Antonie van Leeuwenhoek **58**: 157–161, 1990.

The OxyR regulon

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Abstract

Treatment of *Salmonella typhimurium* and *Escherichia coli* cells with low doses of hydrogen peroxide results in the induction of thirty proteins and resistance to killing by higher doses of hydrogen peroxide. The expression of nine of the hydrogen peroxide-inducible proteins, including catalase, glutathione reductase and a novel alkyl hydroperoxide reductase is controlled by the positive regulator *oxyR*. OxyR is homologous to the LysR-NodD family of bacterial regulatory proteins and binds to the promoters of *oxyR*-regulated genes. The oxidized but not reduced form of the OxyR protein activates transcription of *oxyR*-regulated genes in vitro suggesting that oxidation of the OxyR protein brings about a conformational change by which OxyR both senses and transduces an oxidative stress signal to RNA polymerase.

Introduction

Reactive oxygen species including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxy radical (HO·), have been implicated in several degenerative diseases such as cancer, aging and arthritis. The oxidants can be generated by the incomplete reduction of oxygen to water during respiration, by exposure to radiation, light, metals, or oxidation-reduction (redox) active drugs such as paraquat or by release from stimulated macrophages. They can lead to damage of almost all cell components--DNA, lipid membranes, and proteins. The *oxyR*-controlled regulon of hydrogenperoxide-inducible genes in *Salmonella typhimurium* and *Escherichia coli has provided a model for studying how cells defend against oxidative damage.*

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When *E. coli* and *S. typhimurium* cells are treated with low doses (60 M) of hydrogen peroxide, they become resistant to subsequent lethal doses (10 mM) of hydrogen peroxide (Demple, Halbrook, 1983; Christman et al., 1985). This adaptive response requires the induction of protein synthesis since cells treated with low doses of hydrogen peroxide in the presence of the chloramphenicol, a protein synthesis inhibitor, are no longer resistant to the subsequent lethal challenges (Christman et al., 1985). In agreement with the requirement for protein synthesis, treatment with low doses of hydrogen peroxide induces the synthesis of approximately 30 proteins as seen on two dimensional gels (Morgan et al., 1986; VanBogelen et al., 1987). A subset of the hydrogen peroxide-inducible proteins overlap with heat shock proteins and proteins induced by other types of stress. Time-course experiments showed that the 30 hydrogen peroxide-inducible proteins can be grouped into two temporal classes; 12 proteins are maximally induced within the first 10 minutes after treatment with hydrogen peroxide and the other 18 proteins are induced within the subsequent hour (Christman et al., 1985).

The expression of nine of the early hydrogen peroxide-inducible proteins is regulated by *oxyR*. *E. coli* and *S. typhimurium* strains carrying deletions of the *oxyR* gene are hypersensitive to hydrogen peroxide and are unable to induce the expression of the nine proteins. Other mutant strains (*oxyR1* in*S. typhimurium* and *oxyR2* in *E.*

coli) which are more resistant to hydrogen peroxide than wild type strains constitutively overexpress the nine proteins (Christman et al., 1985).

Several of the proteins whose expression is regulated by *oxyR* have been identified (Table 1).

Table 1. The *oxyR* Regulon

Activity		Gene	*E. coli* map position
OxyR		*oxyR*	89.6
Catalase	HPI	*katG*	89.2
Alkyl hydroperoxide reductase	C22, F52	*ahpC, ahpF*	13
Glutathione reductase		*gorA*	77–78

All of these activities can be implicated in a defense against oxidative stress. *E. coli* has two isozymes of catalase that convert hydrogen peroxide to oxygen and water. The expression of one of the catalase activities, denoted HPI (position D69/D71 on two dimensional gels) and encoded by *katG*, is regulated by *oxyR*. Strains carrying the *oxyR1* mutation have 50-fold higher levels of HPI catalase than the corresponding wild type strains (Christman et al., 1985). Glutathione reductase (G35) encoded by *gorA* is also elevated 6-fold in the *oxyR1* mutant strains (Christman et al., 1985). Glutathione reductase probably plays a role in protecting against oxidation by maintaining a pool of reduced glutathione which in turn can serve to maintain the reduced state of cellular proteins.

