Genetics and Molecular Biology, 27, 2, 291-303 (2004)

www.sbg.org.br

Review Article

Several pathways of hydrogen peroxide action that damage the *E. coli* genome

Nasser Ribeiro Asad[1], Lidia Maria Buarque Oliveira Asad[1], Carlos Eduardo Bonacossa de Almeida[3], Israel Felzenszwalb[1], Januário Bispo Cabral-Neto[2] and Alvaro Costa Leitão[2]

[1]*Universidade do Estado do Rio de Janeiro, Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcantara Gomes, Rio de Janeiro, RJ, Brazil.*
[2]*Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro, RJ, Brazil.*
[3]*Comissão Nacional de Energia Nuclear, Divisão de Monitoração Individual, Instituto de Radioproteção e Dosimetria, Rio de Janeiro, RJ, Brazil.*

Abstract

Hydrogen peroxide is an important reactive oxygen species (ROS) that arises either during the aerobic respiration process or as a by-product of water radiolysis after exposure to ionizing radiation. The reaction of hydrogen peroxide with transition metals imposes on cells an oxidative stress condition that can result in damage to cell components such as proteins, lipids and principally to DNA, leading to mutagenesis and cell death. *Escherichia coli* cells are able to deal with these adverse events via DNA repair mechanisms, which enable them to recover their genome integrity. These include base excision repair (BER), nucleotide excision repair (NER) and recombinational repair. Other important defense mechanisms present in *Escherichia coli* are OxyR and SosRS anti-oxidant inducible pathways, which are elicited by cells to avoid the introduction of oxidative lesions by hydrogen peroxide. This review summarizes the phenomena of lethal synergism between UV irradiation (254 nm) and H_2O_2, the cross-adaptive response between different classes of genotoxic agents and hydrogen peroxide, and the role of copper ions in the lethal response to H_2O_2 under low-iron conditions.

Key words: hydrogen peroxide, cross-adaptive response, lethal synergism, copper and iron.

Received: February 13, 2004; Accepted: February 19, 2004.

General Aspects

The appearance of aerobic forms of life was an important step in the evolutionary process, since oxygen consumption leads to the production of ten-fold more energy from glucose than does anaerobic metabolism (Meneghini, 1987). However, this process imposes constraints on cell viability, because of the generation of reactive oxygen species during respiration.

The consecutive univalent reduction of molecular oxygen to water produces three active intermediates: superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). These intermediates, collectively referred to as reactive oxygen species (ROS) are potent oxidants of lipids, proteins, and nucleic acids (Halliwell and Gutteridge, 1984; Mello-Filho and Meneghini, 1985; Meneghini, 1988). Among the oxidative DNA lesions, one of the major classes of DNA damage leads to modification in purine and pyrimidine bases, together with oligonucleotide strand breaks, DNA-protein cross-links and abasic sites. Increasing evidence suggests that the cumulative damage caused by ROS contributes to numerous degenerative diseases associated with aging, such as atherosclerosis, rheumatoid arthritis and cancer (Ames *et al.,* 1993; Halliwell and Gutteridge, 1999).

Living organisms have developed specific mechanisms to prevent the production and effects of ROS. The reduction of O_2 by cytochrome oxidase without yielding ROS, the superoxide dismutase catalysis of $O_2^{-\bullet}$ into H_2O_2 through a dismutation reaction, the decomposition of H_2O_2 by catalase and peroxidases, and the scavenging of ROS by some vitamins comprise part of the set of cellular antioxidant defenses (Halliwell and Gutteridge, 1999). Synthesis of the enzymes that catalyze these reactions is a part of the adaptive response triggered by the stress posed by ROS.

Send correspondence to Dr. Alvaro C. Leitão. Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Bloco G, Ilha do Fundão, 21949-900 Rio de Janeiro, RJ, Brasil. E-mail: acleitao@biof.ufrj.br.

Despite these cellular defenses, many lesions can be produced in cellular targets, mainly in the DNA molecule, leading to mutagenesis and cell death. However, cells are able to deal with these adverse events via DNA repair mechanisms, which enable them to recover their integrity. These include base excision repair (BER), nucleotide excision repair (NER) and recombinational repair (Miles and Sancar, 1989).

Several studies indicate that the killing of *E. coli* cells exposed to H_2O_2 is mainly due to damage to DNA (Imlay and Linn, 1986; Imlay and Linn, 1988; Hagensee and Moses, 1989; Asad and Leitão, 1991) and a wide variety of DNA lesions are formed (Halliwell and Gutteridge, 1999) as a by-product of the H_2O_2 reaction. Some of these lesions are miscoding acting as an important source of mutagenesis in aerobically growing cells.

Participation of $OH^{\bullet}$ as the main damaging agent has been suggested by studies using scavengers of $OH^{\bullet}$. Repine *et al*. (1981) and Brakely *et al*. (1990) have demonstrated that dimethyl sulfoxide partially inhibits DNA base damage by H_2O_2, and Brandi *et al*. (1989) have noted that *E. coli* bacteria are partially protected against the lethal effects of H_2O_2 by pretreatment with ethanol, dimethyl sulfoxide, or thiourea.

The induction of DNA damage by H_2O_2 in *E. coli* as well as in mammalian cells can be either impaired or enhanced by the presence of transition metal ion chelators (Mello-Filho and Meneghini, 1985: Asad and Leitão, 1991). In addition, H_2O_2 can cause membrane lesions through lipid peroxidation and, by promoting alterations in several amino acids can lead to the inactivation of enzymes (Farr and Kogona 1991).

Recent reviews on the mechanisms of oxidative DNA damage and repair have focussed on prevention and repair (Cooke *et al.*, 2003), biochemical features (Cadet *et al.*, 2003), substrate specificities for glycosylases (Dizdaroglu, 2003), biological consequences (Wallace, 2002: Bjelland and Seeberg, 2003) and the role of iron (Kruszewski, 2003).

In this review, we discuss several aspects of DNA damage and cellular inactivation induced by hydrogen peroxide; the importance of transition metals such as iron and copper in this context; DNA repair pathways involved in the cellular response to H_2O_2, and related antioxidant cell defenses.

H_2O_2 and transition metals

H_2O_2 reacts with $O_2^{-\bullet}$ resulting in $OH^{\bullet}$ production through the so-called Haber-Weis cycle. However, this reaction depends on the presence of transition metals such as Cu^{+} and/or Fe^{2+}, which work as reducing agents, according to the reactions below (Kehrer, 2000).

$$Fe^{3+} + O_2^{-\bullet} \rightarrow Fe^{2+} + O_2 \quad \text{eq. (I)}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH^{\bullet} \quad \text{eq. (II)}$$

$$\text{Net reaction : } O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + OH^{-} + OH^{\bullet} \quad \text{eq. (III)}$$

H_2O_2, per se, is considered a weak oxidant agent. Nevertheless, it easily crosses the cellular membrane and reacts with transition metals, generating $OH^{\bullet}$. Evidence for the importance of transition metals comes from studies with chelators such as dipyridyl, phenanthroline and desferioxamine (Fe chelators) and neocuproine (a Cu chelator) (Brandi *et al*., 1989; Brakely *et al*., 1990; Hallywell and Gutteridge, 1999). Pretreatment with iron chelators protects both prokaryotic and eukaryotic cells against the lethal effects of H_2O_2 (Mello-Filho and Meneghini, 1985; Imlay and Linn, 1988; Asad and Leitão, 1991). Additionally, cultures of *Staphylococcus aureus* are more sensitive to H_2O_2 when iron is added to the culture media (Repine *et al*., 1981) and in *E. coli* cultures the same is observed (Touati *et al*., 1995).

In *E. coli* the metabolism of iron is involved in the cellular antioxidant response. A transcription factor denoted Fur, the global repressor of ferric ion uptake, regulates about 30 genes implicated in iron uptake from the environment (Braun, 1997; Braun *et al*., 1998). Most Fur-regulated genes are derepressed in low iron concentrations and repressed when a high concentration of iron is present (Hantke, 1981; Hantke, 2002). The finding that *fur* mutants are sensitive to H_2O_2 and that they suffer an increase in oxidative DNA damage leading to mutations under aerobic conditions supports the hypothesis that Fur has a role in the defense against oxidative stress (Touati *et al*., 1995). Furthermore, the regulators of *E. coli* responses to oxidative stress, OxyR and SoxRS, activate the expression of Fur and suggest that control of iron metabolism in *E. coli* is an integral part of the antioxidant response (Zheng *et al*., 1999)

DNA Repair Pathways and Antioxidant Defense Systems

Base excision repair (BER)

Lesions produced by H_2O_2 are typically repaired by base excision repair (BER) mechanisms. This kind of DNA repair is initiated by DNA glycosylases, enzymes that recognize the modified bases and act by cleaving the glycosylic bond, thereby removing the damaged base from the sugar phosphate backbone and, as a result, producing an apurinic/apyrimidinic (AP) site (Sancar and Sancar, 1988).

