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Prooxidant States and Tumor Promotion

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In cellular prooxidant states the intracellular concentration of activated forms of oxygen is increased, presumably because cells either overproduce these reactive substances or are deficient in their ability to destroy them. The major forms of active oxygen are superoxide, O_2^-, and its conjugate acid the hydroperoxy radical, $HO_2\cdot$; singlet oxygen, O_2^{1*}; the hydroxyl radical, ·OH; and hydrogen peroxide, H_2O_2. Prooxidant states vary, depending on the type of target cell and on the induction mechanism, and can result in ubiquitous cell damage through readily oxidizable target molecules. Major reactions include initiation of autoxidation chain processes by hydroxyl and hydroperoxy radicals and of branching reactions by alkoxy radicals, addition of hydroxyl radicals and singlet oxygen to double bonds, hydrogen abstraction from allylic carbon atoms by hydroxyl radicals, and oxidation of sulfhydryl, thioether, and amino functions (*1*). The biological consequences are mutations, sister chromatid exchanges, chromosomal aberrations, cytotoxicity, carcinogenesis, and cellular degeneration related to aging. In carcinogenesis active oxygen appears to play a role mostly in the promotion phase, during which gene expression of initiated cells is modulated by affecting genes that regulate cell differentiation and growth. In a subsequent step, usually referred to as progression, mostly benign neoplasms are stimulated to more rapid growth and malignancy. Active oxygen is known to induce chromosomal aberrations with high efficiency and could play a role in progression (*2*).

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Evidence for a Role of Prooxidant States in Carcinogenesis

The human hereditary diseases ataxia telangiectasia, Fanconi's anemia, and Bloom's syndrome are characterized by increased cancer incidence and spontaneous chromosomal breakage (*3*). There are indications in all three diseases of abnormalities in oxygen metabolism (*4*). Cultured skin fibroblasts from patients with these diseases are hypersensitive to agents that induce prooxidant states. For example, fibroblasts from patients with ataxia telangiectasia are hypersensitive to x-rays, bleomycin, and neocarcinostatin; those from patients with Fanconi's anemia show sensitivity to mitomycin C and psoralen; and those from patients with Bloom's syndrome are sensitive to near-ultraviolet radiation. This last agent also induces excessive DNA strand breakage in fibroblasts from Bloom's syndrome patients. Increased oxygen tension causes excessive amounts of chromosomal aberrations in Fanconi's anemia. Serum from patients with ataxia telangiectasia and Bloom's syndrome contains clastogenic factors (CF's), and cultured fibroblasts release CF's into the culture medium. These factors break chromosomes in test cultures of lymphocytes from healthy donors. The CF from Bloom's syndrome fibroblasts was inhibited by CuZn superoxide dismutase (SOD), indicating the intermediacy of O_2^- in the clastogenic process (*5*). The

increase in the specific activity of SOD found in Bloom's syndrome fibroblasts could be a consequence of a prooxidant state in these cells (*6*). Spontaneous chromosomal aberrations and sister chromatid exchange frequencies in these fibroblasts can be decreased by the addition of protease inhibitors (*7*). It is conceivable that a chronic prooxidant state in these chromosomal breakage disorders exerts a promotional effect (*4*).

Further support for a role of active oxygen in carcinogenesis derives from the observation that dioxygen (O_2), O_2^-, and certain organic hydroperoxides are tumor promoters (and weak complete carcinogens). For example, high oxygen tensions increase the transformation frequency of mouse embryo cells that have been irradiated with fluorescent light (*8*); O_2^- promotes radiation- or chemically initiated transformation of mouse embryo fibroblasts; H_2O_2, peroxyacetic acid, benzoyl peroxide, and other organic peroxides promote chemically initiated transformation of mouse epidermal cells (*8–10*).