oxyR1 mutant strains were also found to overproduce a novel alkyl hydroperoxide reductase activity (20-fold) (Christman et al., 1985). This activity was purified to homogeneity and found to be a flavoprotein comprised of two identical 57 kDa subunits (F52) encoded by *ahpF* .and a 22 kD protein (C22) encoded by *ahpC* (Jacobson et al., 1989). Recent sequence analysis of the *ahpCF* operon revealed that the *ahpF* encoded protein (F52) is highly homologous to the *E. coli* thioredoxin reductase protein, particularly in the active-site region, suggesting that the alkyl hydroperoxide reductase is a disulfide oxidoreductase (Tartaglia et al., 1990). The purified enzyme is capable of reducing physiological hydroperoxides such as thymine hydroperoxide and linoleic hydroperoxides to their corresponding alcohols, and mutant strains that lack the alkyl hydroperoxide reductase are extremely sensitive to killing by organic hydroperoxides (Jacobson et al., 1989; Storz et al., 1989). These results suggest that the alkyl hydroperoxide reductase detoxifies damaging peroxides generated by oxidative stress.

OxyR-mediated defenses against oxidative damage

The *oxyR*-regulated catalase, glutathione reductase and alkyl hydroperoxide reductase activities all protect against oxidation. This raises the question of when might bacteria encounter oxidants? Enteric bacteria are most often found in the intestinal tracts of mammals where they live under predominantly anaerobic conditions. Nevertheless, bacteria can move outside of the intestinal tract where they may encounter an oxidative burst of superoxide radicals and hydrogen peroxide released by macrophages when the macrophages encounter bacteria. Preliminary studies have shown that *oxyR* mutant strains are less virulent in mice than the corresponding wild type strains suggesting that the *oxyR* regulon has a role in defending against macrophages (Fields et al., 1986). Bacteria have also been isolated from sewage and soil. In these latter environments the bacteria depend on aerobic respiration and consequently must be able to defend against oxidants. The following studies have shown that *oxyR* plays an important role in preventing oxidative damage that occurs during aerobic growth.

Both *E. coli* and *S. typhimurium* strains carrying deletions of *oxyR*, have significantly increased frequencies of mutagenesis especially under aerobic conditions (Storz et al., 1987; Greenberg, Demple, 1988). The increased mutation frequency is most pronounced under aerobic conditions (Figure 1).

When the mutations generated in the *oxyR*$^+$ and the *oxyR* deletion strains were characterized, it was found that the type of mutation whose frequency was most elevated was T:A to A:T transversions, the type of mutation most frequently caused by oxidative mutagens. These mutations may in part be generated as a consequence of SOS induction, a response induced by