Some DNA glycosylases also display a class I AP lyase activity that incises the phosphodiester linkage on the 3' side of the AP lesion and generates a 5'-phosphate group and a 3'-terminus that needs removal by a class II AP endonuclease/3'-diesterase prior to repair synthesis and ligation (Piersen *et al*., 2000). The cleavage of AP sites can also be catalyzed by class II AP endonucleases which incise the 5' side of the AP site, leaving a 3'-OH terminus and a 5'-abasic residue that is removed by a deoxyribophosphodiesterase (dRPase) (Mol *et al*., 2000).

In *E. coli* two enzymes are representative of the class I AP lyases, the Fpg/MutM DNA glycosylase, product of the

fpg/mutM gene, which recognizes primarily oxidized purines, and the DNA endonuclease III, product of the *nth* gene, which recognizes primarily oxidized pirimidines. Interestingly, the *E. coli* repair-deficient *fpg/mutM*, as well as the *nth* mutants, are not more sensitive to killing by H_2O_2 than wild-type cells (Cunningham and Weiss, 1985; Boiteux and Huisman, 1989). Subsequently, endonuclease VIII and IX were purified from endonuclease III-deficient *E. coli* cells (Wallace *et al*., 1988; Melamede *et al*., 1994). Endonuclease VIII was found to exhibit a thymine glycol DNA glycosylase activity as well as an AP lyase activity. Endonuclease IX recognizes urea residues and β-ureidoisobutyric acid in DNA; however, DNA containing thymine glycol or dihydrothymine is not a substrate for this enzyme (Friedberg *et al*., 1995). Later, the gene for endonuclease VIII (*nei*) was isolated (Jiang *et al*., 1997; Saito *et al*., 1997). Endonuclease VIII is present in extracts of *E. coli* at 5 to 10% of the level of endonuclease III and is responsible for the repair of 10% of the thymine glycol in *E. coli* (Wallace *et al*., 1988; Wallace, 1988).

The *nth nei* double mutants are hypersensitive to H_2O_2 (Jiang *et al*., 1997). Moreover, endonuclease VIII can recognize 8-oxo-7,8-dihydroxyguanine (8-oxoG) lesions, a kind of lesion also recognized by Fpg protein (Blaisdell *et al*., 1999). The multiplicity of DNA glycosylases that recognize and attack sites of oxidative damage in DNA confirms the importance of this form of base damage.

Class II AP endonucleases of *E. coli* are mainly represented by the exonuclease III, the *xthA* gene product, and endonuclease IV, the *nfo* gene product.

The role of exonuclease III in repairing oxidative damage was highlighted by the demonstration that *xth* mutants are extremely sensitive to H_2O_2 (Demple *et al*., 1983). On the other hand, *nfo* mutants are not sensitive to H_2O_2 (Cunningham *et al*., 1986), and deletion of *nfo* increases the killing of *xth* mutants to H_2O_2, indicating that many of the repair activities of exonuclease III and endonuclease IV overlap.

Endonuclease IV normally represents about 10% of the total endonuclease activity; superoxide-generating agents induce a 10- to 20-fold increase in the level of this enzyme through the *soxRS* response (Chan and Weiss, 1987).

Nucleotide excision repair (NER) in response to H_2O_2

In *E. coli*, a complex of proteins encoded by the *uvrA*, *uvrB* and *uvrC* genes is required for lesion recognition and the dual incisions. This complex eliminates DNA lesions that cause significant distortions in the phosphodiester backbone of the molecule (Friedberg *et al*., 1995; Hanawalt, 2001).

This kind of repair is of fundamental importance for the correction of UV (254 nm) lesions, mainly cyclobutane pyrimidine dimers. It appears not to be related to the repair of H_2O_2 lesions, since the *uvrABC* mutants are not sensitive to H_2O_2 (Imlay and Linn, 1987).

The finding that the triple mutant *uvrA nfo xthA* cannot be constructed, despite the fact that the double mutants are viable (Saporito *et al*., 1989), has raised the question of the connection between BER and NER DNA repair pathways. Additionally, the UvrABC complex is able to remove AP sites generated by oxidative lesions in the DNA molecule (Lin and Sancar, 1989). So, these results suggest that both repair systems correct the lesions produced by oxidant agents produced during cellular respiration.

These findings suggest that some intermediate products of base excision repair may be substrates for the UvrABC complex. This hypothesis was confirmed by Kow *et al*. (1990), who demonstrated the role of this complex in the repair of thymine glycols in the DNA of replicative form of phage ΦX174. The survival of phage containing thymine glycols is lower in the *nth uvrA* double mutant than in the *nth* single mutant.

SOS response

The best-studied transcriptional response to DNA damage is the SOS response (Friedberg *et al*., 1995; Walker, 1996). Single-stranded DNA produced by several DNA-damaging agents and repair mechanisms can be bound by RecA protein, resulting in conversion of this protein to its activated form. Once activated, RecA interacts with LexA protein, the repressor of the SOS genes (Wagner *et al*., 1999). This interaction triggers the autocatalytic cleavage of LexA and consequent destruction of its ability to function as a repressor, which results in the derepression of SOS genes (Mustard and Little, 2000; Fernandez De Henestrosa *et al*., 2000). By using DNA microarray techniques Courcelle *et al*. (2001) have shown that in *E. coli* the expression of 43 genes is controlled by LexA.

The expression of LexA-controlled genes allows the increased phenotypic expression of mutagenesis (*umuDC* genes), nucleotide excision repair (*uvrA* and *uvrB* genes), genetic recombination (*recA*, *recN*, *recQ* and *recD* genes), cellular filamentation (*sulA* and *sulB* genes), and survival and mutagenesis of irradiated phages (W-reactivation and W-mutagenesis) (Friedberg *et al*., 1995).

A low concentration of H_2O_2 (1-3 mM) results in SOS gene induction in wild-type cells (Imlay and Linn, 1987; Goerlich *et al*., 1989). However, H_2O_2 can induce some SOS responses without SOS induction. An example is the cell filamentation induced by H_2O_2 in *sulA* and *recA* mutants and the mutagenesis that occurs at the same level in wild-type and *umuC* mutant cells treated with H_2O_2 (Imlay and Linn, 1987). On the other hand, the induction of SOS by H_2O_2 is an important event, since *recA* and *recBC* mutant cells are very sensitive to H_2O_2 treatment probably due to the lack of recombinational repair necessary for the repair of H_2O_2-induced lesions (Imlay and Linn, 1987). Additionally, Konola *et al*., (2000) have shown that the *ruvA*

mutants are 10- to 15-fold more sensitive to H_2O_2 (1-3 mM) than the wild-type cells. Together with RuvB, the RuvA protein generates the RuvAB complex, which stimulates strand migration in the Holliday junctions (West, 1996).

Other inducible events: anti-oxidant and cross-adaptive responses triggered by H_2O_2

Most genes encoding DNA repair enzymes that act on oxidative damage appear to be expressed constitutively in actively growing cells. This is presumably because oxidative DNA damage is continuously produced by ROS, which are normal by-products of aerobic metabolism (Demple and Harrison, 1994; Henle and Linn, 1997). However, in order to deal with elevated levels of peroxide in the environment, cells have evolved changes in metabolism that help to protect DNA from ROS.

Most inducible genes that respond to oxidative damage prevent, rather than repair DNA damage. However, a notable exception is endonuclease IV, the *nfo* gene product, an AP endonuclease that repairs 3' phosphate residues to 3'OH groups that can prime DNA synthesis (Chaudhry *et al*., 1999; Izumi *et al*., 2000).

Two key protective responses have been described in *E. coli* - one controlled by *soxRS* genes and the other by *oxyR* (Tsaneva and Weiss, 1990; Storz and Imlay, 1999; Gonzalez-Flecha and Demple, 2000).

The SoxRS

Low concentrations of superoxide-generating compounds such as paraquat and menadione render the cells resistant to higher doses of these agents (Geenberg and Demple, 1989) in a manner dependent on the integrity of the *soxRS* locus.

The *soxRS* regulatory system acts in two steps, with SoxR serving both as a sensor and as an activator protein. When activated by the univalent oxidation of the 2Fe-2S clusters of the protein through a not yet explained mechanism (Storz and Imlay, 1999) SoxR induces transcription of *soxS*, a positive regulator that stimulates transcription of more than 16 other superoxide responsive genes (Wu and Weiss, 1992; Hidalgo *et al*., 1995). Although this system responds to oxidative stress when cells are exposed to superoxide radical-generating agents, it is not induced by H_2O_2 (Chan and Weiss, 1987; Tsaneva and Weiss, 1990; Hidalgo *et al*., 1997). However, it was demonstrated that in some conditions H_2O_2 as well as singlet molecular oxygen could activate the SoxRS regulon *in vivo* (Manchado *et al*., 2000; Agnez-Lima *et al*., 2001).

