The Role of the Cellular Antioxidant Defense in Oxidant Carcinogenesis

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Oxidant carcinogens interact with multiple cellular targets including membranes, proteins, and nucleic acids. They cause structural damage to DNA and have the potential to mutate cancer-related genes. At the same time, oxidants activate signal transduction pathways and alter the expression of growth- and differentiation-related genes. Indeed, the carcinogenic action of oxidants results from the superposition of these genetic and epigenetic effects. All cells possess elaborate antioxidant defense systems that consist of interacting low and high molecular weight components. Among them, superoxide dismutases (SOD), glutathione peroxidases (GPx), and catalase (CAT) play a central role. Our studies with mouse epidermal cells demonstrate that the balance between several antioxidant enzymes rather than the activity of a single component determines the degree of protection. Unexpectedly, increased levels of Cu,Zn-SOD alone in stable transfectants resulted in sensitization to oxidative chromosomal aberrations and DNA strand breaks. However, a concomitant increase in CAT or GPx in double transfectants corrected or overcorrected the hypersensitivity of the SOD clones depending on the ratios of activities CAT/SOD or GPx/SOD. The cellular antioxidant capacity also affected oxidant induction of the growth-related immediate early protooncogene c-*fos*. Increases in CAT or SOD reduced the accumulation of c-*fos* message, albeit for different reasons. The cellular antioxidant defense also affects the action of UVB light (290–320 nm) that represents the most potent carcinogenic wavelength range of the solar spectrum. UVB light is known to exert its action in part through oxidative mechanisms. Increases in CAT and GPx protected mouse epidermal cells from UVB-induced DNA breakage. An increase in GPx enhanced the induction of c-*fos* by UVB probably because it diminished DNA breaks. DNA breaks appear to exert a long-range effect on chromatin confirmation, which is incompatible with efficient transcription. — Environ Health Perspect 102(Suppl 10):123–130 (1994)

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Introduction

It is well recognized that oxidants play a role in several stages of carcinogenesis. They are ubiquitous in our natural environment but they are also formed in the tissue by endogenous cellular mechanisms (*1–3*). Oxidants can introduce structural changes in cancer-related genes in the form of chromosomal aberrations and point mutations. Indeed, point mutations in the *ras*-family protooncogenes (*4*) and in the *p53* tumor suppressor gene (*5,6*) represent the most frequent genetic changes in human malignancies, and at least some of them may be caused by oxidants. In addition to these genotoxic effects, oxidants activate signal transduction pathways that lead to the modulation of the expression of entire families of growth- and differentiation-related genes (*7–9*). However, unlike polypeptide growth factors, oxidants always induce macromolecular damage, cytotoxicity, and cell killing. All the effects of oxidants are influenced by the cellular antioxidant defenses (*10,11*). This multilayer system consists of low molecular weight components and several antioxidant enzymes. It is evident that the biological consequences of the exposure to an oxidant carcinogen, e.g., growth stimulation, growth inhibition, differentiation, or cell death is not readily predictable. It may vary with the dose, the type of the oxidant, and the tissue because it is the result of the superposition of effects on the multiple cellular targets mentioned above.

Oxidants are likely to play an important role in lung carcinogenesis. Chronic tissue injury by physical and chemical irritants frequently results in inflammation accompanied by the infiltration of phagocytic leukocytes (*12–15*). The inflammatory reaction is particularly striking in bronchial tissue that had been exposed to particulates and irritants in tobacco smoke and to mineral fibers (*13*). The mechanisms by which inflammatory leukocytes exert their carcinogenic effects have not been elucidated but some likely pathways can be proposed. Phagocytic leukocytes produce a highly complex mixture of growth and differentiation factors as well as biologically active arachidonic acid metabolites (*16*). In addition, they possess the capacity to release large amounts of active oxygen (AO) in an oxidative burst (*17*). Current evidence suggests that AO and arachidonic acid metabolites are important in tumorigenesis. Low molecular weight antioxidants, antioxidant enzymes, and antiinflammatory agents that inhibit arachidonic acid metabolism are anticarcinogenic in several experimental systems (*18–20*). The notion that AO from phagocytes may be an important carcinogen is supported by the finding that an extracellular burst of AO produced by xanthine/xanthine oxidase (X/XO) is a potent promoter for initiated mouse embryo 10T1/2 fibroblasts and mouse epidermal JB6 cells (*21,22*). Furthermore, the carcinogenic effect of cocultured neutrophils on 10T1/2 cells has been directly demonstrated (*23*). While hydrogen peroxide (H_2O_2) alone is a weak promoter for initiated mouse skin, several xenobiotic organic endo- and hydroperoxides possess considerable potential as promoters and progressors (*18,24,25*).

