Free radicals, peroxides and the control of gene expression

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Reactive oxygen intermediates such as superoxide radical ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) are produced in all living cells as byproducts of enzymatic reactions and by exposure to light, radiation, metals, and redox-active drugs. Phagocytes generate $\bullet O_2^-$ and H_2O_2 as well as nitric oxide (NO•) and hypochlorous acid (OCl^-) as a defense against bacterial infection. Other types of radicals and peroxides arise when these oxygen intermediates react with each other or with lipids, proteins, and other small molecules such as glutathione. Reactive oxidants can lead to the damage of almost all cellular components, and may also mediate responses such as programmed cell death. Therefore, cells need receptors that sense the presence of reactive oxygen intermediates and induce a change in the expression of the appropriate genes either directly or via other transcription factors.

Two transcriptional activators that both sense an oxidative signal and transduce its effects are the SoxR and OxyR proteins of *Escherichia coli*. The bacterial cells adapt to the toxic effects of $\bullet O_2^-$ by synthesizing a group of defense enzymes. The expression of at least nine $\bullet O_2$-inducible proteins, including manganese superoxide dismutase, is controlled by SoxR together with a second transcriptional regulator, SoxS. In response to increased levels of $\bullet O_2^-$ or NO• but not H_2O_2, SoxR activates transcription of the *soxS* gene and thereby increases the production of SoxS protein (see Fig. 1). The SoxS protein then induces the transcription of the nine target genes. Two forms of SoxR, an apoprotein (apo-SoxR) and a form containing a redox-active iron-sulfur cluster (Fe-SoxR), have recently been purified. While both the apo-SoxR and Fe-SoxR proteins bind to the *soxS* promoter, only the iron-sulfur form is able to activate transcription *in vitro*. These results suggest that the iron-sulfur cluster in the SoxR protein is modified upon exposure to superoxide, causing SoxR to alter the *soxS* promoter structure and activate transcription [1].

Bacterial cells can also adapt to the presence of H_2O_2, and the expression of several H_2O_2-inducible proteins is under the control of the transcriptional activator protein, OxyR. *In vitro* experiments showed that purified OxyR was able to activate transcription under oxidizing conditions (in the presence of air and low concentrations of dithiothreitol) but not under reducing conditions (in the presence of nitrogen or high concentrations of dithiothreitol), suggesting that oxidation of OxyR causes a conformational change in the protein, which allows it to transduce directly the oxidative stress signal to RNA polymerase [2]. Mutational analysis of the protein has implicated one essential cysteine residue as the redox-active site in OxyR. The critical cysteine may be oxidized reversibly to sulfenic acid, but this hypothesis needs to be tested by additional biochemical experiments.

A number of genes in eukaryotic cells are induced by exposure to $\bullet O_2^-$, H_2O_2 or other reactive oxygen intermediates, implying the presence of regulators that sense the oxidants. Although the direct sensors have not yet been identified, the activities of two important mammalian transcription factors, NF-κB and AP-1 (Jun/Fos), are modulated when cells or extracts are treated with oxidants.

Oxidative stress appears to be a major contributor to degenerative diseases; thus, the characterization of additional redox-sensitive transcription factors or receptors for the reactive oxygen intermediates will be an important direction for future research. Many intriguing questions remain to be addressed. What are the cellular concentrations and the subcellular distributions of the peroxides and free radicals? Are the receptors or transcription factors oxidized directly, or do the regulators respond to secondary effects such as changes in glutathione levels? For the proteins that are oxidized, what chemical modifications take place, and how does oxidation induce a conformational change? SoxR and OxyR use different chemical switches to sense the presence of reactive oxygen species; it is likely that the reactive oxygen species can induce several distinct chemical reactions that affect gene expression.