The products of the induced *soxRS* regulon include: Mn-superoxide dismutase (*sodA*), DNA repair endonuclease IV (*nfo*), glucose-6-phosphate dehydrogenase (*zwf*), aconitase (*acnA*), stable fumarase (*fumC*), ferredoxin reductase (*fpr*), toxin and antibiotic efflux pumps (*acrAB*), an antisense RNA for the ompF porin mRNA (*micF*) and an iron-binding repressor of iron transport (*fur*) (Amabile-Cuevas and Demple, 1991;Gaudu and Weiss, 1996; Gaudu *et al*., 1997; Pomposiello and Demple, 2001).

Regulation of the OxyRS response to oxidative damage

Bacterial cells possess an adaptive response to oxidizing agents, which means that exposure to low levels of H_2O_2 allows bacterial cells to survive further toxic doses of H_2O_2 (Demple and Halbrook, 1983; Demple, 1991). The expression of nine proteins induced by H_2O_2 treatment is under the control of the *oxyR* gene (Christman *et al*., 1985). Several proteins whose expression is regulated by *oxyR* have been identified, including catalase and an alkyl hydroperoxide reductase (Morgan *et al*., 1986; Storz *et al*., 1990).

The level of OxyR protein does not change with H_2O_2 treatment, indicating that it is activated post-translationally (Storz *et al*., 1990). H_2O_2 activates the transcriptional activity of OxyR by oxidizing two of its cysteine residues (Zheng *et al*., 1998; Aslung *et al*., 1999; Storz and Toledano, 1999). When activated, OxyR activates transcription of genes that include *katG* (catalase hydroperoxidase I), *ahpCF* (alkylhidroperoxide-NADPH oxido-reductase), *grxA* (glutaredoxin), *gorA* (glutathione reductase), *dps* (a protein that protects DNA from peroxide damage) (Altuvia *et al*., 1994; Martinez and Kolter, 1997) and *fur* (an iron-binding repressor of iron transport). Under oxidative-stress conditions with the influence of OxyR and SoxRS, the number of Fur molecules per cell increases from 5,000 to about 10,000 (Zheng *et al*., 1999).

In addition, OxyR activates the synthesis of *oxyS* (Altuvia *et al*., 1997; Zhang *et al*., 1997), which encodes an untranslated mRNA that appears to regulate as many as 20 additional genes, possibly by an antisense mechanism (Altuvia *et al*., 1997; Argaman and Altuvia, 2000). The *oxyS* gene product is independent of OxyR in limiting the endogenous production of H_2O_2 in *E. coli* (Gonzalez-Flexa and Demple 1999).

Through random transcriptional fusions some new genes, the expression of which require OxyR, have been detected. These include *henF* (coproporphyrinogen III oxidase), which participates in the synthesis of photoheme IX, which is required for activity of both HPI (*katG*) and HPII (Mukhopadhyay and Schellhorn, 1997). DNA microarray techniques have detected several other new OxyR-activated genes including the *henH* heme biosynthetic gene; the six-gene *suf* operon, which may participate in Fe-S cluster assembly of repair; and four genes of unknown function (Zheng *et al*., 2001).

Cross-Adaptive Responses

Cross-adaptive response occurs when cells exposed to doses of a sub-lethal agent develop resistance against challenging doses of another lethal agent. For instance, it is

well known that *E. coli* cells exposed to low doses of H_2O_2 develop resistance against heat shock, ethanol (Jenkins *et al*., 1988), ultraviolet A (UVA) (Tyrrell, 1985), formaldehyde (Nunoshiba *et al*., 1991), menadione and cumene hydroperoxide (Christman *et al*., 1985). In contrast, prior exposure of *E. coli* to low doses of H_2O_2 has shown little or no effect on the resistance of these cells to UVC or alkylating agents (Demple and Halbrook, 1983). This section is meant to provide a general view into the cross-adaptive response induced by H_2O_2 against effects of UV (254 nm), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and cumene hydroperoxide.

Cross-adaptive response between H_2O_2 and UV (254 nm)

Demple and Halbrook (1983) have shown that prior exposure of *E. coli* to low doses of H_2O_2, in the micromolar range, engenders little or no effect on the resistance of these cells to UV. However, pretreatment with 2.5 mM H_2O_2 protected wild-type cells against UV-irradiation. This protection is independent of the SOS response, since it is also observed in a *lexA* mutant, and is dependent on DNA excision repair, since this protection is not observed in an *uvrA* mutant (Asad *et al*., 1994). The possibility that DNA repair activities other than those known to be involved in SOS response could be induced during H_2O_2 pretreatment was verified by examining the ability of H_2O_2-pretreated cells to repair incoming damaged DNA. In this way, experiments similar to those described by Weigle (1953), but involving treatment of the cells with 2.5 mM H_2O_2 instead of UV, were carried out with UV-irradiated phages. An enhanced survival of UV-irradiated phages in the wild-type, H_2O_2-pretreated cells has been shown. The same results were obtained with the *lexA1* mutant, indicating that the response is not dependent on the SOS system; nevertheless RecA and UvrA proteins are involved, since there is no UV-damaged phage reactivation in *recA* and *uvrA* mutant cells pretreated with 2.5 mM H_2O_2. The *oxyΔ3* mutant, in which the adaptive response to H_2O_2 does not occur (Imlay and Linn, 1987), presented similar results to those observed in wild-type and the *lexA1* mutant, indicating that the *oxyR* regulon does not participate in the UV-damaged phage repair which takes place in H_2O_2-treated cells.

The induction of some proteins after UV irradiation in *lexA* (Def) mutants has been observed (Lesca *et al*., 1991), and these proteins may act in mutagenesis and in the DNA repair of UV-irradiated bacteriophage lambda (Calsou *et al*., 1987). In fact, Petit *et al*. (1993) have characterized an *E. coli* gene, *dinY*, whose induction does not require the cleavage of LexA repressor, although it may be considered a member of the SOS regulon (Petit *et al*., 1993; Friedberg *et al*., 1995). The protective effect induced by H_2O_2 against UV is shown to be independent of the induction of *dinY* gene or other genes under the same control as *dinY* (Asad *et al*., 2000). Besides, this cross-protection response is not induced when the SOS regulon is constitutively expressed. The most consistent view of these observations seems to be that the induction of this response may be related to the induction of genes that are not under the control of LexA, and are inhibited by the expression of SOS genes (Asad *et al*., 2000).

Cross-adaptive response between H_2O_2 and MNNG

The Ada and Ogt proteins are involved in the DNA repair of some lesions caused by alkylating agents, as O^6-methylguanine and O^4-methylthymine. Moreover, Ada induces the expression of different genes such as *ada*, *alkA* and *aidB* in cells treated with alkylating agents, leading to the development of adaptive responses induced by pretreatment with sublethal concentrations of alkylating agents (Friedberg *et al*., 1995).

A cross-adaptive response against lethal effects caused by alkylating agents does not occur when cells of *E. coli* are exposed to low doses of H_2O_2 (micromolar) (Demple and Halbrook, 1983), but on the other hand, pretreatment with 5 mM H_2O_2 protects the wild-type strain, as well as *ada, ogt*, *ada-ogt, alkA* and *aidB* mutants against the lethal effect of MNNG (Assad *et al*., 1997). Since H_2O_2 is able to oxidize thiols (Halliwell and Gutteridge, 1999), which are necessary to convert MNNG into the mutagenic methylnitrosamine (Sedgwick and Robins, 1980), similar experiments were performed with N-nitroso-N-methylurea and N-nitroso-N-ethylurea, which do not require activation by thiols, and the results were similar to those obtained with MNNG (Assad *et al*., 1997). This protection is accompanied by a reduction in the mutation frequency in the wild-type cells and in the *ogt* mutant, but not in the *ada* mutant. So, Ada protein is able to decrease the mutagenic lesions induced by MNNG in H_2O_2-pretreated cells. Horsfall *et al*. (1990) have shown that the DNA context influences both the distribution and reparability of alkylation damage. This observation may provide information on the cross-adaptive response between H_2O_2 and MNNG, since the DNA alkylation pattern induced by MNNG can be altered when the DNA has already been oxidized.

Cross-adaptive response between H_2O_2 and cumene hydroperoxide

Ahp protein expression is under the control of OxyR protein. Ahp plays an important role in protecting bacterial cells against alkyl hydroperoxides, such as cumene hydroperoxide (Storz *et al*., 1989), and the active enzyme requires the presence of two subunits with molecular weights of 22 kDa (AhpC) and 57 kDa (AhpF).

It has been shown that 2.5 mM H_2O_2 pre-treatment protects wild-type cells against cumene hydroperoxide killing (Asad *et al*., 1998). Unexpectedly, this protection is observed in the *oxyΔ* 3 mutants, meaning that it is an OxyR-independent phenomenon. The protection is mediated by Ahp protein and there is no requirement for novel

protein synthesis (Asad *et al*., 1998). Electrophoretic profile studies of proteins from wild-type cells treated with H_2O_2 (2.5 mM) for 20 min have shown that a 22 kDa protein (possibly Ahp protein) observed in untreated wild-type cells is faintly visible in H_2O_2-treated cells. In the latter, instead of the 22 kDa band a 24 kDa band appears. This modification was not observed when wild type was treated with lower concentrations of H_2O_2 (micromolar).