Polycyclic aromatic hydrocarbons (PAHs) represent important etiologic agents in lung cancer induced by tobacco

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smoke. They are metabolically activated to epoxy intermediates that form covalent adducts to DNA. In addition, PAH metabolites and other aromatic compounds that have the potential to form quinoid intermediates can induce oxidative DNA damage. This was shown for benzo[*a*]-pyrene, which caused the formation of 5-hydroxymethyluracil at a high rate in the DNA of human epithelioid lung cells A549 (*26,27*).

As mentioned above, the cellular antioxidant defenses are bound to play a role in oxidant carcinogenesis. Epidemiologic studies on serum antioxidants and diet suggest that an elevated level of vitamins E and β-carotene reduce mortality due to cancer in the lung and colon (*28,29*). On the other hand, levels of antioxidant enzymes in tumors have yielded inconclusive results, although it had been observed that the activity of Mn-superoxide dismutase was often below that of normal tissues (*30*). In view of the multiple stages and targets where oxidants can act in carcinogenesis, it may not be astonishing that the effect of the antioxidant defense depends on the cell type and tissue. For example, high antioxidant capacity is expected to protect the DNA from oxidative damage and mutagenesis but at the same time it may protect "initiated" cells from excessive oxidant toxicity and favor their clonal expansion in tumor promotion (*11,31,32*). Furthermore, the cellular antioxidant system consists of multiple interacting and interdependent components (*10*). Therefore, the biological consequences of the variation of a single component "out of the cellular context" is difficult to predict.

In this article we review progress made in our laboratory in the understanding of the role of the antioxidant enzymes Cu, Zn-superoxide dismutase (SOD), catalase (CAT), and Se-glutathione peroxidase (GPx) in oxidant carcinogenesis. To evaluate their individual contributions inside the cell, we prepared single and double transfectants with moderately increased levels of one or two antioxidant enzymes. With the help of these transfectants, we defined their role in the protection from oxidant-induced chromosomal aberrations, DNA strand breakage, growth inhibition, and the transcriptional inducibility of the growth- and differentiation-related protooncogene c-*fos*.

Increased Constitutive Antioxidant Defense in Promotable Mouse Epidermal Cells

A first indication that the cellular antioxidant defense affects the capacity of oxidants to stimulate the growth of epithelial cells was obtained in a study comparing promotable and nonpromotable mouse epidermal cells JB6. When we measured the specific activities of Cu,Zn-SOD, CAT, and GPx in monolayer cultures of JB6 cells, we discovered that the promotable clone 41 contained approximately twice the activity of SOD and CAT relative to the nonpromotable clone 30, whereas the activities of GPx were comparable. The activity data were confirmed by Western blots that indicated elevated protein levels of SOD and CAT in clone 41 cells. Northern blots indicated that the higher amounts of CAT and SOD in clone 41 were due to increased stationary concentrations of mRNAs for these genes. We concluded that the antioxidant defense of JB6 clone 41 is superior to that of clone 30. The difference between the two clones is particularly remarkable, because the two antioxidant enzymes SOD and CAT are increased coordinately in clone 41. Because the product of the action of SOD is H_2O_2, an increase in its activity is only beneficial to the cell if it is counterbalanced by a sufficient capacity for the destruction of H_2O_2. This is apparently accomplished in clone 41 by an increase in CAT (*32*). It should be mentioned that SOD and CAT may mutually protect each other from inactivation by active oxygen (*33*). The difference in the constitutive antioxidant defense of the two epidermal cell clones may play a role in their promotability by oxidants (*31,32*). In support of this concept, we recently found that oxidants produced by X/XO caused higher cytotoxicity and cell death in nonpromotable clone 30 cells (*22*). A rise in cytosolic ionized calcium $(Ca^{2+})_i$ preceding bleb formation was sustained for 8 to 10 min longer in nonpromotable relative to promotable cells according to digital imaging fluorescence microscopy. We concluded that the superior antioxidant defense of promotable clone 41 protects it from more severe deregulation of $(Ca^{2+})_i$ and, as a consequence, from excessive cytotoxicity following exposure to oxidant promoters (P Jain, S Chang, I Berezesky, P Cerutti, B Trump, unpublished data).

