

# Adaptive Response and Oxidative Stress

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The ability of a cell, tissue, or organism to better resist stress damage by prior exposure to a lesser amount of stress is known as adaptive response. It is observed in all organisms in response to a number of different cytotoxic agents. One of these agents, oxidative stress, is known to induce an adaptive response in bacteria that is accompanied by the induction of many proteins. *De novo* protein synthesis is required for adaptive response to oxidative and other types of stress, indicating that newly synthesized protective proteins are necessary for adaptation. Adaptive response to oxidative stress also has been observed in mammalian cells. Several studies suggest it is necessary to first preexpose mammalian cells to a somewhat toxic oxidative stress in order to observe significant resistance to a subsequent highly lethal dose of oxidant. Cross-resistance of oxidatively stressed cells to other toxic agents including  $\gamma$ - and X-irradiation, heat shock, aldehydes, heavy metals, MNNG, *N*-ethylmaleimide, and heme also has been reported. Understanding oxidant adaptive response in more detail and identifying the protective proteins involved may prove to be of clinical benefit. — Environ Health Perspect 102(Suppl 10):25–28 (1994)

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## Introduction

Adaptive response refers to the ability of cells or organisms to better resist the damaging effects of a toxic agent when first pre-exposed to a lower dose. It is a widespread phenomenon that has been observed in prokaryotes, yeast, mammals, and plants. Many different types of damaging agents, including alkylating agents, heat stress, oxidant stress, radiation, and heavy metals have been reported to induce an adaptive response. In general, adaptation in response to these damaging agents appears to involve the modulation of expression of many genes.

The main physiologic benefit of adaptive response is clear: to protect cells and organisms from high doses of a toxic agent. Such a protective response also indicates that the cell, once exposed to the toxin, expects, or at least is prepared for, a subsequent lethal dose. Although adaptive response studies primarily have involved acquired resistance to high levels of toxic agents, adaptive responses of physiologic relevance probably are often of a more subtle nature. For example, it has been shown that exercise training, which involves an oxidative stress (1), leads to a reduction in the amount of lipid peroxidation produced during acute exercise (2). Also, the lymphocytes of people occupationally exposed to low levels of ionizing radiation show an enhanced repair capacity (3).

## Adaptive Responses in Prokaryotes

Valuable information on the adaptive response process and the enzymes mediating this response were obtained originally from studies in bacteria. An important early study by Samson and Cairns nicely demonstrated a strong adaptive response in *Escherichia coli* following exposure to alkylating agents (4). In this study, cells were first exposed to relatively low levels of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) followed by a normally toxic level. After the lower initial exposure (pretreatment) dose, a significant increase in resistance to the toxic dose was observed. Importantly, chloramphenicol inhibited this acquired resistance, indicating the necessity for *de novo* protein synthesis. These results suggested that pretreatment with MNNG induced the synthesis of protective proteins in the bacteria that protected them from a subsequent toxic dose. Also of interest was the observation that MNNG provided protection against other alkylating agents (5). This "cross-resistance" has subsequently been observed in a number of adaptive response systems, both prokaryotic and eukaryotic.

Two other well-characterized adaptive responses also have been described in bacteria: responses to heat shock and oxidative stress. Heat shock, characterized by the induction of a small group of genes following heat exposure, is known to protect cells against subsequent toxic levels of heat and other stresses (6). A number of other toxic agents also induce these heat shock proteins, and in very rapid fashion. It appears that the induction of heat shock proteins is a global response to stress. Oxidative stress can also

induce adaptive responses. This was initially studied in *E. coli* where pretreatment of growing bacteria with a relatively low concentration of hydrogen peroxide ( $H_2O_2$ ) substantially reduced the toxicity of a subsequent high dose (7,8). Once again, *de novo* protein synthesis was required for this adaptation, and cross-resistance was observed following  $H_2O_2$  pretreatment. For example, Nunoshiba et al. (9) reported that a sublethal concentration of  $H_2O_2$  made *E. coli* resistant to lethal amounts of several different aldehydes. It should be pointed out that all of these agents are thought to induce an adaptive response separate from the error-prone SOS system of DNA repair.