In contrast, many antioxidants are anticarcinogens (*11*). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) inhibit the transformation of mouse skin cells promoted by phorbol-12-myristate-13-acetate (PMA) (*12*) and benzoyl peroxide (*10*). Their antipromotional activity is probably due to their antioxidant capacity. Vitamins C and E inhibit primarily the late steps in carcinogenesis, and they antagonize PMA promotion of 7,12-dimethylbenz-[*a*]anthracene–initiated transformation of mouse skin cells. Vitamin C reverts the phenotype of transformed mouse embryo 10T1/2 cells at early stages, and vitamin E succinate inhibits the growth of mouse melanoma and L cells (*12*). The constitutive levels and the inducibility of the antioxidant enzymes SOD, glutathione peroxidase (L-γ-glutamyl-L-cysteinylglycine peroxidase), and catalase (CAT) vary for different tissues. Therefore, it is not surprising that the effects of the exogenous addition of these enzymes depend on the particular cell system. For example, SOD but not CAT inhibited the transformation of hamster embryo cells by x-rays and bleomycin as well as their promotion by PMA, whereas both enzymes were only moderate antipromoters in analogous experiments with mouse embryo fibroblasts. Similarly, cupric 3,5-diisopropyl-salicilic acid, a biomimetic analog of SOD, suppressed PMA promotion of initiated mouse skin cell transformation (*13*). Exogenous SOD also inhibited the promotion by PMA of JB6 mouse epidermal cell transformation to anchorage-independent growth (*14*).

Promotion of mouse skin transformation appears to encompass at least two stages, and there is evidence for the involvement of active oxygen in both. Hydrogen peroxide and benzoyl peroxide induce dark basal keratinocytes, which represent a reliable marker for stage I promotion. The protease inhibitor L-1-tosylamide-2-phenylethylchloromethyl ketone and the antioxidant vitamin E are stage I inhibitors. Mezerein elicits a strong oxidative burst in human polymorphonuclear leukocytes; it is a specific stage II promoter (*10*, *15*, *16*). Covalently linked retinoic acid and PMA (that is, 12-*O*-retinoylphorbol-13-acetate) is a stage II promoter that completes promotion when applied several weeks after a single treatment of skin with PMA. The antioxidants BHT and BHA are stage II inhibitors (*10*, *16*). Stage II promotion is accompanied by numerous biochemical changes, many of which are related to the stimulation of cell proliferation. For example, the induction by PMA of ornithine decarboxylase, which is a key enzyme in polyamine synthesis, is associated with stage II promotion. Ornithine decarboxylase induction is suppressed by SOD and CAT in mouse mammary tumor cells and by BHT in mouse epidermal cells, suggesting the intermediacy of active oxygen in the induction process (*17*).

Mechanisms Inducing Prooxidant States

The characteristics of prooxidant states depend on the inducing agent and the responding cell; that is, the quantity, type, and intra- and extracellular distribution of the active oxygen species may differ. There is a variety of agents and mechanisms that can induce prooxidant states.

Hyperbaric oxygen tension. Oxygen tensions exceeding approximately 40 percent inhibit macromolecular synthesis and cell division and are cytotoxic, mutagenic, clastogenic (*18*), and tumor promoting (*19*). This is probably due to the generation of small amounts of active oxygen species.

Radiation. Aerobic ionizing radiation is the prototype of a prooxidant. It causes ubiquitous oxidative damage to the cellular macromolecules (including DNA) and is mutagenic, clastogenic, and carcinogenic (*20*). The chromosomal aberrations that it causes are suppressed by SOD (*21*). It has been speculated that the peroxidation of membrane lipids plays a role in radiation carcinogenesis (*22*). The mode of action of ultraviolet radiation is wavelength-dependent, so that the near-ultraviolet range is responsible for the formation of active oxygen and contributes to macromolecular damage. Radiation in the near-ultraviolet range is mutagenic, clastogenic, and carcinogenic (*8*, *23*, *24*) for cultured cells, and the epidemiology of nonmelanoma skin cancer clearly implicates solar radiation as the causative agent. Thus the generation of a prooxidant state by near-ultraviolet radiation may exert a promotional effect in tumorigenesis.

Xenobiotic metabolism and Fenton-type reactions. Certain xenobiotics induce the formation of active oxygen in the course of their metabolism (*25*). The active oxygen and xenobiotic radicals formed in these reactions can cause macromolecular damage. Such agents are radiomimetic in the true sense; that is, their biological effects resemble those of ionizing radiation.

