects of superoxide-generating agents, low levels of hydrogen peroxide (H_2O_2) trigger peroxide resistance in *E. coli*[27]. Much of this inducible resistance depends on the *oxyR* gene, which governs a set of genes that constitute the *oxyR* regulon[28]. The OxyR protein belongs to the LysR family of transcription factors[29], and it can also respond to nitrosothiols[30]. The OxyR-activated genes include *dps* (a DNA- and iron-binding protein), *gorA* (GSH reductase), *grxA* (glutaredoxin), *katG* (peroxidase), *ahpCF* (alkyl hydroperoxide-NADPH oxido-reductase), and *fur* (an iron-binding repressor of iron transport). Many of these functions have clear roles in antioxidant defense of the cell, such as removal of H_2O_2 by catalase and the protection of DNA from oxidative attack by the Dps protein.

In addition, OxyR activates the synthesis of the small, noncoding oxyS RNA. This molecule allows OxyR to regulate as many as 20 additional gene

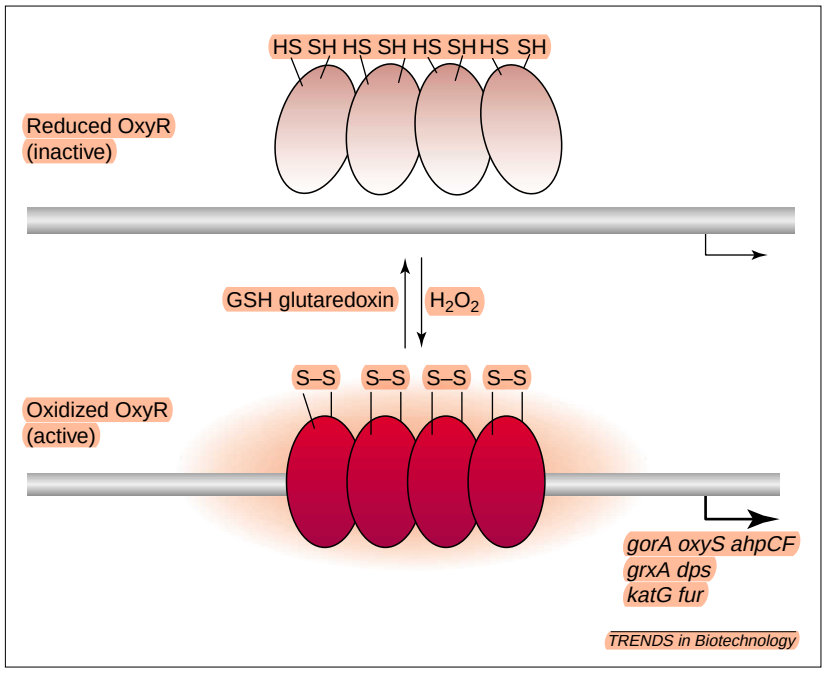

Fig. 3. The *oxyR* regulon. The OxyR protein is produced constitutively and is oxidized by hydrogen peroxide (H_2O_2). The oxidized form of OxyR binds to promoter regions of target genes and activates transcription by protein–protein contact with RNA polymerase. OxyR-activated genes have direct and indirect antioxidant functions. Each subunit of the OxyR tetramer contains two cysteine residues that form intramolecular disulfide bonds upon exposure to H_2O_2. The disulfide bonds are re-reduced by glutathione, which in turn is re-reduced by glutathione reductase. The expression of the glutathione reductase and glutaredoxin genes is under transcriptional control of OxyR, and thus the response is self-regulated. Abbreviation: GSH, glutathione.

products indirectly, by affecting mRNA stability or translation efficiency[31]. The *oxyS* gene has role(s) that are independent from *oxyR* in limiting the endogenous production of H_2O_2 in *E. coli*[32]. Post-transcriptional regulation via *oxyS* applies to two other genes that themselves encode regulatory proteins: *flhA* (activator of the *hyp* operon[33]) and *rpoS* (stationary-phase sigma factor[34]). The regulation of other regulatory functions by SoxR protein, OxyR protein and oxyS RNA is a theme that complicates the interpretation of the phenotypes conferred by these systems. Such multi-layered control might allow both amplification and fine-tuning of the action of these regulators in response to oxidative stress.