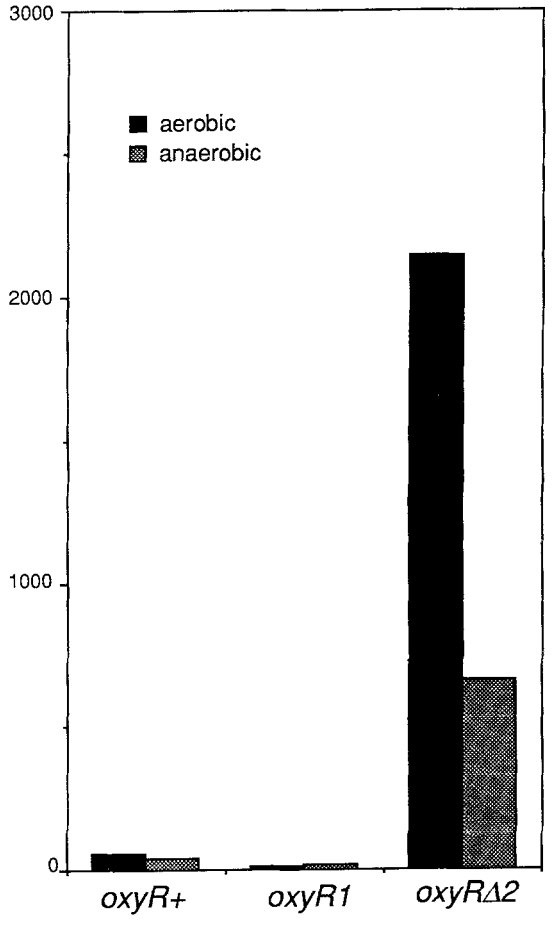

Fig. 1. Spontaneous mutation frequencies in *oxyR*$^+$, *oxyR1*, and *oxyR*Δ2 strains (carrying the pKM101-encoded *mucA* and *mucB* genes) under aerobic and anaerobic conditions. The mutation frequencies were assayed as the number of revertants from His- auxtrophy to His+ prototrophy per plate (Storz et al., 1987).

several types of DNA damage (Goerlich et al., 1989). In agreement with this conclusion, the greatest increase in mutagenesis is seen in strains that also carry the pKM101 plasmid. pKM101 encodes the *mucA* and *mucB* genes, analogs of the *E. coli umuC* and *umuD* genes (members of the SOS regulon) that make strains more susceptible to mutagenesis by a number of mutagens. The high frequency of mutagenesis in *oxyR* deletion strains was suppressed by multi-copy plasmids expressing high levels of catalase (*katG*), alkyl hydroperoxide reductase (*ahpCF*) or superoxide dismutase (*sodA*) activity (Storz et al., 1987) and by suppressor mutations that caused increased expression of *katG* and *ahpCF* genes (Greenberg, Demple, 1988). These observations provide evidence that the *oxyR* regulon plays an important role in protecting against oxidative DNA damage that is otherwise converted into mutations by the SOS response and the products of the *mucA* (*umuB*) and *mucB* (*umuD*) genes. In addition, studies on the uptake of labelled lactose, guanosine, uracil, or methylglucopyranoside have shown that *oxyR* and *katG* protect against a loss of proton motive force-dependent (lactose, guanosine) and -independent (methylglucopyranoside) transport after oxidative stress (Farr et al., 1988).

Mechanism of OxyR regulation

The mechanism by which *oxyR* senses oxidative stress and regulates the increased expression of these nine proteins is well understood. The *oxyR* gene was cloned on the basis of its ability to complement the hydrogen peroxide sensitivity of a strain carrying a deletion of the *oxyR* locus (Christman et al., 1989). Sequence comparisons showed that OxyR is homologous to an expanding family of bacterial regulators including LysR (*E. coli* regulator of *lysA* involved in lysine biosynthesis), NodD (*Rhizobium* regulator of genes involved in the formation of nodules), IlvY (*E. coli* regulator of *ilvC* and other genes in the isoleucine-valine biosynthetic pathway), CysB (*E. coli* and *S. typhimurium* regulator of a cysteine regulon), MetR (*S. typhimurium* regulator of *metE* and *metH*) and AmpR (*Enterobacter cloacae* regulator of the *ampC* gene which confers resistance to cephalsporin) (Bölker, Kahmann, 1989; Christman et al., 1989; Tao et al., 1989). The evolutionary significance of the homology among these proteins is not clear but it may be noteworthy that the fixation of nitrogen in nodules is very sensitive to oxygen and the amino acids, cysteine, methionine, and isoleucine are all especially susceptible to oxidation. All of the above regulators serve as activators of heterologous genes, and many, including OxyR, have also been found to negatively autoregulate their own expression.

Dot blots of total cellular RNA isolated from *oxyR*+ and *oxyR1* "constitutive" mutant strains probed with the cloned *katG* gene showed that the level of the *katG* message is elevated 50-fold in the *oxyR1* mutant strain (Morgan et al., 1986).

