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OXIDATIVE STRESS AND SUPEROXIDE DISMUTASE IN DEVELOPMENT, AGING AND GENE REGULATION

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Abstract

Free radicals and other reactive oxygen species are produced in the metabolic pathways of aerobic cells and affect a number of biological processes. Oxidation reactions have been postulated to play a role in aging, a number of degenerative diseases, differentiation and development as well as serving as subcellular messengers in gene regulatory and signal transduction pathways. The discovery of the activity of superoxide dismutase is a seminal work in free radical biology, because it established that free radicals were generated by cells and because it made removal of a specific free radical substance possible for the first time, which greatly accelerated research in this area. In this review, the role of reactive oxygen in aging, amyotrophic lateral sclerosis (a neurodegenerative disease), development, differentiation, and signal transduction are discussed. Emphasis is also given to the role of superoxide dismutases in these phenomena.

INTRODUCTION

Oxygen free radicals are believed to play a fundamental role in a wide variety of pathologies and other biological phenomena including aging (1-5). At a molecular level, free radicals modify proteins and inactivate enzymes (6-13), damage DNA (14-19), the cellular transcriptional machinery (20, 21) and initiate the chain reactions that peroxidize lipids (22-24). Damage inflicted by reactive oxygen species is believed to be one underlying cause of ischemic damage (25-27), to increase the incidence of neoplastic transformation (28-30) and to promote metastasis (31). Survival in the presence of oxygen is thus dependent on prevention of oxidative damage by enzymes such as the superoxide dismutases (SOD), which eliminate the superoxide radical ($\cdot O_2^-$) and produce H_2O_2 (32), and catalase and peroxidases, which catalyze removal of H_2O_2 (33, 34). Non-enzymatic low molecular weight antioxidants such as glutathione, ascorbate, and carotenoids are also believed to play an important role in protecting cells from toxic oxidation reactions (34). Evidence derived from a number of studies supports the hypothesis that shifts in the cellular oxidant/antioxidant equilibrium may also influence developmental pathways in a variety of tissues from phylogenetically diverse organisms (35, 36). In fact, there is limited evidence to suggest that oxidants drive a molecular clock that controls the timing of certain cellular events during both development and aging *via* modulation of normal physiological pathways (3, 35, 37). Nevertheless, the existing evidence supports the view that most free radical reactions produce pathological lesions rather than useful physiological effects (1, 2, 14, 38).

The seminal work in free radical biology and aging-related studies was the discovery of superoxide dismutase activity (32). Three forms of SOD are known to exist in mammalian tissues (39). SOD-1 is a dimeric copper and zinc-containing form that appears to be largely localized to peroxisomes (40, 41), while SOD-2 is tetrameric and contains manganese in all 4 of its subunits; it is localized primarily in mitochondria (42). SOD-3 is a tetrameric extracellular form of the enzyme that also contains copper and zinc (39, 43). This discussion will provide a brief overview of several current areas of focus in free radical biology, and will also illustrate the overwhelming importance of superoxide dismutases in the shaping of free radical biology as well as a number of related disciplines.

OXIDATIVE STRESS IN AGING

The "Free Radical Theory of Aging" as first presented by Harman (1) postulated that oxygen radicals generated in metabolic pathways damaged cells and increased their vulnerability to death. It also postulated that it is the incessant accumulation of structural damage that disrupts functions at a macromolecular level and is the underlying cause of aging. Since it was first proposed, there have been many modifications to this theory (3, 44). From a number of studies, it has also become apparent that neither gross structural damage to cellular components, nor decreased repair capacity can completely account for cellular dysfunction and death (3, 45-47). However, even if free radical reactions do not account for all aspects of aging, they appear to underlie many aspects of aging and to play a major role in the onset and progression of many human diseases (44, 48). Free radical reactions probably account for certain aspects of adult respiratory distress syndrome (49, 50), age-associated diseases such as diabetes (51-53), ischemic injury associated with organ transplant, stroke and heart disease (26, 54-67), and various late-onset neurodegenerative diseases (see discussion below).