Effect of the Antioxidant Defense on Oxidant-induced Chromosome and DNA Breakage

The genome represents one of the most vulnerable targets to oxidants which cause permanent structural damage to DNA as well as transient changes in gene expression. The sensitivity of the genome to oxidants is modulated by the cellular antioxidant defense. To study the effect of the major antioxidant enzymes on genome vulnerability we constructed genetic variants of promotable mouse epidermal cells JB6 clone 41 by transfection with cDNAs coding for human Cu,Zn-SOD, CAT, and bovine Se-GPx. Different resistance cassettes were used to construct each of the three expression vectors, allowing the preparation of single- and double transfectants with increased complements of one or two enzymes. This represents a crucial feature of our experimental design since it allows dissection of the individual contributions of these interacting enzymes to the overall antioxidant defense. To stay in the physiologic range, we chose stable transfectants with moderate 2- to 3-fold increases in antioxidant enzyme activities for in-depth characterization. These clones were completely analyzed on the molecular and biochemical level [(*11,34*); P Amstad, R Moret, P Cerutti, unpublished data].

Northern blots of total RNA from these clones revealed increased expression of Cu,Zn-SOD, CAT, or GPx, respectively, and Southern blots indicated the presence of the transfected DNA. Western blots with antibodies against the human proteins showed the presence of bands corresponding to the human enzymes in addition to the cross-reacting endogenous mouse proteins. For the antioxidant enzyme activities of the transfectants, we refer to the original report (*34*) from our laboratory. Below we describe the effects of the modulation of the antioxidant defense on oxidant-induced chromosome and DNA damage.

Cu,Zn-SOD Transfectants Are Sensitized to Oxidant-induced Chromosomal Damage while CAT and GPx Transfectants Are Protected. Cytogenetic analysis revealed that the Cu,Zn-SOD transfectants SOD3 and SOD15 were sensitized to chromosomal damage induced by oxidant. Two- to threefold higher SOD activities resulted in a 3- to 5-fold increase in total chromosomal aberrations after exposure to X/XO relative to the parent strain (scored: breaks, iso-breaks, gaps, iso-gaps, exchanges; Y Oya, P Cerutti, unpublished data). Similarly, the SOD transfectants were sensitized to X/XO-induced DNA strand breaks as measured by the alkaline elution method (*34*).

In contrast, stable transfectants with increased levels of CAT were protected from X/XO-induced chromosome and DNA breakage. For example, in transfectant CAT4 with approximately 3-fold increased CAT activity, the oxidant-

induced total chromosomal aberration frequency was reduced 4-fold relative to the parent strain (Y Oya, P Cerutti, unpublished data). As expected, CAT transfectants also were more resistant to oxidant-induced DNA strand breakage. Interestingly, a SODCAT double transfectant derived from CAT4 was more resistant than the parent strain to DNA breakage. These results indicate that the balance between SOD and CAT plays a crucial role for the overall vulnerability of the genome to a mixture of $O_2^{\cdot-}$ and H_2O_2 produced extracellularly by X/XO (*34*). As a follow-up to our observation, Mao et al. (*35*) studied the efficacy of conjugates of Cu,Zn-SOD and CAT as protectors from ischemia-reperfusion damage. They found that the enzyme conjugate supplied better protection than Cu,Zn-SOD alone.