Primer extension studies showed the same to be true for the *ahpC* transcript and allowed identification of the start of the *oxyR*-regulated *katG* and *ahpC* transcripts (Tartaglia et al., 1989). Deletion analysis of sequences directly upstream of the *ahpC* promoter defined a region (–67 to –46 relative to the start of transcription) required for *oxyR* activation. A fragment carrying 141 bp of upstream *katG* sequence could confer *oxyR*-dependent hydrogen peroxide induction to a marker *lacZ* gene. The regions found to be important for *oxyR* activation upstream of the *ahpC* and *katG* genes were found to be protected from DNase I digestion by purified OxyR protein. These results suggested that OxyR activates the expression of *katG* and *ahpC* by binding to promoter sequences upstream of the hydrogen peroxide-inducible genes and increasing the rate of transcription initiation. Intriguingly, the sequences protected by OxyR show very little homology (Tartaglia et al., 1989).

The mechanism by which the cell senses hydrogen peroxide and activates transcription was illuminated by studies on the ability of OxyR-overproducing extracts and purified OxyR to activate the expression of the *oxyR*-regulated *ahpCF* and *katG* genes in vitro (Storz et al., 1990) (Figure 2).

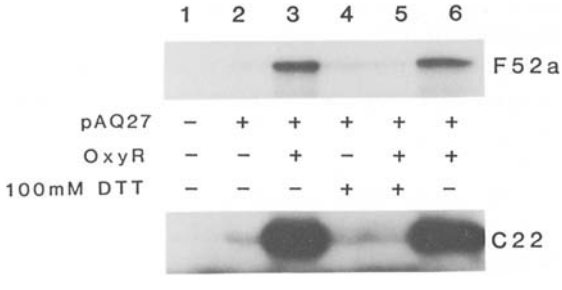

Fig. 2. Effect of OxyR on the expression of the components comprising the alkyl hydroperoxide reductase (F52a and C22, encoded by pAQ27) in in vitro transcription-translation assays. The addition of extracts of OxyR-overproducing cells greatly increased the expression of the two proteins (lane 3 compared to lane 2). The ability of OxyR to induce expression was inhibited by 100 mM DTT (lane 5) though 100 mM DTT did not reduce the basal level of expression (lane 4 compared to lane 2). OxyR could be reactivated if the 100 mM DTT was removed by dialysis (lane 6).

Initially this finding was surprising since neither the extracts not the purified protein had been treated with hydrogen peroxide. However, subsequent experiments showed that OxyR was activated solely by the release from the reducing environment of the *E. coli* cell. If the extracts from the overproducing strains were prepared under argon, OxyR no longer activated expression, though exposure to air immediately converted OxyR into a transcriptional activator. The extracts and purified OxyR could also be inactivated by high concentrations of dithiothreitol and subsequently reactivated if the dithiothreitol was removed. The reducing conditions did not denature the OxyR protein, since OxyR was still capable of repressing its own expression under the reducing conditions and still bound the *katG* and *ahpC* promoter regions. The DNase I protection patterns for purified OxyR under oxidizing and reducting conditions are significantly different suggesting that OxyR undergoes a conformational change. These findings indicate that OxyR senses oxidative stress directly by becoming oxidized, and that oxidation brings about a conformational change by which OxyR transduces the oxidative stress signal to RNA polymerase.

Perspectives

Studies on the *oxyR* regulon have shown that bacterial cells can defend against oxidative DNA and membrane damage by inducing a regulon of defense genes. The expression of these genes is under the control of the OxyR protein which senses the oxidative stress directly by becoming oxidized. As additional prokaryotic and eukaryotic responses to oxidative stress are characterized it will be interesting to compare them to the *oxyR*-regulated response. The above studies have also given rise to several additional questions. Only four of the *oxyR*-regulated proteins have been identified. What are the roles of the unidentified proteins? Secondly, Bölker and Kahmann have found that OxyR corresponds to the *mom* gene repressor (MomR), the methylation-dependent regulator of the *mom* gene (encoding a DNA modification function) of phage Mu (Bölker, Kahmann, 1989). Why does the regulator of hydrogen peroxide-inducible genes also act to repress phage gene expression? Since the sequences bound by OxyR show very little homology, what is the basis for OxyR-DNA recognition? Finally, what is the nature of the redox active center in OxyR which is

capable of sensing treatment with 60 M hydrogen peroxide?