Included in this group are potent carcinogens and carcinostatic drugs. (i) Quinoid molecules (xenobiotics with quinoid structures) can participate in redox cycles in which semiquinone intermediates are oxidized to quinones with concomitant reduction of O_2 to O_2^- (*26*). Examples are daunorubicin, streptonigrin, adriamycin, mitomycin C, and certain polycyclic aromatic hydrocarbons. The last three also form covalent DNA adducts. The observation that SOD inhibits in vitro the transformation of mouse embryo 10T1/2 fibroblasts by mitomycin C suggests the participation of active oxygen in the transformation process (*13*). Antioxidants also suppress lipidperoxidation by benzanthraquinones and carcinogenicity by daunomycin and polycyclic aromatic hydrocarbons (*26*). A prooxidant state resulting from quinone metabolism can lead to a decrease in the concentration of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and in the release of mitochondrial Ca^{2+}. Disturbance of Ca^{2+} homeostasis can change cytoskeleton function and structure (*27*). (ii) Radical intermediates formed in the reductive metabolism of nitroaromatic drugs such as the carcinogen 4-nitroquinoline-*N*-oxide (4-NQO) can transfer an electron to O_2 as they revert to starting material (*25*, *28*). That glutathione reduces the cytotoxicity of 4-NQO supports the notion that radicals are involved in this carcinogen's mechanism of action (*28*). (iii) Fenton-type reagents, ferrous (Fe^{2+}) and cuprous (Cu^+) ions plus H_2O_2 or organic

hydroperoxides, can produce hydroxyl and alkoxy radicals that initiate or propagate oxidation chain reactions. The anticancer drug bleomycin acts by this mechanism (*29*), and its capacity to transform hamster embryo cells in vitro is suppressed by SOD (*13*, *29*). (iv) Miscellaneous reagents, such as azo anion and hydrazyl and bipyridylium radicals, can also participate in the univalent reduction of O_2 to O_2^- (*25*) and cause lipidperoxidation and hemolysis. Typical representatives are sulfonazo III, phenylhydrazine, and paraquat, the last of which promotes urethane tumorigenesis in mouse lung (*30*). The trichloromethyl radical of carbon tetrachloride initiates lipidperoxidation and is a potent hepatotoxin and hepatopromoter and a complete hepatocarcinogen. The environmental toxin 7,8-tetrachlorodibenzo-*p*-dioxin has similar activities (*31*).

Modulation of the cytochrome electron-transport chain. The microsomal and mitochondrial electron-transport chain can release moderate amounts of active oxygen under physiological conditions and large amounts when perturbed by inhibitors, uncouplers, inducers, and pseudosubstrates (*32*). The resulting prooxidant states can cause damage to lipids and cytochromes (*33*). The respiration inhibitor rotenone is hepatocarcinogenic (*34*), and the P-450 inducer phenobarbital is a hepatopromoter (*35*). However, there is no direct correlation between the ability of many compounds to act as promoters of liver cell transformation and their ability to induce mixed-function oxidases (*36*). Whether the tumor promoter PMA affects mitochondrial respiration may depend on the cell type (*37*).

Peroxisome proliferators. Structurally unrelated xenobiotics—such as clofibrate, nafenopin, and di(2-ethylhexyl)phthalate—stimulate the biosynthesis of peroxisomes, organelles that contain H_2O_2-generating oxidases, CAT, and enzymes involved in the β-oxidation of fatty acids. Peroxisome proliferators induce overproduction of H_2O_2; thus the formation of lipofuscin in the liver after prolonged exposure to peroxisome proliferators is a measure of lipidperoxidation caused by a prooxidant state. Peroxisome proliferators are hepatocarcinogenic and hepatopromoters (*38*) and may induce chromosomal damage by indirect action. Di(2-ethylhexyl)phthalate may produce a prooxidant state by stimulating peroxisome synthesis or by inhibiting the electron-transport chain through its metabolite, monoethylhexylphthalate (*39*).