The level of OxyR protein does not change in cells treated with H_2O_2, an indication that it is activated post-translationally[35]. *In vitro* experiments demonstrated that OxyR can exist in either oxidized or reduced forms. Only the oxidized form binds DNA at the promoters of *katG*, *dps*, *gorA*, and so on, where activated OxyR stimulates transcription by interacting with the carboxyl-terminal domain of the α-subunit of RNA polymerase. Detailed footprinting studies[36] indicate that oxidized OxyR binds its target promoters as a tetramer (Fig. 3). Uniquely, at the *oxyR* promoter OxyR binds as a repressor in either the oxidized or the reduced state[36], which serves to limit the amount of this regulatory protein.

Most information concerning OxyR has come from studies of the *E. coli* system, although OxyR homologs had been reported in at least 15 other bacterial species, including Mycobacteria and other Gram-positives. The expression of OxyR in *E. coli* varies during growth of liquid cultures, and the level of OxyR is adjusted to endogenous H_2O_2 levels. Under the control of the cyclic-AMP-activated Crp protein, transcription of the *oxyR* gene increases during exponential growth, and decreases upon transition to stationary phase under *rpoS* control[37]. The amount of OxyR, as monitored using western blotting, parallels the transcription of the *oxyR* gene. Thus, *oxyR* is involved in a web of regulation, both in its control of other regulators (*fur*, *fhlA* and *rpoS*) and in the control systems that operate on its expression (*cya*, *crp* and *rpoS*).

The transcriptional activity of OxyR also varies during normal cell growth, which increases the activities of catalase, encoded by *katG*, and alkyl hydroperoxide reductase, encoded by *ahpFC*, during early exponential growth[38]. This upregulation is a consequence of increased H_2O_2 output by aerobic metabolism under these conditions and constitutes a homeostatic control of this dangerous oxidant. Thus, OxyR functions under normal physiological conditions and not only in response to stress.

Redox sensing by OxyR: activation by intramolecular disulfide bond formation

The OxyR polypeptide has a mass of 34 kD, and forms tetramers in solution (Fig. 3). *In vivo* transcriptional activation by OxyR in response to H_2O_2 depends on two cysteine residues (Cys199 and Cys208). Mass spectrometry analysis and proteolytic studies showed that, upon treatment of purified OxyR with H_2O_2, Cys199 and Cys208 form a disulfide bond[39]. Because oxidized OxyR does not seem to form covalently crosslinked multimers[35], the Cys199–Cys208 disulfide is intramolecular. *In vitro*, the disulfide bond in oxidized OxyR is re-reduced by compounds such as dithiothreitol, or by the small dithiol protein glutaredoxin in the presence of GSH, and this inactivates OxyR (Ref. 39).

Reversible disulfide bond formation governing OxyR activity has been confirmed *in vivo* by taking advantage of thiol chemistry[40]. A bulky iodoacetamide derivative, which alkylates thiols but not disulfides, reacts to add ~1 kD of mass to reduced OxyR, and the various alkylated forms of OxyR can be separated using gel electrophoresis. Alkylation assays revealed that during normal aerobic growth, the Cys199 and Cys208 residues are maintained as thiols. Cellular exposure to 100–1000 μM H_2O_2 results in the oxidation of these cysteines to form the intramolecular disulfide bond. The *in vivo* oxidation reaction is fast; the reaction is complete within ~30 s. OxyR remains in the oxidized form for up to 5 min after removal of H_2O_2 from the growth medium. Transcription of *oxyR*-regulon genes occurs in parallel with the appearance and maintenance of the oxidized form of OxyR *in vivo*.

The GSH-glutaredoxin-1 system contributes to restoring OxyR to the reduced state following oxidative stress[40]. Mutations in the genes that encode either glutaredoxin-1 (*grxA*) or GSH reductase (*gor*) slow the rate of OxyR re-reduction in *E. coli*. It is noteworthy that both genes are members of the *oxyR* regulon[3], which gives the system an autoregulatory capacity during oxidative stress. During aerobic growth, simultaneously disrupting both GSH metabolism and thioredoxin-1

(in *gorA trxA* or *gshA trxA* double mutants) partially activates OxyR in the absence of added H_2O_2, but mutating both glutaredoxin-1 and thioredoxin-1 (in a *grxA trxA* strain) has only a small effect[40]. Thus, OxyR is sensitive both to H_2O_2 and to changes in the cellular thiol status, but multiple systems might contribute to keeping the protein in the reduced state.

H_2O_2 is the best characterized activator of OxyR. However, there is at least one report of OxyR activation by a different class of agent, nitrosothiols[30]. These compounds can release NO or transfer it to other molecules and it was suggested that treatment of *E. coli* with nitrosothiols generates a distinct form of activated OxyR. The details of this reaction have not yet been elucidated, and contrary observations have been reported[39].