References

1. Hildalgo, E. & Demple, B. (1994). An iron-sulfur center essential for transcriptional activation by the redox-sensing SoxR protein. *EMBO J.* **13**, 138–146.
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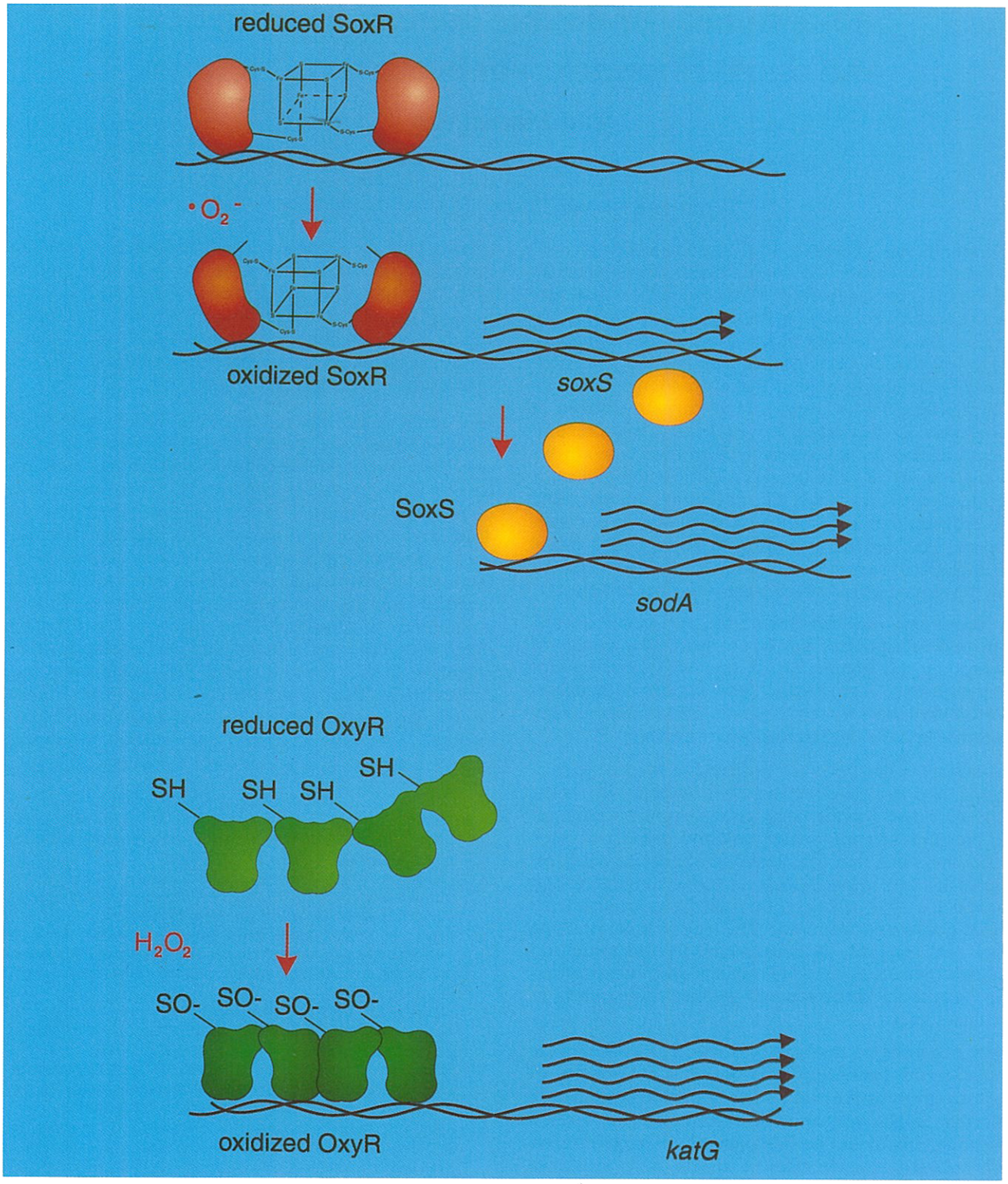

Fig. 1. Models for SoxR and OxyR activation. **(a)** Upon exposure to $\cdot O_2^-$ or NO•, SoxR is oxidized and induces *soxS* expression. The SoxS protein then activates the expression of *sodA*-encoded manganese superoxide dismutase. The iron–sulfur cluster is drawn as a Fe_4S_4 cluster; however, the subunits could also have individual or shared Fe_2S_2 centers. Hildalgo and Demple have proposed that the iron-sulfur cluster is oxidized directly [1], although the SoxR may cycle between an apo-SoxR and Fe-SoxR state. **(b)** When cells are treated with H_2O_2, OxyR is oxidized and activates the expression of the catalase (*katG*) gene. One or more cysteine residues in the OxyR tetramer may be oxidized reversibly to sulfenic acid.