Aging is usually associated with increasing levels of oxidation. Conversely, the antioxidant defenses only rarely increase during aging; they are known to decline in some tissues during aging. In most cases, however, the antioxidant defenses do not change with age (68). It has been demonstrated repeatedly that the relative rate of oxidant generation increases with age, which correlates with age-associated changes in cellular redox state that are also commonly seen during aging (4, 69-71). For example, the rates of superoxide ($O_2^{\cdot}$) (5, 71-76) and H_2O_2 generation (4, 72, 76-79) increase in the cells of aging organisms while glutathione concentration declines progressively with advancing age (69, 70, 80, 81). Furthermore, it has been demonstrated that species longevity correlates inversely with the rate of free radical generation (5, 71) and that overexpression of Cu/Zn SOD (SOD-1) and catalase can extend the lifespan and metabolic potential in *Drosophila* (82, 83). In spite of this, the full extent of oxidative involvement in the regulation of longevity is only beginning to be understood.

The underlying causes of aging-associated increases in oxidative stress are unknown. *In vivo*, age-associated decreases in the activities of cytochrome *c* oxidase, NADH dehydrogenase and to a lesser extent succinate dehydrogenase activities have been reported in a wide variety of mammalian species (84, 85) including humans (86-88). These changes are believed to play an important role in aging-dependent increases in oxidation *in vivo* (78, 84) although they do not necessarily occur in all or even most of the cells of a given tissue (89). Those aging-associated decreases in cytochrome *c* oxidase that occur *in vivo* appear to result from age-dependent changes in lipid-protein interactions (75, 90-94). Furthermore, restoring young levels of mitochondrial membrane cardiolipin in rats by treatment with acetyl-L-carnitine restores cytochrome *c* oxidase activity to the level seen in young animals (93, 94). While the majority of studies show that oxidant generation increases with age, there are some instances in which oxidant generation fails to increase and may even decline during aging (95-97). The reasons for these discrepancies are unknown, but assay conditions appear to be a major factor (95). Considering the effects of membrane changes on the activities of key mitochondrial enzymes, it also seems probable that tissue differences in membrane composition as well as the diets of experimental animals could to some extent determine whether age-associated changes in oxidant generation are observed.

i.) Oxidative Stress, Aging and Neurodegenerative Disease

Of central biological interest to studies of aging are the cellular mechanisms that measure physiological time in order to signal initiation or termination of critical events at various stages of life. An understanding of these mechanisms is crucial to elucidating the mechanisms of late-onset degenerative diseases associated with aging. Although aging is progressive, some age-associated changes and disease states appear to occur suddenly rather than gradually. For example, aging is the major risk factor for late-onset neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS; Lou Gehrig's Disease) (98, 99). Furthermore, even when expressed as a dominant trait, penetrance is rarely seen during the first several decades of life (99-104). This suggests that some disease genes may cause disease only when the level of cellular damage has reached a critical level or when the genetic background in the cells has undergone age-associated changes that are permissive to the disease state.

Interestingly, the late-onset neurodegenerative diseases are frequently associated with impaired function of the mitochondrial respiratory complexes or defects in cellular machinery that removes metabolically-generated oxidants (105-109; for reviews see refs. 98, 99, 110-113). For example, cytochrome *c* oxidase activity is diminished in some cases of ALS and Alzheimer's (106, 107, 109), while NADH dehydrogenase (complex I) is increased by as much as 55% in patents with ALS (99, 108). Changes in the abundance and activity of other respiratory complexes are also associated with ALS, Alzheimer's, Huntington's and Parkinson's disease and are discussed in detail by Bowling and Beal (99). Possibly the most compelling evidence of oxidative stress involvement in neurodegenerative diseases stems from the fact that defects in superoxide dismutase, an enzyme associated with oxidant removal, appear to be the cause of one form of ALS (114).

ii.) SOD-1 in Familial Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis is an adult-onset, progressive, paralytic disorder that leads to paralysis and death largely as a result of degeneration of motor neurons in cortex, brain stem and spinal cord (100, 104, 110, 115). While the majority of ALS cases occur sporadically, about 10-15% are inherited as an autosomal dominant trait (115, 116). About 15-20% of the familial cases (2% of all cases) appear to arise because of mutations in the copper/zinc superoxide dismutase gene (SOD-1; 110, 114, 117-121). A summary of known SOD-1 defects associated with ALS is presented in Table 1. A complete discussion of all of these mutations is beyond the scope of this discussion; however, more detailed discussions do exist (115, 122) and the interested reader is referred to these. Interestingly, no mutations that cause ALS have ever been found in exon 3, although one silent mutation has been observed in exon 3 of a human SOD-1 in transgenic mice (123).