As with SoxR, detailed structural information about OxyR is needed. Important questions that might be addressed by solving a structure for OxyR include defining the mechanism that makes Cys199 particularly sensitive to oxidation by H_2O_2; the linkage between disulfide bond formation and transcriptional activation; and the common features of possible distinct forms of activated OxyR protein.

Biotechnology applications of SoxR and OxyR?

An obvious application of the *soxRS* and *oxyR* systems is for toxicology testing to detect compounds that cause cellular oxidative stress. Indeed, companies now use these systems to test their own products or provide systems to test the products of other companies. Note that this type of application can be somewhat generic, in that a broad array of structurally distinct compounds can generate the *in vivo* signals to activate SoxR. This generality can be useful in providing a 'first-pass' sorting of compounds with the potential to cause cellular damage but it provides no direct information about the mechanism. Conversely, use of OxyR as a reporter has the advantage of sensitivity to low levels of H_2O_2, but might be rather restricted in the types of agents that activate it. For both the *soxRS* and the *oxyR* systems, possible interference by toxic agents with the respective reducing systems might further limit their specificity.

Oxidative stress has been linked to physiological and pathological processes in mammals, ranging from apoptosis to neurodegenative disease and reperfusion damage. Although the signal transduction mechanisms of SoxR and OxyR are facile and sensitive, clear-cut counterparts have not yet been identified in mammalian cells. It is interesting to consider the possibility of harnessing the exquisite sensitivity of SoxR or OxyR to oxidative activation to construct reporters of *in vivo* oxidative stress in mammalian cells. This is easier to envision for OxyR, with its DNA-binding activity regulated by its oxidation state. Thus, the protein might be adapted as a redox-regulated repressor, or with the fusion of a suitable transactivation domain (e.g. VP16) as a redox-regulated activator. The application of SoxR would be more difficult, because the protein binds DNA even in its inactive state, and because SoxR has a rather unusual transcription-activating mechanism. The latter might necessitate synthesizing eukaryotic promoters that are sensitive to the same changes as *soxS*, the natural target of SoxR. For both SoxR and OxyR there is a further potential problem, namely the question of whether mammalian activities would act to maintain them in the reduced state in the absence of stress, and whether such reducing systems would operate in the mammalian nucleus. Although these issues might not be insurmountable, they certainly constitute formidable challenges to broader application of the knowledge obtained from these bacterial oxidative stress sensors.

Finally, the application of SoxR and OxyR in non-biological systems can be considered, in which redox-switching activities might be useful. Imagine, for example, applying the torsional work that might be done by SoxR as a nanoscale switch regulated by the redox state of the surrounding medium. The use of the redox-regulated DNA-binding capacity of OxyR as a part of a chemo-mechanical micromachine can also be imagined. As we learn more about the mechanisms and structures of these proteins, the possibilities for such applications will probably grow.

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Telomerase inhibitors

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There has been a vast increase in telomerase research over the past several years, with many different pre-clinical approaches being tested for inhibiting the activity of this enzyme as a novel therapeutic modality to treat malignancy. In this review, we will provide some basic background information about telomeres and telomerase and then discuss the pros, cons and challenges of the approaches that are currently under investigation, and what we might expect in the future of this emerging field.

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Telomeres are repetitive DNA sequences at the ends of linear chromosomes that protect the termini from being recognized as double-strand breaks. Without telomeres, the ends of the chromosomes would be 'repaired', leading to chromosome fusion and massive genomic instability. In humans, the telomeres typically contain 5–15 kb pairs of repeating TTAGGG sequences. With each cell division, telomeres shorten by 50–200 bp because the lagging strand of DNA synthesis is unable to replicate the extreme 3′ end of the chromosome (the 'end replication problem'; Fig. 1). Normal cultured human cells have a limited replication potential in culture. As first described by Hayflick[1], normal cells in culture replicate until they reach a discrete point at which population growth ceases. This is termed the Phase III or M1 stage and is caused by the shortening of a few telomeres to a size that leads to a growth arrest called 'cellular senescence'. A popular misconception is that cellular senescence leads to cell death rather than to a stable non-dividing state. This stage can be bypassed *in vitro* by abrogation of the function of the *p53* and *pRB* human tumor suppressor genes[2]. The cells can then replicate until the telomeres have become critically shortened, which produces the M2 or crisis stage. As opposed to M1, the net growth arrest in the M2 or crisis stage is caused by a balance between the cell proliferation and cell death rate. At this stage, when most of the telomeres are extremely short, end-to-end fusions and chromosome breakage-fusion cycles cause marked chromosomal abnormalities and apoptosis. Under rare circumstances a cell can escape M2 and

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