We recently evaluated the effect of increasing the cellular complement in GPX on the oxidant sensitivity of the parent strain JB6 clone 41 and its SOD transfectants SOD3 and SOD15. Sensitivity to DNA strand breakage and killing by X/XO was reversely related to the ratio of activities GPx over SOD. A GPx transfectant with a GPx/SOD ratio of 3.8 was very strongly protected. The hypersensitivity of the SOD clones with a GPx/SOD ratio of 0.4 was corrected or overcorrected by secondary transfection with bovine seleno-GPx resulting in increased activity ratios GPx/SOD of 1 to 2.4. Our results indicate that small deviations from the physiologic activity ratios of GPx/SOD have a dramatic effect on the resistance of cells to oxidant-induced damage to the genome and cell killing (P Amstad, R Moret, P Cerutti, unpublished data). X/XO produces a large burst of active oxygen close to the cell surface and it is conceivable that lipid peroxidation in the membrane and the formation of long-lived clastogenic products are on the pathway to DNA breakage (*36–38*). Indeed, the protective action of GPx might be due in part to its capacity to destroy clastogenic lipid hydroperoxides. Our results are in agreement with the recent finding that the transfection of human GPx into human breast carcinoma cells rendered them more resistant to menadione-induced DNA breakage (*39*).

A satisfactory interpretation of these results requires an understanding of the reasons for the toxicity of high levels of Cu,Zn-SOD. The compensatory effect of CAT and GPx suggests that overproduction of H_2O_2 by $O_2^{\cdot-}$ dismutation might be responsible for SOD toxicity (*34,35*). Alternatively, overscavenging of hydroperoxy radical HO_2 (the conjugate acid of $O_2^{\cdot-}$) by excess SOD may reduce radical chain termination and result in increased lipid peroxidation (*40*). Finally, the inherent peroxidatic activity of Cu,Zn-SOD could play a role (*41*). However, the fact that both excess Cu,Zn- and Mn-SOD have been shown to be toxic argues against this possibility since the latter enzyme lacks peroxidatic activity (*40*). While we favor the interpretation that the genotoxicity of elevated levels of Cu,Zn-SOD in cells exposed to $O_2^{\cdot-}$ plus H_2O_2 is due to overproduction of H_2O_2, we do not imply that DNA is the immediate target for attack by H_2O_2 (*42*) or its radical derivatives. The fact that the chelation of intracellular Ca^{2+} strongly inhibits DNA breakage by H_2O_2 suggests that the activation of Ca^{2+}-dependent endonucleases plays a role. It should be noted that evidence in the literature supports the notion that overexpression of SOD can sensitize rather than protect cells from oxidative stress. For example, a 5-fold increase in Mn-SOD or a 10-fold increase in Fe-SOD sensitized *E. coli* to paraquat toxicity (*43,44*). While transfection of Cu,Zn-SOD into HeLa cells resulted in overall resistance to paraquat, the degree of protection was not proportional to the increase in enzyme activity (*45*).

Effect of Antioxidant Defense on Oxidant-induced Growth Inhibition and Cell Killing. In general, oxidants are cytostatic and cytotoxic, although under exceptional circumstances they can promote growth and facilitate the clonal expansion of initiated cells in carcinogenesis (*22,31, 46,47*). Membrane lipid peroxidation, which causes disruption of membrane integrity, increased permeability to ions, and surface bleb formation as well as chromosomal breakage are major mechanisms leading ultimately to growth inhibition and cell death (*3*). Of course, the antioxidant defenses are expected to modulate the sensitivity of cells to these cytopathologic effects of oxidants. As mentioned above, the oxidant promotability of clone 41 of JB6 mouse epidermal cells may in part be due to its elevated content in SOD and CAT. The enhanced antioxidant defense may protect these cells from excessive toxicity but still allow the activation of the signal transduction pathways that are required for growth stimulation (*32*).

We have further explored the role of the major antioxidant enzymes in the protection from inhibition of cell growth and killing with the help of our collection of stable antioxidant enzyme transfectants. We measured the effect of an extracellular burst of oxidant produced by X/XO on growth in monolayer cultures and on the survival of colony forming ability. As a whole, our results are in qualitative agreement with those described above for chromosomal and DNA breakage: *a*) overexpression of Cu,Zn-SOD sensitized to growth inhibition and killing (*34*); *b*) elevated levels of CAT or GPx protected from growth inhibition and killing; *c*) transfection of SOD clones with either CAT or GPx corrected or even overcorrected their hypersensitivity [(*34*); P Amstad, R Moret, P Cerutti, unpublished data).