Inhibitors of the antioxidant defense system. Cells have an elaborate defense system against active oxygen consisting of antioxidant enzymes (SOD, CAT, and glutathione peroxidase) and low molecular weight nonprotein sulfhydryls (*40*). These enzymes help to keep the steady-state concentrations of active oxygen at acceptable levels under physiological conditions, and their inhibition can induce a prooxidant state. Azide, hydroxylamine, and aminotriazole inhibit CAT, and dithiocarbamatic acid inhibits SOD (*41*). The observation that the mutagenicity of these drugs for *Salmonella* could be increased by oxygen and the addition of microsomes plus NADPH supports the notion that they act via the reduction of the antioxidant defense (*42*). The promoter PMA induces a reduction in the amount of SOD and CAT but not glutathione peroxidase in mouse epidermal cells in vivo (*43*) and in promotable (but not in promotion-insensitive) mouse epidermal JB6 cells (*14*). Whether the reduction in manganese-containing SOD in tumor cells represents a cause or effect of malignant transformation remains an open question (*44*).

In addition to these antioxidant enzymes, low molecular weight nonprotein sulfhydryls, most importantly glutathione, cysteine, and cysteinylglycine, play a major role in the cellular defense against active oxygen. Treatment with prothionine- and buthionine-sulfoximine, specific inhibitors of glutathione synthesis, increases the sensitivity of mouse mammary tumor cells to peroxides and of human lymphoid cells to radiation (*45*). Vitamins C and E, β-carotene, and urate are additional physiological molecules that contribute to the natural antioxidant defense (*46*).

Membrane-active agents. Certain agents of xenobiotic and endogenous origin and of different molecular weights and structure share the property of membrane activity. They can affect plasma, nuclear, mitochondrial, Golgi, or endoplasmic reticulum membranes and interact with receptors or perturb membrane conformation in a less specific manner. Examples of membrane-active agents of particular importance to carcinogenesis are peptide hormones; growth factors; lectins; the tumor promotors PMA (*47*, *48*), teleocidin (*49*), and mezerein; complete carcinogens such as aflatoxin B_1 (*50*); benzo[*a*]pyrene (*51*); certain bacteria and viruses (*52*); particulates such as asbestos and silica (*53*); and components of the immune system (*54*). Perturbation of membrane conformation by chaotropic agents may render membrane lipids susceptible to autoxidation (*55*). Other agents stimulate membrane phospholipases (phospholipase C or A_2), resulting in the formation of diacylglyceride, free arachidonic acid, and increased amounts of arachidonic acid metabolites [see in (*56*)].

Biosynthesis of prostaglandins and hydroxyarachidonic acid proceeds via the intermediate formation of the hydroperoxy derivatives prostaglandin G_2, 15-hydroperoxyprostaglandin E_2, and 5,12- or 15-hydroperoxyarachidonic acid. These unstable intermediates react spontaneously or in peroxidase-catalyzed reactions to the corresponding hydroxyl derivatives and release active oxygen. In specialized phagocytic leukocytes, membrane-active agents can elicit an oxidative burst by the activation of a NADPH-dependent oxidase (*56*). The activation of phospholipase A_2 and NADPH-dependent oxidase by membrane-active agents such as PMA may be the consequence of conformational changes in the membrane or of a kinase cascade initiated by protein kinase C (*57*).

Summary. There is convincing evidence that cellular prooxidant states—that is, increased concentrations of active oxygen and organic peroxides and radicals—can promote initiated cells to neoplastic growth. Prooxidant states can be caused by different classes of agents, including hyperbaric oxygen, radiation, xenobiotic metabolites and Fenton-type reagents, modulators of the cytochrome P-450 electron-transport chain, peroxisome proliferators, inhibitors of the antioxidant defense, and membrane-active agents. Many of these agents are promoters or complete carcinogens. They cause chromosomal damage by indirect action, but the role of this damage in carcinogenesis remains unclear. Prooxidant states can be prevented or suppressed by the enzymes of the cellular antioxidant defense and low molecular weight scavenger molecules, and many antioxidants are antipromoters and anticarcinogens. Finally, prooxidant states may modulate the expression of a family of prooxidant genes, which are related to cell growth and differentiation, by inducing alterations in DNA structure or by epigenetic mechanisms, for example, by polyadenosine diphosphate–ribosylation of chromosomal proteins.