It is known that H_2O_2 causes modification in several *E. coli* proteins (Farr and Kogona, 1991). So, these results suggest that millimolar concentrations of H_2O_2 might induce an alteration in the electrophoretic profile of the smaller subunit of AhpC (22 kDa). It is important to bear in mind that inhibition of protein synthesis does not prevent the appearance of the 24-kDa band promoted by H_2O_2. These results are interpreted to mean that AhpC 22-kDa subunit undergoes conversion to different oxidized forms when challenged with higher doses of H_2O_2, resulting in a small alteration of its molecular weight. Since Ahp may prevent oxidative damage and repair lesions caused by active oxygen species (Farr and Kogona, 1991), the AhpC 24 kDa form can be interfering with the repair of lesions caused by cumene hydroperoxide (Asad *et al*., 1998).

DNA damage and repair in *E coli* challenged by H_2O_2 in the presence of iron chelators

The protection conferred by iron scavengers against the effect of H_2O_2 was first demonstrated by Imlay and Linn (1988). In 1991, Asad and Leitão showed that prior treatment with iron chelators such as o-phenanthroline, dipyridyl and desferrioxamine protected the cells against the H_2O_2 lethal effect, but the number of induced DNA-single strand breaks (DNA-SSB) was similar even in the presence of iron chelators. More significantly, the breaks observed after treatment with metal chelators and H_2O_2 are repaired 60 min after H_2O_2 elimination in *xthA* but not *polA* mutant cells (Asad and Leitão, 1991).

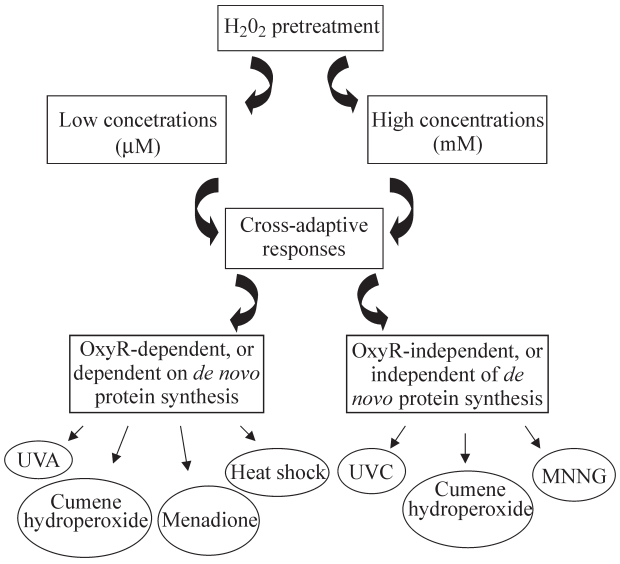

Figure 1 - Cross-adaptive responses induced by low or high H_2O_2 concentration operate through different mechanisms.

Since then, in a series of experiments spanning a full decade, DNA damage and the genetic repair mechanisms of the H_2O_2-induced DNA lesions in a low-iron condition, which means cells previously treated with metal-ion chelators, have been examined. Asad *et al*. (1995) demonstrated a striking hypersensitivity of the *fpg* and *uvrA* mutant strains to H_2O_2 under low-iron conditions, suggesting that the processing of DNA lesions induced in this situation may occur in a different way from that found under physiological iron conditions.

Other investigations have demonstrated common pathways in the cell response to H_2O_2 challenge in a low-iron condition and in physiological iron conditions. SOS and the OxyR pathways are the ones involved in the response of *E. coli* cells subjected to both challenges (Asad *et al*., 1997).

Evidence for the participation of endonuclease IV (the *nfo* gene product) in the repair of DNA lesions generated by H_2O_2 under low-iron conditions came from studies of the processing of DNA strand breaks in different *E. coli* strains (Galhardo *et al*., 2000). Survival experiments with *xth*A, *nf*o, and *xthA nfo* mutant strains in which the cultures were treated with 5 mM H_2O_2 confirm data previously described in the literature, so that the *xthA* mutant was sensitive to H_2O_2 (Demple *et al*., 1983) while the *nfo* strain was not (Cunningham *et al*., 1986), and the *xthA nfo* double mutant was even more sensitive than the *xthA* mutant. Experiments with cultures pretreated with dipyridyl showed a distinct pattern of dependence on AP endonucleases for bacterial survival. Neither the *xthA* nor the *nfo* single mutants were significantly inactivated by H_2O_2 under low-iron conditions. However, the *xthA nfo* double mutant was highly sensitive to this treatment, indicating that both exonuclease III and endonuclease IV may act in the repair of the oxidative lesions generated under such conditions. Analysis of the sedimentation profile in alkaline sucrose gradients also demonstrated that both *xthA* and *nfo* mutants, but not the *xthA nfo* double mutant, could carry out complete repair of DNA-SSB generated by H_2O_2 under low-iron conditions (Galhardo *et al*., 2000). Thus, these findings support the idea that both exonuclease III and endonuclease IV act in the repair of DNA damage induced by H_2O_2 in iron-depleted *E. coli,* and then strongly suggest that the lesions caused by H_2O_2 in the presence of dipyridyl are qualitatively different from those found in the absence of this iron chelator. It is interesting to note that despite the severe repair defect of the *xthA nfo* strain, significant levels of repair were observed under both physiological and low-iron conditions. The exact nature of this repair mechanism remains to be elucidated.

Further studies indicated that the formation of substrates for exonuclease III and endonuclease IV is mediated

by the Fpg DNA glycosylase, since the *fpg* mutation increases cell survival and repair of DNA strand breaks in a null AP endonuclease background (*xthA nfo* double mutant). Recently, Speck *et al*. (2002) showed that the same phenomenon is observed for nitric oxide treatment, that is, in a null AP endonuclease background, *fpg* and *ung* mutations are able to increase cell survival.

The role of copper ions in the lethality induced by H_2O_2 in low-iron conditions

Most of the work on the effects of H_2O_2 in living organisms has reported that DNA damage induced by H_2O_2 can be explained basically by the generation of hydroxyl radicals through the iron-mediated Fenton reaction. Some authors have attributed the same role to copper ions, also present in biological systems (Sagripanti and Kraemer, 1989; Aruoma *et al*., 1991; Dizdaroglu *et al*., 1991). The suggested mechanism for this effect takes into account the formation of a DNA-Cu^{2+} complex in which this metal would be reduced and then the DNA-Cu^{+} would react with H_2O_2 to produce oxidative damage, via Fenton-type reactions (Aruoma *et al*., 1991; Byrnes *et al*., 1992; Lloyd *et al*., 1997; Lloyd and Phillips, 1999). Although copper ions seemed not to participate in the genotoxicity of H_2O_2 in *E. coli* under physiological iron conditions (Asad and Leitão, 1991), these ions have been shown to take part in the genotoxicity of H_2O_2 under conditions of low iron availability. In fact, neocuproine (copper ion chelator) can inhibit cell inactivation and DNA strand breakage caused by H_2O_2 in the presence of iron chelators (Almeida *et al*., 2000). This phenomenon can only be detected in high concentrations of H_2O_2 (15 mM), suggesting that these ions only interact with H_2O_2 in the intracellular environment in the absence of iron and under severe oxidative stress.

Copper-induced DNA damage has been studied in several systems. It has been demonstrated that such damage is targeted preferentially to adjacent polyguanosines (Aruoma *et al*., 1991). Analysis of the oxidative base lesions generated by copper-mediated Fenton reactions showed that purine residues are the preferred targets of the DNA-damaging species (Sagripanti and Kraemer 1989; Dizdaroglu *et al*., 1991; Lloyd and Phillips, 1999], with 8-oxoG being the most abundant lesion formed. Frelon *et al*. (2003) have shown that only 8-oxoG is formed upon incubation of DNA with Cu(II) ions and H_2O_2 and suggested that *in vitro* the Fenton reaction triggered by copper ions generates singlet oxygen as the predominant reactive species, with hydroxyl radical being produced predominantly when the Fenton reaction is triggered by iron ions. In fact, 8-oxoG is the only lesion excised from H_2O_2/Cu^{2+}-treated DNA at detectable levels by the yOgg1 glycosylase from *Saccharomyces cerevisiae* (Karahalil *et al*., 1998). The finding of a remarkable increase in mutagenesis in *uvrA fpg*-strain cultures pretreated with dipyridyl, and its inhibition by neocuproine (Almeida *et al*., 2000), strongly suggest that 8-oxoG is formed in large amounts by such treatment. The data suggesting a significant role for Fpg and UvrA proteins in the repair of DNA lesions correlate well with the hypothesis of copper participation, since it was demonstrated that these proteins are important in the repair of lesions induced by singlet oxygen, another well-known guanine-damaging agent. Taken together these results may indicate the production of singlet oxygen as the predominant ROS when *E. coli* cultures are challenged with H_2O_2 under low-iron conditions.