The effects of these mutations on SOD protein are known. Normally, the copper/zinc form of SOD protein (SOD-1) exists as a dimer; it contains a large β-sheet that consists of 8 strands in an antiparallel arrangement (42). About 50% of the SOD-1 residues are contained in this β-sheet, which, seen in three dimensions, ap-

pears as a cylinder or barrel (β-barrel). There are seven loops in SOD; loops I and V are short β-hairpin connections between adjacent β-strands.

Table 1. Mutations of SOD-1 Found in Familial Amyotrophic Lateral Sclerosis

Exon	Codon	Sequence	Amino Acid	Reference
1	4	GCC→GTC	Ala→Val	124-126
1	4	GCC→ACC	Ala→Thr	127, 128
1	6	TGC→TTT	Cys→Phe	129
1	14	GTG→ATG	Val→Met	130
1	21	GAG→AAG	Glu→Lys	131
2	37	GGA→AGA	Gly→Arg	114, 124
2	38	CTG→GTG	Leu→Val	114, 124
2	41	GGC→AGC	Gly→Ser	114, 124
2	41	GGC→GAC	Gly→Asp	114, 124
2	43	CAT→CGT	His→Arg	114, 124
2	46	CAT→CGT	His→Arg	132, 133
2	48	CAT→CAG	His→Gln	134
4	84	TTG→GTG	Leu→Val	103, 124, 133
4	85	GGC→CGC	Gly→Arg	114, 124, 135
4	90	GAC→GCC	Asp→Ala	101, 136-138
4	93	GGT→GAT	Gly→Asp	139
4	93	GGT→GCT	Gly→Ala	114, 124, 125, 135
4	93	GGT→TGT	Gly→Cys	114, 124
4	93	GGT→CGT	Gly→Arg	124, 140, 141
4	93	GGT→GTT	Gly→Val	122, 142
4	100	GAA→GGA	Glu→Gly	121, 124, 141, 143
4	101	GAT→AAT	Asp→Asn	131, 144, 145
4	101	GAT→GGT	Asp→Gly	146
4	104	ATC→TTC	Ile→Phe	133, 147
4	106	CTC→GTC	Leu→Val	114, 124
4	112	ATC→ACC	Ile→Thr	134, 139
4	113	ATT→ACT	Ile→Thr	114, 119, 120, 124, 134, 141, 148
4	115	CGC→GGC	Arg→Gly	149
5	124	GAT → GTT	Asp→Val	122
5	125	GAC →CAC	Asp→His	134
5	126	TTG → **G	131 stop	122, 150-153
5	133	GAA → ___	Glu___	122
5	139	AAC→AAA	Asn→Lys	154
5	144	TTG→TCG	Leu→Ser	146
5	144	TTG→TTC	Leu→Phe	124
5	145	GCT→ACT	Ala→Thr	146
5	148	GTA→GGA	Val→Gly	124
5	148	GTA→ATA	Val→Ile	133, 155
5	149	ATA→ACT	Ile→Thr	134, 154, 156
Intron 4	-10	T→G	+Phe-Leu-Gln	157

The sequence is highly conserved, but the human protein contains a 2-residue insertion in loop II relative to the bovine sequence. Loops III and VII form two Greek key β-barrel connections (158). The active site channel is formed between the electrostatic loop (loop VII; residues 121-144) and loop IV, which is composed of the disulfide and Zn ligand subloop regions (residues 49-84; see refs 118, 158). Fourteen structurally conserved side chains with highly conserved sequences are located at critical positions within or near the loops. These side chains appear to play an essential role in controlling loop conformation and interactions (158). The 4 zinc ligands are at His 63, His 71, His 80 and Asp 83; they are arranged in an approximate tetrahedral configuration. Unlike the Zn ligands, the 4 Cu ligands at His 46, His 48, His 63 and His 120 form a distorted square planar arrangement. This occurs because the imidizole nitrogen at His 63 does not lie in the same plane as the other 3 histidine residues and the copper (42, 158). All of the mutations in the SOD-1 gene associated with ALS can be grouped into one of four categories:

Mutations that alter the length of the coding sequence such as the two bp deletion at codon 126. This mutation inserts a stop codon at position 131 resulting in the loss of an electrostatic loop and a dimer contact region necessary for enzyme function (150-153). The single bp substitution in intron 4 (10 bp upstream of exon 5) results in an alternatively spliced mRNA containing 3 additional amino acids between exons 4 and 5 (Table 1).