An advantage of using bacteria as a model system for studying adaptive responses is the ready identification of mediators of this response, as compared with eukaryotes. Since many of the early studies used DNA damage as an endpoint, it was suspected that induction of DNA repair enzymes was important in adaptive response. This argument was further strengthened by the need for *de novo* protein synthesis. The involvement of DNA repair enzymes has since been confirmed. Those identified include *adaA* and *adaB* methyltransferases, both isolated from *Bacillus subtilis ada* mutants deficient in the adaptive response to alkylating agents (10); and *AlkA*, a repair glycosylase that repairs methylated DNA bases (11).

## Adaptive Responses in Eukaryotes

Adaptive response is now known to occur in a number of different eukaryotic organisms and cell types. As with bacteria, different toxic agents are able to induce this

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response, including alkylating agents. In a follow-up study to the aforementioned alkylation study in bacteria (4), chronic treatment of CHO fibroblasts and SV40-transformed human skin fibroblasts with nontoxic levels of MNNG induced cell resistance to subsequent high doses (12). This result paralleled what was observed in bacteria and once again suggested an induction of DNA repair.

Probably the most frequently studied adaptive response system in eukaryotic cells involves radiation. These studies were originally carried out by Olivieri et al. (13). These authors reported that human lymphocytes exposed to low level beta-radiation in the form of incorporated tritiated thymidine are more resistant to chromosomal damage caused by high dose X-rays. Since chromosomal damage was used as an endpoint in this study, it was once again assumed that an induction in DNA repair was responsible for this adaptation. It was subsequently demonstrated that the adaptive response was sustained through three cell cycles following the low dose exposure, after which the cells again became sensitive to high dose irradiation (14). Additional studies in human and rabbit lymphocytes indicated that these adaptive responses can be induced at all stages of the cell cycle with the questionable exception of  $G_0$  (15,16) and that, again, the adaptive response was sustained for three cell cycles.

Other observations worthy of note regarding eukaryotic adaptive responses include reports of this adaptation *in vivo* (4,15-17), the variability of this response in human lymphocytes from different donors (18), its requirement for *de novo* protein synthesis (15,16), and the cross-resistance to certain agents (19-21).

Bacteria represent convenient models for identifying genes involved in stress response, mainly due to the easy selection of stress response mutants as well as the smaller number of genes in the bacterial genome. Therefore the mediators of bacterial adaptive response have been better identified and characterized than have eukaryotic mediators. However, a number of mediators of mammalian adaptive response have now been identified. As suspected, induction of DNA repair appears to be involved in at least some instances of adaptive response. Several studies implicate polyADP-ribosylation in this process. PolyADP-ribosylation is associated with the repair of DNA breaks. Experimentally, it has been shown that inhibition of polyADP-ribosylation with 3-aminobenzamide or nicotinamide depletion abolishes adaptive response (21,22).

## Oxidative Stress

As mentioned earlier, it now has been determined that cells can undergo an adaptive response when challenged with sublethal levels of oxidants. This was originally observed in bacteria, but has since been documented in eukaryotes as well (23-27). Three major systems have been studied that clearly demonstrate a protective response that cells undergo when challenged with oxidants. These include the bacterial responses to  $H_2O_2$  and superoxide anion ( $O_2^-$ ); the protective eukaryotic response to oxidants that renders these cells resistant to killing by the same or related oxidants; and the eukaryotic response to oxidants that renders these cells resistant to killing by other toxic agents.

When considering oxidative stress, it is important to realize that cells have two primary lines of defense. The first involves cellular molecules directly involved in preventing oxidative damage to the cell. These include antioxidant enzymes such as the superoxide dismutases (SOD), glutathione peroxidases, and catalase, and low molecular weight antioxidant molecules such as glutathione and ascorbate. The second line of defense consists of repair enzymes. These repair systems remove and/or repair oxidatively damaged macromolecules. DNA repair often involves DNA nucleases and glycosylases.