It is interesting to note that a pronounced shoulder was discernible in the low dose range of the survival curves of the single transfectant GPx20 and of the SOD-GPx double transfectants. These results suggest that GPx strongly protects from the toxicity of $O_2^{\cdot-}$ plus H_2O_2 up to a threshold dose. Beyond this dose, killing was just as efficient as for the parent strain and its SOD transfectants. Interestingly, Kelner and Eagnell (*48*) were able to distinguish groups of rapidly and slowly growing Cu,Zn-SOD transfectants of NIH-3T3 cells. The rapidly growing clones possessed elevated GPx activities, while the slowly growing clones lacked the increase. These data suggest that Cu,Zn-SOD transfectants are under continuous oxidative stress, which limits their growth potential, and that regulatory overexpression of the endogenous GPx gene overcomes the effect. In a separate study, an indication for oxidative stress in Cu,Zn-SOD transfected mouse L-cells was found in an elevated level of lipid peroxidation (*45*).

The toxicity associated with the overexpression of Cu,Zn-SOD could play a role in human disease. This may be the case in familial amyotrophic lateral sclerosis where mutated Cu,Zn-SOD may possess enhanced activity (*49*). The presence of three gene copies of Cu,Zn-SOD in trisomy 21 (Down's syndrome) has been speculated to contribute to the neuropathologic symptoms in this disease (*50,51*). The increase in the dosage of Cu,Zn-SOD genes in trisomy 21 was accompanied by increased GPx activity (*52*).

Antioxidant Defense Affects Induction of the Protooncogene c-*fos* by Oxidants

Mechanism of c-fos Induction by Oxidants. Oxidants have the capacity to transcriptionally induce the growth competence-related protooncogenes c-*fos* and c-*jun* in several cell systems (*7,8*). The induction of these immediate early genes

represents a prerequisite for the stimulation of cell proliferation. In some respects oxidants mimic the action of bona fide growth factors (*9,53*). Growth promotion by oxidants is expected to play a role in inflammation, fibrosis, and tumorigenesis (*1,3*). We performed mechanistic studies with stable transfectants of mouse epidermal cells with a plasmid containing fos 5′ upstream regulatory sequences linked to an HSV-*tk*-chloramphenicol acetyltransferase reporter construct. Our results indicate that the joint dyad symmetry element-AP-1 motifs exert the most potent enhancer effect in response to active oxygen as well as serum. We concluded that the signal transduction pathways used by these different inducers converge to the same 5′ regulatory sequences of c-*fos*.

In contrast to these common features, only c-*fos* induction by active oxygen, but not by serum nor phorbolester, required the polyADP ribosylation of chromosomal proteins. Inhibitors of ADP-ribose transferase suppressed the elongation of the c-*fos* message and the *de novo* synthesis of nuclear factors, among them c-Fos and c-Jun, which bind to the fos-AP1 motif *in vitro* only following stimulation with active oxygen. Only active oxygen, but not serum or phorbolester, induces DNA breakage. We propose that polyADP-ribosylation is required because it participates in the repair of DNA breaks that interferes with transcription (*54*). At the oxidant doses used in our experiments, the probability that a break is located directly in the *fos*-gene or its regulatory sequences is very low. Rather, unrepaired breaks may exert a long-range effect on chromatin conformation, which is incompatible with efficient message elongation. Evidence for this interpretation was obtained in studies of the induction of c-*fos* by ultraviolet B light (UVB, 290–320 nm) described below.

Inducibility by Oxidants of c-fos in Transfectants with Increased Cu,Zn-SOD and CAT. As mentioned above, the antioxidant defense is bound to affect the expression of genes that are induced by oxidants. Therefore, we compared the increase in the stationary concentration of the c-*fos* message in our transfectants with increased activities of Cu,Zn-SOD and/or CAT. We observed a decrease in the inducibility of c-*fos* in all these transfectants. However, the reasons for the decrease in c-*fos* induction are probably quite different for CAT- and Cu,Zn-SOD transfectants. The former are well protected from excessive H_2O_2 toxicity but at the same time the signal that results in c-*fos* induction is attenuated. We had shown previously that H_2O_2 rather than $O_2^{\cdot-}$ represents the active species for the induction of the translocation to the plasma membrane of protein kinase C (*55*) and that CAT rather than SOD inhibited S6-phosphorylation by X/XO in JB6 cells (*56*). In contrast, increases in Cu,Zn-SOD levels alone augment the formation of H_2O_2 and toxic effects on components of the signal transduction pathways may predominate.