On the other hand some interesting results were obtained from mutagenesis assays performed by our group (manuscript in preparation) regarding the nature of lesions produced by H_2O_2 in cells previously treated with dipyridyl. Using an assay based on *lac*$^{-}$ reversion through a single base change in mutated *lacZ* codon (Cupples and Miller, 1989), we found that concentrations of H_2O_2 above 15 mM induce almost exclusively A:T→ T:A transversions. However, in cells previously treated with dipyridyl a massive and significant presence of G:C→ A:T transitions is detected, a clearly different profile of induced mutations. Considering that this transversion is not reported to be induced by 8-oxoG the authors have suggested that H_2O_2 under low-iron conditions may generate the lesion 5-hydroxy-2'-deoxycytidine, a highly mutagenic product of cytosine oxidation that also constitutes a substrate for Fpg repair protein (Hatahet *et al*., 1994; Feig *et al*., 1994).

A qualitative difference exists between the DNA lesions generated by H_2O_2 plus copper (in the presence of iron chelators) and iron (physiologic iron condition), which can be deduced by the different DNA repair requirements for cell survival in these situations. The reason for this difference is not known, and there exists an as yet unsolved question about the participation of hydroxyl radical in the damage to DNA caused by copper plus H_2O_2 (Sagripanti and Kraemer, 1989; Yamamoto and Kawanishi, 1989). Free-radical scavengers partially inhibit *E. coli* inactivation by H_2O_2 (Imlay *et al*., 1988), and we observed that thiourea protects the cells against the lethality produced by H_2O_2 both in the presence of dipyridyl (our unpublished results) and in physiological iron conditions (Asad and Leitão, 1991).

Synergistic lethal interactions

In the last three decades, many studies have been published regarding synergistic lethal effects between H_2O_2 and different physical and chemical agents. Some of them will be described in this section.

Lethal synergism between UV (254 nm) and H_2O_2

Synergistic lethal interaction between UV (254 nm) and X-ray irradiation, a well known system of generating H_2O_2 and DNA strand breaks was described three decades

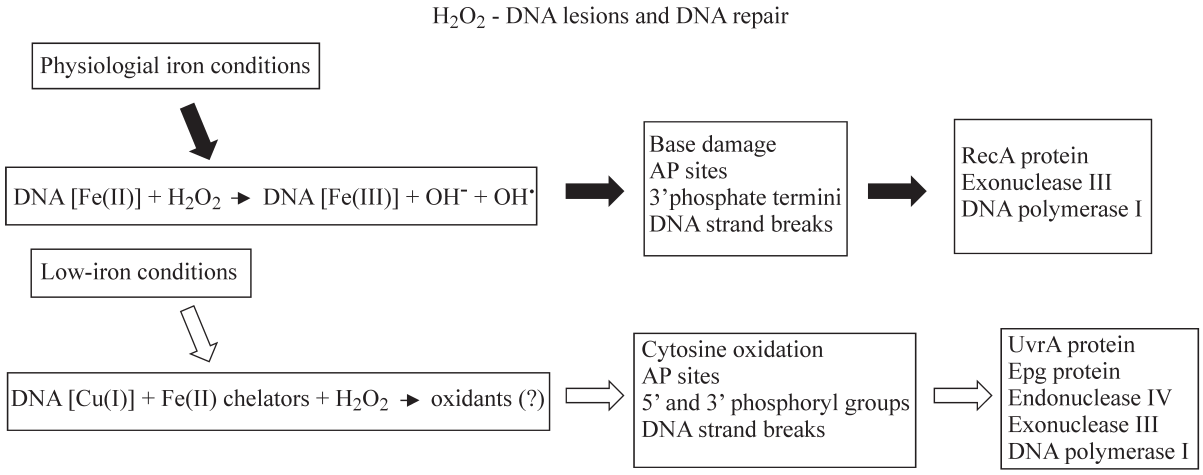

Figure 2 - DNA lesions and DNA repair in physiological and low-iron conditions.

ago in *E. coli* (Haynes, 1966; Martignoni and Smith, 1973). However, synergistic lethal interaction was not observed between UV (254 nm) and H_2O_2 (Hartman and Eisenstark, 1980). On the other hand, synergistic killing of *E. coli* and *S. thyphimurium* by near-UV (300-400 nm) radiation and H_2O_2 has been described (Hartman and Eisenstark, 1978; Hartman and Eisenstark, 1980; Kramer and Ames, 1987).

An analysis of the UV and X-ray effects (Martignoni and Smith, 1973), the results of repair of H_2O_2 lesions in several mutant strains (Ananthaswany and Eisenstark, 1977), and our own results for UV and reductone (an H_2O_2-generating keto-aldehyde) (Leitão *et al*., 1981a; Leitão *et al*., 1981b) suggested that a synergistic lethal interaction between UV and H_2O_2 (254 nm) should be observed. Indeed, Leitão and Carvalho (1988) observed that prior UV (254 nm) irradiation strongly increased the sensitivity to H_2O_2 of wild-type *E. coli* cells, and this synergistic lethal interaction was also observed to a reduced extent in a *polA* mutant, suggesting that UV lesions are potentiated by the additional damage produced by H_2O_2, a result similar to those observed by Martignoni and Smith (1973) for UV and X-rays.

The detection of DNA double-strand breaks by DNA sedimentation on neutral sucrose gradients (Leitão and Carvalho, 1988) clearly indicates a mechanism responsible for the synergistic lethal interaction observed. Although H_2O_2 can produce DNA strand breaks with low efficiency (Demple and Linn, 1982), the repair of UV lesions by the action of the *uvr* gene products generated single-stranded DNA regions and it was supposed that, in the presence of H_2O_2, DNA double-strand breaks might arise in these regions, produced by the action of exonuclease III (the *xthA* gene product). This hypothesis was confirmed by the absence of synergistic lethal interaction between UV (254 nm) and H_2O_2 in *xthA* as well as in *uvrA* mutant strains (Leitão and Carvalho, 1988). It seems that the same kind of mechanism may be operating in the synergisms observed with near-UV and H_2O_2, UV and reductone and UV and X-rays.

Lethal synergism between phenanthrolines and H_2O_2

As described earlier, prior treatment with iron chelators such as desferrioxamine, dipyridyl or o-phenanthroline (1,10-phenanthroline) protects *E. coli* cells against the lethal effects of H_2O_2. However, Asad *et al*. (1994) detected a strong lethal interaction when *xthA* mutant cells were treated simultaneously with H_2O_2 and o-phenanthroline. In the same way Almeida *et al*. (1999) have also detected a lethal synergistic interaction between neocuproine (2,9-dimethyl 1,10-phenanthroline) and H_2O_2. In both cases the phenomenon of synergism was accompanied by an increase in the number of DNA strand breaks.

In the case of the synergism described by Asad *et al*. (1994), it was argued that the formation and dissociation equilibrium of Fe^{2+}-Phe complex, which follows equations IV, V and VI (Lee *et al*., 1948) shown below, would explain the observed phenomenon.

Fe^{2+}	+ Phe	→	$FePhe^{2+}$	rapidly established equilibrium	(eq. IV)
$FePhe^{2+}$	+ Phe	→	$FePhe_2^{2+}$	rapidly established equilibrium	(eq. V)
$FePhe_2^{2+}$	+ Phe	→	$FePhe_3^{2+}$	rate-determining step	(eq. VI)

Since the equilibrium of the bis and mono complexes of Fe^{2+}-Phe are rapidly established and since these bis and mono complexes react quickly with H_2O_2 (Burgers and Prince, 1965), there would be H_2O_2-mono and H_2O_2-bis complex formation and these complexes may be extremely lethal to the cells, therefore justifying the lethal interaction observed. In this case, the ability of o-phenanthroline to penetrate into the DNA duplex, acting as a shuttle for the Fe^{2+} ions would increase the efficiency of OH generation close to DNA (Furtado *et al*., 1997). Recently Furtado (2002) showed that this interaction also occurs *in vitro*. By

using plasmid DNA, he showed that the high number of breaks, as measured by the transformation of supercoiled to relaxed form, is obtained when iron and o-phenanthroline are added to the reaction medium immediately before H_2O_2.

As mentioned above, Almeida *et al*. (1999) showed that prior incubation of *fpg*, *uvrA* and *lexA* mutant strains with neocuproine led to an increased sensitivity to H_2O_2. The chemistry of this synergistic lethal interaction was suggested to occur via the iron-mediated Fenton reaction, which would be responsible for displacement of copper ions from the complex Neo_2Cu^+, as Florence *et al*., (1985) have reported to occur *in vitro*. In fact, neocuproine, as well as other phenanthroline derivatives, have the ability to penetrate into DNA, guiding metal ions to this site, which can contribute to radical formation in the vicinity of this molecule (Furtado *et al*., 1997). Indeed, it is possible that neocuproine can guide copper ions to the DNA molecule, thereby promoting the occurrence of radical generation at this site as H_2O_2 reacts with these ions.