A Role for Lipidperoxidation in Carcinogenesis

Many agents that induce prooxidant states cause lipidperoxidation because the polyunsaturated fatty acid side chains of membrane lipids are particularly sensitive to oxidation (*58*). Generalized lipidperoxidation resulting from cell death should be distinguished from the enzyme-catalyzed reactions of the arachidonic acid cascade. The following observations support the notion that lipidperoxidation plays a role in carcinogenesis. (i) Structurally unrelated classes of prooxidants-promoters cause lipidperoxidation. (ii) Many antioxidants that suppress lipidperoxidation are antipromoters. (iii) Stable endoperoxide analogs of prostaglandins promote 3-methylcholanthrene–initiated transformation of mouse skin cells (*59*). The overproduction of prostaglandin E_2 (*47*) via its hydroperoxy precursors and the concomitant release of active oxygen may play a role in PMA promotion of mouse skin cell transformation. Often, generalized lipidperoxidation is a consequence of this toxicity, and lipid degradation products originating from dying cells could exert a promotional effect. (iv) Promotion of mouse epidermal JB6 cells in vitro by PMA, benzoyl peroxide, and iodate is accompanied by the specific loss of ganglioside GT_1 from the plasma membrane (the sugar portion of GT_1 is probably oxidized). Benzoyl peroxide and iodate probably directly oxidize GT_1, whereas the oxidation subsequent to treatment of cells with PMA may be due to the PMA-induced decrease in the cellular SOD levels (*14*). (v) Lipid hydroperoxides and their degradation products may act as CF's (low molecular weight components which break chromosomes in the same and remote tissues). The CF's may modulate the expression of genes related to tumor promotion and progression by inducing gene rearrangements or by combined epigenetic-genetic mechanisms (see below).

Oxidative Chromosomal Damage

The recognition that the covalent binding to DNA of many carcinogens requires their activation to electrophilic, ultimate metabolites (*60*) was of pivotal importance to carcinogenesis research. This mechanism of induction of DNA damage is often referred to as direct action. Several years ago, a second basic mechanism was distinguished as indirect action (*61*). Agents operating by indirect action produce secondary DNA-damaging agents in reactions with cellular molecules other than DNA. The secondary agents are mostly active oxygen species, lipid hydroperoxides and their radical and aldehydic degradation products (*56*, *58*, *62*), *N*-chloroamines (*63*), and oxidation products of aromatic amino acids, purines, and the like. When released by cells the secondary agents may act as CF's (*56*). Physical and chemical agents that induce a prooxidant state are expected to damage DNA by indirect action. Characteristic DNA lesions are single- and double-strand breaks, apurinic and apyrimidinic sites, products of the 5,6-dihydroxydihydrothymine type, and so forth. Many agents operate by direct and indirect mechanisms at the same time. Unambiguous proof for indirect action, then, requires the demonstration of characteristic base damage, for example, the formation of 5,6-dihydroxydihydrothymine or tritiated water from the ^3H-labeled methyl group of thymine. This has been accomplished for gamma (*20*) and near-ultraviolet (*64*) radiation, cupric ascorbate, and benzo[*a*]pyrene (*51*). Strong evidence for indirect action has also been obtained for aflatoxin B_1 (*50*) and PMA (*65*, *66*) (see below). Many carcinogens that induce prooxidant states cause chromosomal damage by indirect action, but the role of this damage in carcinogenesis remains unclear.

Agents operating by indirect action are usually strong cytotoxins and potent inducers of chromosomal aberrations but weak inducers of sister chromatid exchanges and point mutations. Hyperbaric oxygen induces mutations in bacteria (*50*) and aberrations in eukaryotic cells (*8*, *18*) probably because a small amount of active oxygen is formed by cellular metabolism. Indeed, O_2^- and H_2O_2 weakly mutagenize bacteria (*67*) but efficiently induce DNA breaks and chromosomal aberrations (*68*). Cells have complex constitutive and inducible enzyme systems that accomplish the repair of oxidative DNA damage.