Induction of c-*fos* and other immediate early genes is necessary for the acquisition of growth competence in many types of cells but it is by no means sufficient for growth stimulation. Oxidants are expected to affect multiple pathways that participate in positive and negative growth regulation. Therefore, it is not astonishing that no simple relationship was observed between the inducibility of c-*fos* and growth response to oxidants for the SOD and CAT transfectants studied in our work (*34*). Interestingly, the transcriptional induction of c-*fos* by UVB was enhanced rather than attenuated in transfectants of mouse epidermal cells JB6 with increased activities of GPx (see below).

Oxidant Stress Induced by Ultraviolet B Light Contributes to Its Carcinogenic Effect

The UVB portion in the wavelength range from 290 to 320 nm possesses the highest potency for the induction of skin cancer (*57–59*). In contrast to short wavelength UVC (190–290 nm) that preferentially causes damage to DNA by electronic excitation, UVB interacts with multiple cellular targets and appears at least in part to act by oxidative mechanisms. This notion is supported by the observation that UVB efficiently induces lesions of the 5,6-dihydroxy-dihydrothymine type in DNA as well as single-strand breaks (*60–62*).

The question arises whether UVB and oxidants have the capacity to introduce activating mutations into protooncogenes and inactivating mutations into tumor suppressor genes. For the detection of base pair changes in a minute minority of cells without the selection of phenotypically altered cells, we have developed the restriction fragment length polymorphism/polymerase chain reaction protocol (RFLP/PCR) which measures mutations in restriction endonuclease recognition sequences. This genotypic mutation system is being applied to UV- and oxidant-induced mutagenesis of cancer-related genes in human cells (*63,64*).

The overall biological consequences of UVB are expected to result from the superposition of its genetic and epigenetic effects. In addition to causing DNA damage, it activates signal transduction pathways at the plasma membrane that involve kinases and phosphatases that are known to participate in the mitogenic response to certain growth factors (*59,65,66*). It modulates the expression of numerous growth-related genes, among them the immediate early genes c-*fos* and c-*jun* (*67–70*). We speculate that the reason for the high carcinogenicity of UVB lies in the fact that it induces structural damage to the genome and at the same time stimulates epidermal proliferation. Since UVB induces oxidative stress, it is to be expected that the cellular antioxidant defenses modulate its action.

Mechanism of c-fos Induction by UVB. To understand the growth-stimulatory effect of UVB, we are studying the mechanism of transcriptional induction of immediate early protooncogenes in mouse epidermal JB6 cells and have made the following observations. UVB is a moderate transcriptional inducer of c-*fos* and c-*jun*. It elicits a biphasic response of c-*fos* with an early peak at 30 to 60 min and a second, broader peak at 7 to 8 hr. Only the early phase of expression is suppressed by inhibitors of ADPR-transferase. Stable transfectants with reporter constructs linked to 5′-upstream sequences (–345 to –285) of c-*fos* require the joint DSE-AP1 enhancer motifs for efficient early induction by UVB, and there is no evidence for the presence of unique UV-enhancer elements. We propose that the two phases of c-*fos* induction by UVB occur by quite different mechanisms. The early phase requires polyADP ribosylation of chromosomal proteins for the resealing of UVB-induced DNA breaks which otherwise suppress transcription. Experiments with conditioned media from UVB-irradiated cells suggest that an autocrine factor may be responsible for the late phase of c-*fos* induction. These features of c-*fos* induction are characteristic for UVB and have not been observed for stimulation by serum nor phorbolester.

We conclude that the action of UVB has to be understood as superposition of specific mutational changes in cancer-related genes, general genotoxicity and growth factorlike epigenetic effects (*70*).