Conclusions

The most remarkable outcome from the genetic studies on H_2O_2-mediated genotoxicity is the striking sensitivity of *xthA* mutant *E. coli* cells to H_2O_2. In fact, this observation provides some insights into the nature of H_2O_2-induced lesions. Nevertheless, it is important to realize that the oxidative stress produced by H_2O_2 results in the induction of a diverse set of physiological responses, which include some paradoxical effects. In this context, the most unexpected phenomenon investigated so far is the bimodal pattern of inactivation of *E. coli* by H_2O_2 described by Imlay and Linn (1986). In the same way, it is now evident that although pre-treatment of *E. coli xthA* cultures with iron chelators confers protection against the lethal effects of H_2O_2, DNA lesions can still be formed under these conditions. We have to keep in mind that in contrast with what is observed in the repair of damage induced by H_2O_2 under physiological iron conditions, after treatment with H_2O_2 in low-iron conditions: (i) the lesions observed may be repaired in *xthA* mutant cells (ii) both endonuclease IV and exonuclease III as well as Fpg and UvrA proteins participate in the repair of these lesions. Such a difference in the repair suggests a qualitative difference in the formation of DNA lesions that is independent of the presence of iron. Iron chelators partially inhibit *E. coli* inactivation by H_2O_2. However, the precise mechanism for the genotoxic effect of H_2O_2 under low-iron conditions remains to be elucidated. It is also curious to see that some metal-ion chelators such as o-phenanthroline and neocuproine, which should generally inhibit the lethal effect of H_2O_2 can sometimes enhance cell inactivation. Moreover, the data available so far suggest that H_2O_2 (2.5 mM) can induce protection against UV light, MNNG and cumene hydroperoxide independently of the adaptive response, indicating that H_2O_2 can produce cross-protection responses through various mechanisms that might not involve the induction of *de novo* protein synthesis.

Acknowledgements

We thank C.F.M. Menck, D.P. Carvalho and M. Sorenson for critical reading of this manuscript. Research supported by CNPq, CAPES, FUJB, FAPERJ and PRONEX.

References

Agnez-Lima LF, Di Mascio P, Demple B and Menck CFM (2001) Singlet molecular oxygen triggers the *soxRS* regulon of *Escherichia coli.* Biol Chem 382:1071-1075.

Almeida CEB, Felício DL, Galhardo RS, Cabral-Neto JB and Leitão AC (1999) Synergistic lethal effect between hydrogen peroxide and neocuproine (2,9-dimethyl 1,10-phenanthroline) in *Escherichia coli.* Mutat Res 433:59-66.

Almeida CEB, Galhardo RS, Felício DL, Cabral-Neto JB and Leitão AC (2000) Copper ions mediate the lethality induced by hydrogen peroxide in low iron conditions in *Escherichia coli*. Mutat Res 460:61-67.

Altuvia S, Almiron M, Huisman G, Kolter R and Storz G (1994) The *dps* promoter is activated by OxyR during growth and by IHF and σ^s in stationary phase. Mol Microbiol 13:265-272.

Altuvia S, Weinstein-Fischer D, Zhang A, Postow L and Storz G (1997) A small, stable RNA induced by oxidative stress: role as a pleiotropic regulator and antimutator. Cell 90:43-53.

Amabile-Cuevas CF and Demple B (1991) Molecular characterization of the *soxRS* genes of *Escherichia coli*: two genes control a superoxide stress regulon. Nucleic Acids Res 19:4479-4484.

Ames BN, Shigenaga MK and Hagen TM (1993) Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915-7922.

Ananthaswamy HN and Eisenstark A (1977) Repair of hydrogen peroxide-induced single-strand breaks in *E. coli* deoxyribonucleic acid. J Bacteriol 130:187-191.

Argaman L and Altuvia S (2000) *fhlA* repression by OxyS RNA: kissing complex formation at two sites results in a stable antisense-target RNA complex. J Mol Biol 300:1101-1112.

Aruoma OI, Halliwell B, Gajewski E and Dizdaroglu M (1991) Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem J 273:601-604.

Asad LMBO, Almeida CEB, Silva AB, Asad NR and Leitão AC (1994) Hydrogen peroxide induces the repair of UV-damaged DNA in *Escherichia coli*: A *lexA*-independent but *uvrA*- and *recA*-dependent mechanism. Curr Microbiol 29:291-294.

Asad LMBO, Asad NR, Silva AB, Almeida CEB and Leitão AC (1997) Role of SOS and OxyR systems in the repair of *Escherichia coli* submitted to hydrogen peroxide under low iron conditions. Biochimie 79:359-364.

Asad LMBO, Asad NR, Silva AB, Felzenszwalb I and Leitão AC (1997) Hydrogen peroxide induces protection against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) effects in *Escherichia coli.* Mutat Res 383:137-142.

Asad LMBO, De Carvalho AA, Felzenszwalb I, Leitão AC and Asad NR (2000) H_2O_2-induced cross-protection against UV-C killing in *Escherichia coli* is blocked in a *lexA* (Def) background. J Phochem Photobiol (B) 54:67-71.

Asad NR, Almeida CEB, Asad LMBO, Felzenswalb I and Leitão AC (1995) Fpg and UvrA proteins participate in the repair of DNA lesions induced by hydrogen peroxide in low iron level in *Escherichia coli.* Biochimie 77:262-264.

Asad NR, Asad LMBO, Almeida CEB and Leitão AC (1994) Lethal interaction between hydrogen peroxide and o-phenanthroline in *Escherichia coli.* Braz J Med Biol Res 27:2551-2555.

Asad NR, Asad LMBO, Silva AB, Felzenszwalb I and Leitão AC (1998) Hydrogen peroxide induces protection against lethal effects of cumene hydroperoxide in *Escherichia coli* cells: An Ahp independent but OxyR dependent system? Mutat Res 407:253-259.

Asad NR and Leitão AC (1991) Effects of metal ion chelators on DNA strand breaks and inactivation produced by hydrogen peroxide in *Escherichia coli*: Detection of iron-independent lesions. J Bacteriol 173:2562-2568.

Aslund F, Zheng M, Beckwith J and Storz G (1999) Regulation of OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. Proc Natl Acad Sci USA 96:6161-6165.

Bjelland S and Seeberg E (2003) Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutat Res 531:37-80.

Blaisdell JA, Hatahet Z and Wallace SS (1999) A novel role for *Escherichia coli* endonuclease VIII in prevention of spontaneous G T transversions. J Bacteriol 181:6396-6402.

Boiteux S and Huisman O (1989) Isolation of a formamidopyrimidine-DNA glycosylase (*fpg*) mutant of E*scherichia coli* K-12. Mol Gen Genet 215:300-305.

Brakely WF, Fuciarelli AF, Wegher BJ and Dizdaroglu M (1990) Hydrogen peroxide-induced base damage in deoxyribonucleic acid. Radiat Res 121:338-343.

Brandi G, Cattabeni F, Albano A and Cantoni C (1989) Role of hydroxyl radicals in *Escherichia coli* killing induced by hydrogen peroxide. Free Radic Res Commun 6:47-55.

Braun V (1997) Avoidance of iron toxicity through regulation of bacterial iron transport. Biol Chem 378:779-786.

Braun V, Hantke K and Koster W (1998) Bacterial ion transport: mechanisms, genetics and regulation. In Sigel A and Sigel H (eds) Metal Ions in Biological Systems. Marcel Dekker, New York, NY, pp 67-145.

Burgers J and Prince RH (1965) Kinetics of reactions of ligand-substitued tris-(2-2'-dipyridyl) iron (II) complexes. J Chem Soc 1132:6061-6066.

Byrnes RW, Antholine WE and Petering DH (1992) Oxidation-reduction reactions in Ehrlich cells treated with copper-neocuproine. Free Rad Biol Med 13:469-478.

Cadet J, Douki T, Gasparuto D and Ravanat J-L (2003) Oxidative damage to DNA: Formation, measurement and biochemical features. Mutat Res 531:5-23.

Calsou P, VillaVerde A and Defais M (1987) Activated RecA protein may induced expression of gene that is not controlled by the LexA repressor and whose function is record for mutagenesis and repair of UV-irradiated bacteriophage lambda. J Bacteriol 169:4816-4821.

Chan E and Weiss B (1987) Endonuclease IV of *Escherichia coli* is induced by paraquat. Proc Natl Acad Sci USA 84:3189-3193.

Chaudhry MA, Dedon PC, Wilson DM III, Demple B and Weinfeld M (1999) Removal by human apurinic/apirimidinic endonuclease 1 (Ape1) and *Escherichia coli* exonuclease III of 3'-phosphoglycolates from DNA treated with neocarzinostatin, calicheamicin and gamma-radiation. Biochem Pharmacol 57:531-538.

Christman MF, Morgan RW, Jacobson FS and Ames BN (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium.* Cell 41:753-762.

Cooke MS, Evans MD, Dizdaroglu M and Lunec J (2003) Oxidative DNA damage: Mechanisms, mutation, and disease. FASEB J 17:1195-1214.

Courcelle J, Khoudursky A, Peter B, Brown PO and Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics 158:41-64.

Cunningham RP, Saporito SM, Spitzer SG and Weiss B (1986) Endonuclease IV (*nfo*) mutant of *Escherichia coli.* J Bacteriol 168:1120-1127.