Membrane-mediated chromosomal damage. As discussed above, certain membrane-active agents can induce a prooxidant state. The exact mechanism of formation of a prooxidant state and, as a consequence, its quality and extent depend on the membrane-active agent and the responding cell. This class of structurally diverse agents can cause membrane-mediated chromosomal damage by indirect action (*56*). The tumor promoter PMA is a typical representative of this class, and other membrane-active agents have already been mentioned [see above and (*7*)]. PMA activates protein kinase C, which acts as a receptor (*57*), but also exerts a less specific chaotropic effect on membrane conformation (*48*). Although it does not bind covalently to DNA it causes strand breakage, sister chromatid exchange, and chromosomal aberrations in human leukocytes (*65*, *66*). PMA also induces aberrations in mouse epidermal cells (*69*) and aneuploidy in yeast (*56*). The observation that PMA rapidly stimulates polyadenosine diphosphate (polyADP)–ribosylation of chromosomal proteins in human monocytes also implicates DNA damage (*70*). DNA containing strand breaks is known to stimulate poly(ADP-ribose) synthetase (*71*). A second mechanism of delayed induction of polyADP-ribosylation by PMA in fibroblasts apparently does not require DNA strand breakage (*70*). PMA also facilitates the amplification of dihydrofolate reductase genes in mouse 3T6 cells and of the metallothionein I gene in Chinese hamster lung cells. Similar amplification of the dihydrofolate reductase gene is also observed by bona fide DNA-damaging agents that operate at least in part by indirect action (*72*).

As already mentioned, PMA induces chromosomal aberrations in PHA-stimulated human lymphocytes. Antioxidants suppress the clastogenic action of PMA, which indicates the intermediacy of active oxygen (*65*, *66*). The anticlastogenicity of inhibitors of the arachidonic acid cascade suggests the involvement of arachidonic acid metabolism. It should be kept in mind that many such inhibitors are also potent antioxidants. PMA stimulates phospholipase A_2 (and perhaps C), possibly as a consequence of a kinase cascade initiated by protein kinase C. This results in the stimulation of arachidonic acid metabolism and the increased formation of the hydroperoxyarachidonic acid intermediates described above. Active oxygen is produced when these unstable products react to the corresponding hydroxyl derivatives. The resulting prooxidant state may cause chromosomal damage in the PMA-stimulated cell.

The formation of CF's in human monocytes, polymorphonuclear leukocytes, and regular lymphocyte preparations (*66*) (which are contaminated with monocytes and platelets) is induced by PMA. Only a weak CF is produced from pure lymphocytes. CF's may represent intra- and extracellular signals that translate events from the cell surface to the genome, a concept that is supported by the results of cocultivation experiments. CF released by stimulated phagocytic leukocytes could exert a promotional effect on carcinogen-initiated neighbor-

ing tissue (*56, 73*). The composition of CF's may vary with the cell type from which they originate; for example, they may consist of H_2O_2, arachidonic acid peroxides and their (aldehydic) degradation products, free arachidonic acid and unknown metabolites (*56*, *58*, *62*), *N*-chloroamines (*63*), and possibly oxidation products of aromatic and sulfur-containing amino acids. Ethylacetate extracts of the culture medium of PMA-treated human monocytes contained increased amounts of thromboxane B_2, 12-L-hydroxy-5, 8, 10-heptadecatrienoic acid (HHT), hydroxyl derivatives of arachidonic acid, and free arachidonic acid (*74*). It is evident that PMA stimulates both the cyclooxygenase and lipoxygenase pathways of the arachidonic acid cascade. The biosynthetic formation of thromboxane B_2 and HHT proceeds via the intermediacy of prostaglandin G_2 and is accompanied by the release of active oxygen and malondialdehyde. Prostaglandin G_2, hydroperoxyarachidonic acid, hydrogen peroxide, and hydroxyl radicals produced in Fenton-type reactions may start autoxidation chain reactions in the target cell and cause chromosomal damage. Chromosomal damage in mouse epidermal cells may be induced by a similar mechanism due to PMA-stimulated synthesis of prostaglandin E_2 and release of arachidonic acid. The formation of prostaglandin E_2 from its hydroperoxy precursors is accompanied by the release of active oxygen (*47*). In analogy to active oxygen formed as a consequence of quinone metabolism, overproduction of arachidonic acid hydroperoxides induced by PMA may stimulate Ca^{2+} release from mitochondria. Ca^{2+} mobilization appears to play an integral role in the action of receptor-dependent and -independent membrane-active agents.