Effect of Antioxidant Defense on DNA Strand Breakage and Transcriptional Induction of c-fos. To evaluate the capacity of the antioxidant defense to protect the genome from UVB-induced damage, we compared the sensitivities to DNA

strand breakage of parent JB6 cells and its stable CAT transfectants. As is evident from the alkaline elution curves shown in Figure 1, both clone CAT4 and CAT12 with 3- and 4-fold increased CAT activities were strongly protected. Similar results were obtained with the bovine GPx transfectant GPx20, which possesses a 3-fold elevated GPx activity relative to the parent strain. In contrast, the two Cu,Zn-SOD transfectants SOD3 and SOD15 possessed sensitivities that were comparable to the parent strain. On the basis of the enzymatic specificities of the transfected enzymes we conclude that UVB-induced DNA breakage is at least in part mediated by the formation of H_2O_2 and possibly organic hydroperoxides, but that O_2^- is not directly involved (R Ghosh, P Amstad, P Cerutti, unpublished data).

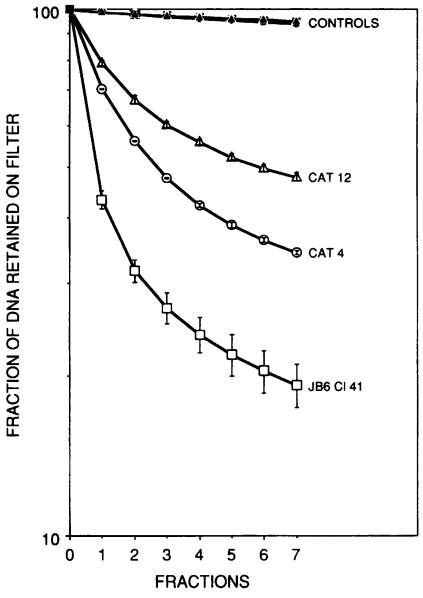

Figure 1. Sensitivity of catalase transfectants of mouse epidermal cells JB6 to ultraviolet B induced DNA strand breakage. Monolayer cultures of transfectants CAT4 and CAT12 (*34*) were irradiated with 9 KJ/m^2 UVB light under a thin layer of dyeless medium supplemented with 0.25% fetal calf serum at 37°C. DNA breakage was measured by the alkaline elution method. Means of two elution curves with error bars are given.

As discussed above, unrepaired DNA breaks may be incompatible with efficient transcription of c-*fos*. Indeed, the fact that UVB possesses only moderate capacity to induce c-*fos* may be the consequence of its genotoxic effect that is superimposed on its potential to activate the necessary signal transduction pathways. According to this model, reduced strand breakage in the CAT and GPx transfectants would be expected to enhance the transcriptional induction of c-*fos*. In the experiment shown in Figure 2, we compared the increase in stationary c-*fos* message induced by UVB and serum between the parent JB6 clone 41 and its transfectants GPx20, SOD3, and SOD15 by Northern analysis. In agreement with our model, c-*fos* expression by UVB was enhanced 3-fold in GPX20 but slightly reduced in the SOD clones SOD3 and SOD 15. It is evident that the antioxidant status had no significant effect on c-*fos* expression by serum (R Ghosh, P Amstad, P Cerutti, unpublished data).

UVB-induced DNA Breaks Exert a Long-range Effect on Chromatin Structure that Suppresses c-fos Transcription. As already mentioned, polyADP ribosylation of chromosomal proteins is required for the efficient resealing of DNA breaks, and consequently its inhibition with 3-aminobenzamide (3-AB) suppressed c-*fos* induction by UVB and oxidants. However, the *fos* gene and its regulatory sequences represent a very small target and are not expected to contain a significant number of breaks at moderate UVB or oxidant doses (*70*). It appears more likely that unrepaired breaks exert a long-range effect on chromatin conformation that is incompatible with efficient transcriptional induction. We have tested this model in experiments comparing the effect of ADPR-transferase inhibition on the UVB induction of the endogenous c-*fos* gene, of a stably integrated p*fos*-cat (chloramphenicol acetyltransferase) construct containing the full length 5′-regulatory sequences of c-*fos*, and of the same transiently transfected p*fos*-cat construct. In contrast to the stably integrated vector, the transiently transfected extra chromosomal vector does not assume a native, higher order chromatin structure and is not susceptible to long-range effects by DNA breaks. The preparation of stable transfectants containing the fos regulatory sequences linked to the bacterial cat-reporter gene has been described (*59*). Serum starved cultures were either irradi-