Cunningham RP and Weiss B (1985) Endonuclease III (*nth*) mutants of *Escherichia coli.* Proc Natl Acad Sci USA 82:474-478.

Cupples C and Miller, J (1989) A set of *lacZ* mutations in *Escherichia coli* which allow rapid detection of each of the six base substitutions. Proc Natl Acad Sci USA 86:5345-5349.

Demple B (1991) Regulation of bacterial oxidative stress genes. Annu Rev Genet 25:315-337.

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

Demple B, Halbrook J and Linn S (1983) *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. J Bacteriol 153:1079-1082.

Demple B and Harrison L (1994) Repair of oxidative DNA damage to DNA: Enzymology and biology. Annu Rev Biochem 63:915-948.

Demple B and Linn S (1982) 5,6-saturated thymine lesions in DNA: Production by ultraviolet light and hydrogen peroxide. Nucleic Acid Res 10:3781-3789.

Dizdaroglu M (2003) Substract specificities and excision kinetics of DNA glycosylases involved in base-excision repair of oxidative DNA damage. Mutat Res 531:109-126.

Dizdaroglu M, Rao G, Halliwell B and Gajewski E (1991) Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions, Arch Biochem Biophys 285:317-324.

Farr SB and Kogoma T (1991) Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium.* Microbiol Rev 55:561-585.

Feig DI, Sowers LC and Loeb LA (1994) Reverse chemical mutagenesis: Identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. Proc Natl Acad Sci USA 91:6609-6613.

Fernandez De Henestrosa AR, Ogi T, Aoyagi S, Chafin D, Hayes JJ, Ohmori H and Woodgate R (2000) Identification of additional genes belonging to the *lexA* regulon in *Escherichia coli.* Mol Microbiol 35:1560-1572.

Florence TM, Stauber JL and Mann KJ (1985) The reaction of copper-2,9-dimethyl-1,10-phenanthroline with hydrogen peroxide. J Inorg Biochem 23:243-254.

Frelon S, Douki T, Favier A and Cadet J (2003) Hydroxyl radical is not the main reactive species involved in the degradation of DNA bases by copper in the presence of hydrogen peroxide, Chem Res Toxicol 16:191-197.

Friedberg EC, Walker GC and Siede W (1995) DNA Repair and Mutagenesis. Academic Press, New York, 698 pp.

Furtado FAC (2002) Caracterização adicional do fenômeno de interação letal entre 1,10-fenantrolina e peróxido de hidrogênio em *Escherichia coli.* PhD Thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

Furtado FAC, Asad NR and Leitão AC (1997) Effects of 1,10-phenanthroline and hydrogen peroxide in *Escherichia coli*: Lethal interaction. Mutat Res 385:251-258.

Galhardo RS, Almeida CEB, Leitão AC and Cabral-Neto JB (2000) Repair of DNA lesions induced by hydrogen peroxide in the presence of iron chelators in *Escherichia coli*: Participation of endonuclease IV and Fpg. J Bacteriol 182:1964-1968.

Gaudu P, Moon N and Weiss B (1997) Regulation of the *soxRS* oxidative stress regulon. Reversible oxidation of the Fe-S centers of SoxR in vivo. J Biol Chem 272:5082-5086.

Gaudu P and Weiss B (1987) SoxR a [2Fe-2S] transcription factor, is active only in its oxidized form. Proc Natl Acad Sci USA 84:3189-3193.

Geenberg JT and Demple B (1989) A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. J Bacteriol 171:3922-3939.

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.

Gonzalez-Flecha B and Demple B (2000) Genetic responses to free radicals. Homeostasis and gene control. Ann New York Acad Sci 899:69-87.

Hagensee ME and Moses RE (1989) Multiple pathways for repair of hydrogen peroxide-induced DNA damage in *Escherichia coli.* J Bacteriol 171:991-995.

Halliwell B and Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. J Biochem 219:1-14.

Halliwell B and Gutteridge JMC (1999) Free Radicals In Biology and Medicine. Oxford University Press, New York, 936 pp.

Hanawalt PC (2001) Controling the efficiency of excision repair. Mutat Res 485:3-13.

Hantke K (1981) Regulations of ferric ion transport in *Escherichia coli* K12: Isolation of a constitutive mutant. Mol Gen Genet 182:288-292.

Hantke K (2002) Members of the Fur protein family regulate iron and zinc transport in *E. coli* and characteristics of the Fur-regulated fhuF protein. J Mol Microbiol Biotechnol 4:217-222.

Hartman PS and Eisenstark A (1978) Synergistic killing of *Escherichia coli* by near-UV radiation and hydrogen peroxide. Distinction between RecA-repairable and RecA-non-repairable damage. J Bacteriol 133:769-774.

Hartman PS and Eisenstark A (1980) Killing of *Escherichia coli* K-12 by near-ultraviolet radiation in the presence of hydrogen peroxide: Role of double-strand breaks in absence of recombinational repair. Mutat Res 72:31-42.

Hatahet Z, Kow YW, Purmal AA, Cunninghan RP and Wallace SS (1994) New substrates for old enzymes. 5-hydroxy-2'-deoxicytidine and 5-hydroxy-2'-deoxyuridine are substrates for *Escherichia coli* endonuclease III and formamidopyrimidine DNA N-glycosylase, while 5-hydroxy-2'-deoxyuridine is a substrate for uracil DNA N-glycosylase. J Biol Chem 269:18814-18820.

Haynes RH (1966) The interpretation of microbial inactivation and recovery phenomena. Radiat Res Suppl 6:1-29.

Henle ES and Linn S (1997) Formation, prevention and repair of DNA damage by iron/hydrogen peroxide. J Biol Chem 272:19095-19098.

Hidalgo E, Bollinger Jr JM, Bradley TM, Walsh CT and Demple B (1995) Binuclear [2Fe-2S] clusters in the *Escherichia coli* SoxR protein and role of the metal centers in transcription. J Biol Chem 270:20908-20914.

Hidalgo E, Ding H and Demple B (1997) Redox signal transduction mutations shifting [2Fe-2S] centers of SoxR sensor-regulated to the oxidized form. Cell 88:121-129.

Horsfall MJ, Gordon JE, Burns PA, Zielenska M, Van der Viet GME and Glickman BW (1990) Mutational specificity of alkylating agents and the influence of DNA repair. Environ Mol Mut 15:107-122.

Imlay JA, Chin SM and Linn S (1988) Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro.* Science 240:640-642.

Imlay JA and Linn S (1986) Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. J Bacteriol 166:519-527.

Imlay JA and Linn S (1987) Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J Bateriol 169:2967-2976.

Imlay JA and Linn S (1988) DNA damage and oxygen radical toxicity. Science 240:1302-1309.

Izumi T, Hazra TK, Boldogh I, Tomkinson AE, Park MS, Ikeda S and Mitra S (2000) Requeriment for human AP endonuclease 1 for repair of 3'-blocking damage at DNA single-strand breaks induced by reactive oxygen species. Carcinogenesis 21:1329-1334.

Jenkins DE, Schultz JE and Martin A (1988) Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli.* J Bacteriol 170:3910-3914.

Jiang D, Hatahet Z, Blaisdell JO, Melamede RJ and Wallace SS (1997) *Escherichia coli* endonuclease VIII: Cloning, sequencing, and overexpression of the *nei* structural gene and characterization of *nei* and *nei nth* mutants. J Bacteriol 179:3773-3782.

Karahalil B, Girard PM, Boiteux S and Dizdaroglu M (1998) Substrate specificity of the Ogg1 protein of *Saccharomyces cerevisiae*: excision of guanine lesions produced in DNA by ionizing radiation-or hydrogen peroxide/metal ion-generated free radicals. Nucleic Acids Res 26:1228-1232.

Kehrer JP (2000) The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 149:43-50.

Konola JT, Sargent KE and Gow JB (2000) Efficient repair of hydrogen peroxide-induced DNA damage by *Escherichia coli* requires SOS induction of RecA and RuvA proteins. Mutat Res 459:187-194.

Kow YW, Wallace SS and Van Houten B (1990) UvrABC nuclease complex repairs thymine glycol, an oxidative DNA base damage. Mutat Res 235:147-156.

Kramer GF and Ames BN (1987) Oxidative mechanisms of toxicity of low-intensity near-UV light in *Salmonella typhimurium.* J Bacteriol 169:2259-2266.

Kruszewski M (2003) Labile iron pool: the main determinant of cellular responses to oxidative stress. Mutat Res 531:81-92.

Lee TS, Kolthoff IM and Leusing DL (1948) Reaction of ferrous and ferric ions with 1,10-phenanthroline. II. Kinetics of formation and dissociation of ferrous phenanthroline. J Am Chem Soc 70:3596-3600.

Leitão AC, Caldas LR and Alcantara Gomes R (1981a) Inhibition of DNA repair and production of single-strand breaks in *Escherichia coli* by reductone. Photochem Photobiol 34:49-53.