The adaptive response to oxidative stress in bacteria was described earlier. Pretreatment with a nontoxic level of  $H_2O_2$  renders these organisms much more resistant to a subsequent lethal dose (7,8). The same has been observed for  $O_2^-$  (28-30). As with other damaging agents, some cross-resistance is observed (e.g., heat shock and aldehydes).

In eukaryotes, adaptive response to oxidative stress has also been demonstrated. Using  $H_2O_2$  as an oxidant, Spitz et al. observed a greater resistance to a normally lethal dose of peroxide following pretreatment of CHO fibroblasts with the same agent (23). In this study, it was necessary to pretreat the cells with a somewhat toxic dose of peroxide in order to observe protection. This seeming paradox of having to "clonogenically inactivate" or even kill some of the cells in order to observe protection of the rest appears to be more characteristic of adaptive response to oxidative stress studies in eukaryotic systems than prokaryotic. This same study reported a slight resistance to heat shock damage as well following the peroxide pretreatment. Also, pretreatment with heat shock protected the cells somewhat from subsequent

oxidative stress. Therefore, some cross-resistance occurs.

Similar results have been described by others in CHO and rat H4 hepatoma cells (24) and bovine endothelial cells (25). In the CHO and rat cell study,  $H_2O_2$  and xanthine-plus-xanthine-oxidase were used as sources of oxidants. Xanthine-plus-xanthine-oxidase produces both  $O_2^-$  and  $H_2O_2$ . Pretreatment of the cells with these oxidants for 1 hr conferred resistance to subsequent challenge to  $H_2O_2$  as well as  $\gamma$  rays. The mutagenic effect of  $\gamma$ -irradiation was decreased by the xanthine-plus-xanthine-oxidase pretreatment. Bovine vascular endothelial cells also became more resistant to oxidative damage when preexposed to lower levels of  $H_2O_2$  (25). In this case, a toxic level of  $H_2O_2$  was used as the oxidative stress challenge. There also has been another report of  $H_2O_2$  protection at low dose against a toxic peroxide dose in CHO cells (31). This study, however, was carried out under conditions in which the toxic challenge dose was only 10 min after pretreatment. It is not yet clear how a significant adaptive response as was observed could be effected in this short time, although early response repair enzymes may be involved. Again, significant cross-resistance was reported, this time to MNNG and  $\gamma$ -irradiation but not UV light. The same investigator also found a cadmium-induced adaptive response that appears to act primarily through an oxidative stress mechanism (32). It is known that heavy metals increase cellular lipid peroxidation by decreasing the availability of essential thiol groups. In this study, cadmium pretreatment protected cells against oxidative damage.

It has also been demonstrated that pre-exposure of eukaryotic cells to oxidative stress can render these cells resistant to killing by other toxic agents. Cortes (20) reported the induction of an adaptive response in human lymphocytes by  $H_2O_2$  that protected these cells against subsequent lethal X-irradiation. Reports in the literature describe a number of similar cross-resistant examples. These studies demonstrate that pretreatment with  $H_2O_2$  decreased the damage caused by subsequent toxic challenge with organic peroxide, gamma rays, heat, *N*-ethylmaleimide, aldehydes, and X-rays, but not UV irradiation nor MNNG.

The macromolecules mediating adaptive response to oxidative stress have been partially identified. In bacteria, exposure to levels of peroxide that stimulate a protective response are accompanied by the

induction of 30 to 40 proteins as assessed by 2D gel electrophoresis. Some of these proteins have been identified as antioxidant enzymes, including catalase, alkyl hydroperoxide reductase, and glutathione reductase, and are regulated by OxyR (8). Increased DNA repair has also been observed. Exposure to O<sub>2</sub><sup>-</sup> also induces many proteins, including antioxidant enzymes such as manganese-SOD and glucose-6-phosphate dehydrogenase (30). DNA repair enzymes have also been implicated in O<sub>2</sub><sup>-</sup> response (29) and some, such as endonuclease IV, identified (33). SoxR plays an important part in regulating the expression of proteins in response to O<sub>2</sub><sup>-</sup>. Ozone has also been shown to induce catalase and SOD levels in *E. coli* (34).