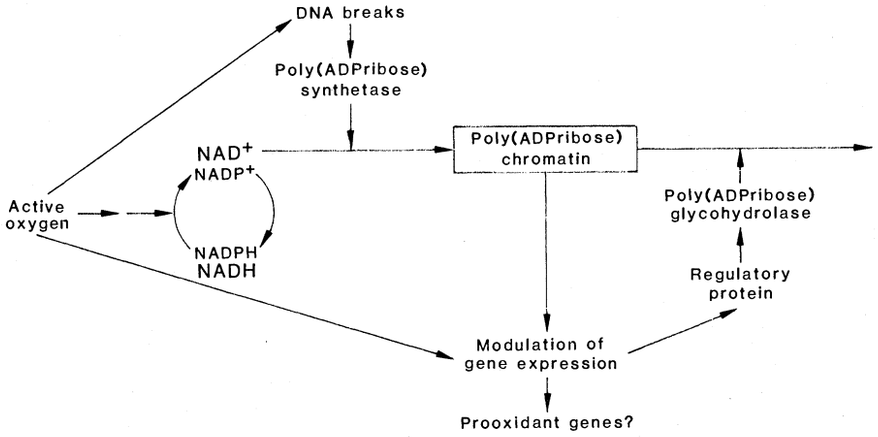

Fig. 1. Active oxygen–induced modulation of gene expression.

Modulation of Gene Expression by Prooxidant States

Tumor promotion encompasses the modulation of the expression of genes related to growth and differentiation of initiated cells. This results in the formation of tumors by selection and clonal expansion. The evidence indicates that many agents that induce a prooxidant state have promotional activity. Active oxygen may represent an intermediate in the induction of promotion-related genes. Indeed, antioxidants suppress the induction by PMA of ornithine decarboxylase in mouse epidermis and mouse mammary tumor cells (*17*). Catalase also suppresses the induction by PMA of exogenous copies of mouse mammary tumor virus in the tumor cells (*75*).

The elucidation of the mechanism or mechanisms by which a prooxidant state modulates gene expression is a major goal in tumor promotion research. Chromosomal damage could play a role. Even though the observation that (early) effects of tumor promoters are reversible originally argued against the involvement of structural genomic damage, doubts have arisen about the degree of the reversibility. Two-state promotion experiments on initiated mouse skin indicate that a single application of PMA induces a long-lasting change. Promotion could be completed many weeks later by the stage II promoter 12-*O*-retinoylphorbol-13-acetate (*76*). PMA also induces an irreversible effect on cultured preneoplastic mouse epidermal JB6 cells, promoting them to the transformed phenotype. PMA alone can induce malignant tumors on noninitiated mouse skin (*77*). Even a large degree of reversibility of the biological effects does not eliminate the possibility that oxidative genetic damage plays a role in promotion. Although oxidative DNA damage is repaired rapidly and completely (*78*), the cumulative effect of a small fraction of DNA lesions that remains unrepaired during a prolonged prooxidant state may cause permanent changes.

The processing of indirectly induced genetic damage by repair and constitutive and damage-induced mechanisms of replication and recombination can lead to permanent alterations in DNA sequences, such as mutations, amplification of certain sequences, or intra- and interchromosomal rearrangements of blocks of sequences. Because DNA-damaging agents that operate by indirect action are strong clastogens but weak mutagens, they may preferentially induce sequence rearrangements. Examples of these mechanisms of modulation of gene expression have arisen from oncogene research (*79*). Promoter-induced rearrangement of oncogenes that had been mutated by an initiator may represent a sequence of events in carcinogenesis.

In addition to causing structural genetic changes, active oxygen may participate in epigenetic mechanisms that result in altered gene expression. The modification of chromosomal proteins by polyADP-ribosylation may play a role. The intermediacy of polyADP-ribosylation in gene expression is suggested by the observation that its inhibition suppresses the mitogen-induced activation of human lymphocytes, the induction of two fetal functions in cultured rat hepatocytes, and the differentiation-specific increase in creatine phosphokinase in chick myoblasts (*80*). Poly(ADPribose) synthetase is unique because it is stimulated by DNA strand breaks (*71*). In a prooxidant state the demand for detoxification of active oxygen and organic radicals is increased, and as a result levels of glutathione decrease (for example, as a consequence of the action of glutathione peroxidase) and the levels of oxidized pyridine nucleotides increase. NAD^+ is the substrate for poly(ADPribose) synthetase; therefore, synthetase stimulation by active oxygen–induced DNA breaks coupled with a ready supply of NAD^+ may result in the rapid polyADP-ribosylation of chromosomal proteins and, consequently, in the modulation of gene expression. Later, when poly(ADPribose) synthesis is proceeding at a high rate, the concentration of NAD^+ may drop. Other scenarios can be envisaged; for example, a regulatory protein rather than DNA that contains strand breaks might stimulate poly(ADPribose) synthetase or inhibit poly(ADPribose) degradation. Indeed, PMA-induced polyADP-