References

Bölker, M. and Kahmann, R. (1989) The *Escherichia coli regulatory protein OxyR discriminates between methylated and unmethylated states of the phage Mu mom* promoter. EMBO J. 8, 2403–2410.

Christman M. F., Morgan R. W., Jacobson, F. S. and Ames, B.N. (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium.* Cell 41, 753–762.

Christman, M. F., Storz, G. and Ames, B N (1989) OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli and Salmonella typhimurium,* is homologous to a family of bacterial regulatory proteins. Proc. Natl. Acad. Sci. USA 86, 3484–3488.

Demple, B. and Halbrook, J. (1983) Inducible repair of oxidative DNA damage in *Escherichia coli. Nature 304, 466–468.*

Farr, S. B., Touati, D. and Kogoma, T. (1988) Effects of oxygen stress on membrane functions in Escherichia coli: Role of HPI catalase. J. Bacteriol. 170, 1837–1842.

Fields, P. I., Swanson, R. V., Haidaris, C. G. and Heffron F. (1986) Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. Proc. Natl. Acad. Sci. USA 83, 5189–5193.

Goerlich, O., Quillardet, P. and Hofnung, M. (1989) Induction of the SOS response by hydrogen peroxide in various *Escherichia coli* mutants with altered protection against oxidative DNA damage. J. Bacteriol. 171, 6141–6147.

Greenberg, J. T. and Demple, B. (1988) Over production of peroxide-scavenging enzymes in *Escherichia coli suppresses spontaneous mutagenesis and sensitivity to redox-cycling agents in oxyR*$^-$ mutants. EMBO J. 7, 2611–2617.

Jacobson, F. S., Morgan, R. W., Christman, M. F. and Ames, B. N. (1989) An alkyl hydroperoxide reductase from *Salmonella typhimurium* involved in the defense of DNA against oxidative damage: Purification and properties. J. Biol. Chem. 264, 1488–1496.

Morgan, R. W., Christman, M. F., Jacobson, F. S., Storz, G. and Ames, B. N. (1986) Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA 83, 8059–8063.

Storz, G., Christman, M. F., Sies, H. and Ames, B N. (1987) Spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium.* Proc. Natl. Acad. Sci. USA 84, 8917–8921.

Storz, G., Jacobson, F. S., Tartaglia, L. A., Morgan, R. W., Silveira, L. A. and Ames, B. N. (1989) An alkyl hydroperoxide reductase induced by oxidative stress in *Salmonella typhimurium* and *Escherichia coli: Genetic characterization and cloning of ahp.* J. Bacteriol. 171, 2049–2055.

Storz, G., Tartaglia, L. A. and Ames, B. N. (1990) Transcriptional regulator of oxidative stress-inducible genes: Direct activation by oxidation. Science 248, 189–194.

Tartaglia, L. A., Storz, G. and Ames, B. N. (1989) Identification and molecular analysis of *oxyR*-regulated promoters important for the bacterial adaptation to oxidative stress. J. Mol. Biol. 210, 709–719.

Tartaglia, L. A., Storz, G., Brodsky, M. H., Lai, A. and Ames, B N. (1990) Alkyl hydroperoxide reductase from *Salmonella typhimurium*: Sequence and homology to thioredoxin reductase and other flavoprotein disulfide oxidoreductases. J. Biol. Chem 265, 10535–10540.

Tao, K., Makino, K., Yonei, S., Nakata, A., Shinagawa, H. (1989) Molecular cloning and nucleotide sequencing of *oxyR*, the positive regulatory gene of a regulon for an adaptive response to oxidative stress in *Escherichia coli*: Homologies between OxyR protein and a family of bacterial activator proteins. Mol. Gen. Genet. 218, 371–376.

VanBogelen, R. A., Kelley, P. M. and Neidhardt, F. C. (1987) Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli.* J. Bacteriol. 169, 26–32.