Leitão AC and Carvalho RES (1988) Synergistic killing of *Escherichia coli* K-12 by UV (254nm) and H_2O_2. Int J Radiat Biol 53:477-488.

Leitão AC, Motta HC and Alcantara Gomes R (1981b) Kinetics of DNA-induced breaks by reductone treatment: *in vitro* and *in vivo* studies. Photochem Photobiol 34:745-748.

Lesca C, Petit C and Defais M (1991) UV induction of *lexA* independent proteins which could be involved in SOS repair. Biochimie 73:407-409.

Lin JJ and Sancar A (1989) A new mechanism for repairing oxidative damage to DNA: (A)BC exonuclease removes AP sites and thymine glycols from DNA. Biochemistry 28:7979-7984.

Lloyd DR and Phillips DH (1999) Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. Mutat Res 424:23-36.

Lloyd DR, Phillips DH and Carmichael PL (1997) Generation of putative intrastrand cross-links and strand breaks in DNA by transition metal ion-mediated oxygen radical attack. Chem Res. Toxicol 10:393-400.

Manchado M, Michán C and Pueyo C (2000) Hydrogen peroxide activates the SosRS regulon *in vivo*. J Bacteriol 182:6842-6844.

Martignoni KD and Smith KC (1973) The synergistic action of ultraviolet and X-radiation on mutants of *Escherichia coli* K-12. Photochem Photobiol 18:1-8.

Martinez A and Kolter R (1997) Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. J Bacteriol 179:5188-5194.

Melamede RJ, Hatahet Z, Kow IW, Ide H and Wallace SS (1994) Isolation and characterization of endonuclease VIII from *Escherichia coli.* Biochemistry 33:1255-1264.

Mello-Filho AEC and Meneghini R (1985) Protection of mammalian cells by o-phenanthroline from lethal and DNA-damaging effects produced by active oxygen species. Biochim Biophys Acta 847:82-89.

Meneghini R (1987) A toxicidade do oxigênio. Ci Hoje 5:57-62.

Meneghini R (1988) Genotoxicity of active oxygen species in mammalian cells. Mutat Res 195:215-230.

Miles GM and Sancar A (1989) DNA repair. Chem Res Toxicol 2:197-226.

Mol CD, Hosfield DJ and Tainer JA (2000) Abasic site recognition by two apurinic/apyrimidinic endonucleases families in DNA base excision repair: the 3' ends justify the means. Mutat Res 460:211-229.

Morgan RW, Christman MF, Jacobson FS, Storz G and Ames BN (1986) Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc Natl Acad Sci USA 83:8059-8063.

Mukhopadhyay S and Schellhorn HE (1997) Identification and characterization of hydrogen peroxide-sensitive mutants of *Escherichia coli*: Genes that require OxyR for expression. J Bacteriol 179:330-338.

Mustard JA and Little JW (2000) Analysis of *Escherichia coli* RecA interactions with LexA, lambda CI, and UmuD by site-directed mutagenesis of *recA.* J Bacteriol 182:1659-1670.

Nunoshiba T, Hashimoto M and Nishioka H (1991) Cross-adaptive response in *Escherichia coli* caused by pretreatment with H_2O_2 against formaldehyde and other aldehyde compounds. Mutat Res 255:265-271.

Petit C, Cayrol C, Lesca C, Kaiser P, Thompson C and Defais M (1993) Characterization of *dinY*, a new *Escherichia coli* DNA repair gene whose products are damage inducible even in a *lexA* (Def.) background. J Bacteriol 175:642-646.

Piersen CE, McCullough AK and Lloyd RS (2000) AP lyases and dRPases: commonality of mechanism. Mutat Res 459:43-53.

Pomposiello PJ and Demple B (2001) Redox-operated genetic switches: the SoxR and OxyR transcription factors. Trends Biotechnol 19:109-114.

Repine JE, Fox RB and Berger EM (1981) Hydrogen peroxide kills *Staphylococcus aureus* by reacting with staphylococcal iron to form hydroxyl radical. J Biol Chem 256:7094-7096.

Repine JE, Pfenniger OW, Talmage DW, Berger EM and Pettijohn DE (1981) Dimethyl sulfoxide prevents DNA nicking mediated by ionizing radiation or iron/hydrogen peroxide-generated hydroxyl radical. Proc Natl Acad Sci USA 78:1001-1003.

Sagripanti JL and Kraemer KH (1989) Site-specific oxidative DNA damage at polyguanosines produced by copper plus hydrogen peroxide. J Biol Chem 264:1729-1734.

Sancar A and Sancar GB (1988) DNA repair enzymes. Ann Rev Biochem 57:29-67.

Saporito SM, Gedenk M and Cunningham RP (1989) Role of exonuclease III and endonuclease IV in repair of pyrimidine dimers initiated by bacteriophage T4 pyrimidine dimmer-DNA glycosylase. J Bacteriol 171:2542-2548.

Sayto Y, Uraki F, Nakajima S, Asaeda A, Ono K, Kubo K and Yamamoto K (1997) Characterization of endonuclease III (*nth*) and endonuclease VIII (*nei*) mutants of *Escherichia coli* K-12. J Bacteriol 179:3783-3785.

Sedgwick B and Robins P (1980) Isolation of mutants of *Escherichia coli* with increased resistance to alkylating agents: Mutants deficient in thiols and mutants constitutive for the adaptive response. Molec Gen Genet 180:85-90.

Spek EJ, Vuong LN, Matsuguchi T, Marinus MJ and Engelward BP (2002) Nitric oxide-induced homologous recombination in *Escherichia coli* is promoted by DNA glycosylases. J Bacteriol 184:3501-3507.

Storz G, Jacobson FS, Tartaglia LA, Morgan RW, Silveira LA and Ames BN (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 and Imlay JA (1999) Oxidative stress. Curr Opin Microbiol 2:188-194.

Storz G, TartagliaLA and Ames BN (1990) Transcriptional regulator of oxidative stress-inducible genes: Direct activation by oxidation. Science 248:189-194.

Storz G ands Toledano MB (1994) Regulation of bacterial gene expression in response to oxidative stress. Methods Enzymol 236:196-207.

Touati D, Jacques M, Tardat B, Bouchard L and Despied S (1995) Lethal oxidative damage and mutagenesis are generated by iron in *fur* mutants of *Escherichia coli*: Protective role of superoxide dismutase. J Bacteriol 177:2305-2314.

Tsaneva IR and Weiss B (1990) *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J Bacteriol 172:4197-4205.

Tyrrell RM (1985) A common pathway for protection of bacteria against damage by solar UV(A) (334 nm, 365 nm) and an oxidizing agent (H_2O_2). Mutat Res 145:129-136.

Wagner J, Cruz P, Kim SR, Yamada M, Matsui K, Fuchs RP and Nohmi T (1999) The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis. Mol Cell 4:281-286.

Walker GC (1996) The SOS responses of *Escherichia coli.* In: Curtis III R, Ingraham JL, Lin ECC, Low KB, Magasanick B, Reznicoff WS, Riley M, Schaechter M and Umbarger HE (eds) *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. v. 1, American Society for Microbiology, Washington DC, pp 1400-1416.

Wallace SS (1988) Ap endonucleases and DNA glycosylases that recognize oxidative DNA damage. Environ Mol Mutagen 12:431-477.

Wallace SS (2002) Biological consequences of free radical-damaged DNA bases. Free Rad Biol Med 33:1-14.

Wallace SS, Ide H, Kow YM, Laspia MF, Melamede RJ, Petrullo LA and LeClerc E (1988) Processing of oxidative DNA damage in *Escherichia coli.* In: Fridberg EC and Hanawalt PC (eds) Mechanisms and Consequences of DNA Damage Processing. Allan R. Liss, Inc., New York, pp 151-157.

Weigle JJ (1953) Induction of mutation in a bacterial virus. Proc Natl Acad Sci USA 39:628-636.

West SC (1996) The RuvABC proteins and Holliday junctions processing in *Escherichia coli.* J Bacteriol 178:1237-1241.

Wu J and Weiss B (1992) Two-stage induction of the *soxRS* (superoxide response) regulon of *Escherichia coli.* J Bacteriol 174:3915-3920.

Yamamoto K and Kawanishi S (1989) Hydroxyl radical is not the main active species in site-specific DNA damage induced by copper (II) ion and hydrogen peroxide. J Biol Chem 264:15435-15440.

Zhang A, Altuvia S and Storz G (1997) The novel *oxyS* RNA regulates expression of the sigma s subunit of *Escherichia coli* RNA polymerase. Nucleic Acids Symp Ser 36:27-28.

Zheng M, Aslund F and Storz G (1998) Activation of OxyR transcription factor by reversible disulfide bond formation. Science 279:1718-1721.

Zheng M, Doan B, Schneider TD and Storz G (1999) OxyR and SoxRS regulation of *fur.* J Bacteriol 181:4639-4643.

Zheng M, Wang X, Templeton LR, Smulski DR, Larossa RA and Storz G (2001) DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J Bacteriol 183:4562-4570.

EditorAssociado: Carlos F.M. Menck