ribosylation in mouse and human fibroblasts occurs in 2 to 3 hours in the absence of detectable amounts of DNA strand breaks. De novo RNA and protein synthesis are required and the antioxidants SOD, CAT, and BHT are inhibitory, indicating the intermediary role of active oxygen in the induction process (*70*). It is conceivable that prooxidant states induce a group of prooxidant genes reminiscent of SOS functions in bacteria. The following genes and regulatory sequences may belong to the prooxidant category: ornithine decarboxylase, plasminogen activator, poly(ADPribose) synthetase, genes of the antioxidant defense system, heat shock genes, and intracistronic A-particle and long terminal repeat–type sequences. Figure 1 shows a heuristic scheme of active oxygen–induced modulation of gene expression by polyADP-ribosylation of chromosomal proteins. The role of polyADP-ribosylation in malignant transformation remains to be clarified. Inhibitors of poly(ADPribose) synthetase suppress (*80, 81*) or stimulate (*82*) neoplastic transformation in different systems.

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Safety Concerns and Genetic Engineering in Agriculture

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Federal agencies are considering various regulations to protect the public from environmental and health problems that might arise from the release of genetically engineered organisms. Concern has been expressed because several agricultural practices, such as the widespread use of DDT in past decades (*1*), have caused serious problems that were unintended and unexpected. Also, movement of weeds and insect pests into new environments has created problems that have become difficult to control. Examples include kudzu, hydrilla, the gypsy moth, and the Japanese beetle. Because of these experiences, it is necessary to consider the potential effects of releasing organisms containing genes from related and unrelated genera. This article will focus on the safety issues involved in using genetically engineered plants and microorganisms (bacteria and fungi) to benefit agriculture. Other applications to which the same principles should hold with respect to safety issues include the use of genetically engineered organisms for mining, waste treatment, and detoxifying chemical spills.

The economic and environmental benefits expected to accrue from agricultural use of recombinant organisms are great (*2*) and should be considered in relation to the potential risks. By splicing foreign genes into plant chromosomes it may be possible to create plants resistant to a wide array of pests. The hope and expectation is that they will lead to decreased use of chemical fungicides and insecticides, many of which are toxic to man. Recombinant DNA techniques may be used to develop plants that utilize fertilizers more efficiently, thereby minimizing fertilizer runoff into streams and lakes. In many crop species a relatively narrow base of germplasm is being used to develop varieties. There is concern that this has created genetic vulnerability to disease (*3*). Genetic engineering can be used to introduce new genes and thereby increase genetic variability for the future. The time it takes to develop new plant varieties should be greatly decreased by this new technology.

Genetically engineered bacteria and fungi also have potential value. For example, *Rhizobium* strains isolated from many locations around the world are being applied to soils in large numbers so that legumes can produce high yields without needing expensive nitrogenous fertilizers. Several approaches are being considered to increase legume yields with genetically engineered *Rhizobium* (*4*). Other microbes, such as mycorrhizae, *Pseudomonas*, and *Frankia* (*5*), are also promising candidates for use in agriculture, and there is a good chance that the value of these organisms can be increased through recombinant DNA technology as well as traditional mutation and recombination techniques. As in traditional agriculture, the value of the new plants and microbes can be assessed only after they have been tested under a variety of field conditions. This article will discuss ways to predict the safety level of an organism that has received several foreign genes.

Of particular concern in the introduction of new organisms is the potential to self-perpetuate and spread. For the purpose of this discussion, however, a problem plant that gets no farther than the next field is not defined as a serious problem. Nor is a microbe that unexpectedly kills plants that it was sprayed on but does not damage plants in a neighboring field.

Plants

Plants have been crossed (traditional "genetic engineering") by man for centuries. New variants resulting from such breeding have not caused serious problems. Most of our high-yielding crops,

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