In eukaryotes, a number of different classes of protein have been implicated in protection against the toxic effects of oxidative stress. As expected, one class is the antioxidant enzymes. In bovine vascular endothelial cells, activities of total SOD, catalase, and glutathione peroxidase were all reported to increase following exposure to adaptive response pretreatment levels of H<sub>2</sub>O<sub>2</sub> (25). A modest increase in SOD was observed in xanthine-plus-xanthine-oxidase, but not H<sub>2</sub>O<sub>2</sub>-treated cells, at adaptive pretreatment doses (24). This same study also reported an induction in a member of the heat shock protein (HSP) 70 family. Several other studies have also reported HSP induction following oxidative stress, including the response of Chinese hamster fibroblasts to H<sub>2</sub>O<sub>2</sub> (23), reoxygenation of CHO cells after anoxia (35), and reperfusion injury in rat hearts (36). Interestingly, the protective response to O<sub>2</sub><sup>-</sup> pretreatment in bacteria induced synthesis of heat shock-inducible proteins

including DnaK (8). It is important to note that heat shock has been reported to increase intracellular oxidative damage (37), and a possible mechanism to explain this effect (involving increased O<sub>2</sub><sup>-</sup> generation) has been proposed (38).

There also is evidence for the protective effects of heme oxygenase and ferritin. Heme oxygenase is strongly induced by oxidative stress and free heme. Porcine aortic endothelial cells pretreated with heme become more resistant to subsequent oxidative stress (39). Ferritin appeared to be the ultimate protectant in this system and may protect the cells against hydroxyl radical formation. It has been suggested that these two proteins may act in sequence to protect cells against oxidative stress: heme oxygenase activity results in the release of free iron that then induces the cytoprotectant ferritin (40).

### Concluding Remarks

Most of our understanding of adaptive response to oxidative stress comes from studies of bacteria. Of particular note is the identification of the OxyR and SoxR regulons. Many oxidative stress-inducible sequences have not yet been identified, both in prokaryotes and eukaryote. Their modulation following adaptive response-inducible pretreatment have been observed on two-dimensional protein gels and cDNA library screens following subtractive hybridization and differential display. The identification and characterization of those sequences functionally involved in protection will further enhance our understanding of adaptive response. Furthermore, although we have limited our discussion of repair to DNA repair enzymes, a number of different repair systems exist for every

major cellular macromolecule damaged by oxidative stress. These include protein repair by proteasome (41,42) and membrane repair by phospholipase and glutathione peroxidase (43). Non-DNA repair systems such as these may also prove to be important in adaptive response.

One of the more interesting questions to be answered in the field is the underlying mechanism(s) behind cross-resistance. Presumably, cross-adaptive response is due to an overlap in responsive genes to different types of damage. It may be that already identified stress response genes induced by general stress are involved in resistance to different toxic agents. One such class of stress genes are the growth arrest and DNA damage or GADD genes. These genes are induced by alkylation, UV irradiation, and oxidative stress (44). Their induction leads to growth arrest, which is already known to be a general mechanism by which cells minimize cytotoxic damage. The protooncogenes *c-fos* and *c-jun* also are induced by stress, among other things, and may protect cells against general stress damage (45). NF-κB, activated by oxidative and other types of stress, may also be involved.

Finally, understanding adaptive response may also prove to be of clinical benefit. There is already evidence for this. Ischemic reperfusion injury, that is known to have a significant oxidative damage component, can be decreased by ischemic preconditioning (46). Since cross-resistance is observed between oxidative and heat stress, it would also be expected that hyperthermia, at least in theory, may be protective against oxidative stress-related injuries such as ischemia reperfusion.

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