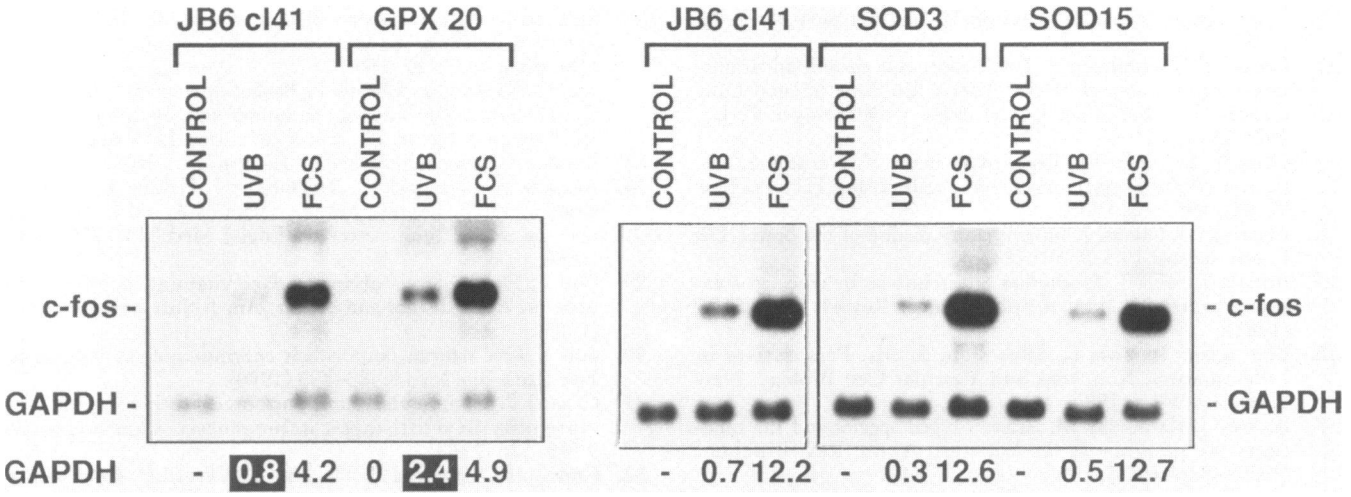

Figure 2. Accumulation of c-*fos* message after ultraviolet B irradiation in Se-glutathione peroxidase transfectant GPx20 and Cu,Zn-SOD transfectants SOD3 and SOD15 of mouse epidermal cells JB6. Serum starved monolayer cultures were irradiated with 12KJ/m^2 UVB light under a thin layer of dyeless medium containing 0.25% fetal calf serum at 30°C and then incubated at 37°C for 60 min. Total RNA was extracted and its content in c-*fos* and GAPDH message determined by Northern blot as described previously (*34*). Ratios of densitometer readings for the c-*fos* and GAPDH bands are listed.

ated with UVB or treated with serum in the presence or absence of 3-AB and total RNA extracted after 60 min. RNAse protection analysis of the concentration of cat-RNA indicated that 3-AB suppressed the induction of the cat-reporter gene by UVB but not serum in the stable p*fos*-cat transfectants. It should be noted again that serum does not cause DNA strand breakage in JB6 cells. In contrast, 3-AB had no effect on the induction of the identical transiently transfected p*fos*-cat construct. As expected from previous results mentioned above, 3-AB reproducibly suppressed the UVB induction of the endogenous c-*fos* gene in the stably and transiently transfected cultures. These results support our proposition that DNA breaks exert a long-range effect on chromatin conformation that interferes with fos transcription (*71*). It is well documented that chromatin undergoes conformational changes in regions of active transcription (*72,73*). In the case of the c-*fos* gene, it has been demonstrated that a transient gradient of increased DNaseI sensitivity extends hundreds of basepairs upstream and downstream from the SRE enhancer motif when HeLa cells are stimulated with serum (*74,75*). It is conceivable that this type of conformational change of chromatin cannot be established in the presence of unrepaired DNA breaks.

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