Mutations in the active site channel of the enzyme. These mutations affect the conformation of the electrostatic loop and destabilize the packing of the core (118, 158).

Mutations at Cu binding sites. It is the Cu component of the enzyme that catalyzes the dismutation of $\cdot O_2^-$ to H_2O_2. It is stabilized by the 4 His residues at His 46, His 48, His 63 and His 120. His 48 and His 120 also seem to play an important role in loop conformation and interactions (118, 158). Interestingly, mutations have been found at His 46 (132, 133) and His 48 (134), but not at His 63 or His 120 (118).

Mutations that affect enzyme structure. These include any of a group of mutations that alter loop conformation, packing structure, hydrogen bonding, backbone conformation, disrupt dimer interactions and destabilize of the β-barrel structure (118).

A complete description of each of these categories is beyond the scope of this discussion; however, the reader is referred to refs 158 and 118 for a detailed discussion of SOD structure and mutation effects.

The reason(s) that defects in SOD result in ALS remains unknown. Most of the known SOD-1 mutations that cause ALS affect the β-barrel fold (114, 118) or dimer contact (124). Many of the mutations associated with ALS decrease SOD activity (108, 124, 159, 160). However, loss of activity is not solely responsible for ALS. First, the amount of the decrease in SOD activity

differs greatly between individuals (108, 124, 159, 160), but does not necessarily correlate with the age of onset or duration of survival (161, 162). In fact, some mutations that result in ALS do not decrease activity (163). Second, treatment with antioxidants has little effect on survival of ALS patients (164, 165) or transgenic animals (166), although some treatments appeared to delay onset (165, 166). Third, transgenic animals that overexpress human ALS mutations develop ALS-like symptoms even though they continue to express their own normal gene (147, 167-169). Indeed, the total SOD activity in their tissues exceeds that found in control animals (167).

Dominantly inherited mutations are usually associated with a gain rather than a loss of function (112). If a gain in function occurs as a result of SOD-1 mutations, the precise nature of that function remains unclear. Beckman *et al.* (170) proposed that SOD mutations permit greater access of peroxynitrite ($ONOO^-$) to the SOD copper. Although $ONOO^-$ is normally used as a subcellular signal, the copper core of SOD catalyzes the formation of an intermediate nitronium-like species (NO_2^+) from $ONOO^-$ that can nitrate phenolics including tyrosine residues in proteins (171). The nitronium intermediate nitrates light neurofilaments (NF-L; 172, 173), and stimulates reactions leading to increased cellular Ca^{2+} (173), as well as excessive stimulation of the *N*-methyl-D-aspartic acid (NMDA) receptor in neurons (174, 175). Other possibilities exist. In at least one study, SOD-1 mutations were found to stimulate apoptotic cell death, while the normal gene prevents activation of this pathway (176). The copper core of the SOD-1 enzyme is seated at the bottom of a long electrostatically charged funnel (42, 158) which, due to its small diameter and charge, limits access of larger molecules to the enzyme core. Mutations that alter this funnel may make the metal core of the enzyme molecule more accessible to some molecules. The consequences of this type of alteration can be highly deleterious. For example, changes in the kinetics of H_2O_2 release from SOD after dismutation of $\cdot O_2^-$ may stimulate the formation of hydroxyl radicals ($\cdot OH$; 112, 177, 178). It has been demonstrated that some ALS mutations alter the K_M of the enzyme in a manner that increases the probability of a Fenton-type reaction (179, 180).

SUPEROXIDE DISMUTASE AND OXIDATIVE STRESS IN DEVELOPMENT AND DIFFERENTIATION

Early biologists observed that regional variations in metabolic rate influenced development and regeneration (181-185). Furthermore, variations in ambient oxygen concentration strongly modulate the developmental fate of embryonic tissues in both vertebrate and invertebrate species (36, 186-188). However, the reason for these effects remained unclear. A seemingly plausible link between oxidative metabolism and developmental effects was the generation of oxidants in metabolic pathways and the subsequent effects of these reactive species on cellular redox state and gene expression. Supportive of this view was the observation that hyperoxia induced differentiation in neuroblastoma even in the presence of enough cyanide to abolish aerobic metabolism (189). Although respiratory inhibitors decrease the rate of oxygen utilization, they also promote electron stacking in cytochromes and thereby stimulate oxygen free radical generation (190). The rate of ROS generation in cells is also strongly modulated by ambient oxygen concentration (191). Of course, if free radical generation changes during differentiation, then it is also reasonable to expect concomitant changes in antioxidant defense levels (192). Since mitochondria and peroxisomes are the major sites of cellular free radical generation, it also follows that the removal of these active oxygen species by SOD is the pivotal step in regulating cellular steady state levels of oxidants. In fact, total SOD activity has been reported to increase during human fetal development in liver (193), blood (194) and placenta (195-197), and during differentiation of monocytes (198) as well as during the development of many other phylogenetically diverse organisms (35, 36). Table 2 provides a summary of the organisms and tissues in which developmental increases in SOD have been observed.

The increases observed in SOD activity during late gestation could also reflect changes in the levels of cytokines. Both IL-1 and TNF-α have been shown to produce rapid accumulation of SOD-2 mRNA through increased transcription of the SOD-2 gene (254-257), although TNF-α does not necessarily affect protein abundance (256). TNF expression has also been shown to occur in fetal skin and to increase during development (258). Skin fibroblasts derived from old individuals have been reported to exhibit higher IL-1 expression in fetal foreskin fibroblasts (259). However, because of the small number of donors and the different sites of origin of the fibroblasts used in these studies, the observation of an age-dependent increase in IL-1 needs to be confirmed.

Differences in fetal and postnatal levels of SOD activity could stem partly from changes in activity of trans-acting factors that can influence both transcription and mRNA stability. Human (260), bovine (261) and rat (262) SOD-2 genes have no obvious TATA box; however, they do contain multiple copies of an Sp1 binding site, which can act as a surrogate TATA box and recruit TFIID (263). In the bovine SOD-2 gene the Sp1 sites have been shown to be necessary for basal promoter function, but not sufficient for conferring responsiveness to lipopolysaccharide. Furthermore, the Sp1 transcription factor has been shown to be regulated developmentally (264). It is, for example, known that Sp1 sites regulate the developmental expresssion of both the mouse secretory protease inhibitor p12 (265) and the murine deaminase gene (266).

The physiological relevance of the developmental increase in SOD activity remains unclear, since despite

Table 2. Development-Associated Increases in SOD activity

Organism	Tissue	SOD Determined	Comparison	Reference
Plants				
Soybeans	seeds	MnSOD	germination	199
Slime molds				
Didymium iridis	Whole Organism	Total	Differentiation	200
Physarum polycephalum	Whole Organism	MnSOD	Differentiation	201-205
Nematodes				
Caenorhabditis elegans	Whole Organism	Total	Dauerlarvae Formation	206
Insects				
Ceratitis capitata	Mitochondria	MnSOD	Pupae/Adult	207
Drosophila melanogaster	Whole Organism	Total	First Instar/Adult	208
Drosophila melanogaster	Whole Males	Total	Third Instar/Adult	209
Musca domestica	SOD Isoforms			
	Whole Males	MnSOD RNA	Larval Stages/ Adult	210
Amphibians				
Discoglossus pictus	Whole Organism	Total	Stage V/Stage XIV	211
Rana ridibunda	Whole Organism	Total	Stage III/Stage XIV	
			Stage V/Stage XIV	211
Xenopus laevis	Oocytes	Cu/Zn SOD	Oogenesis	212
Birds				
Chicken	Liver	Cu/Zn, MnSOD	Development (days 6-18)	213
	Brain	Cu/Zn, MnSOD	Development (days 6-18)	213
Mammals				
Rabbit	Lung	Total	Fetal/Neonate	214, 215
		Total	Neonate/Adult	216
		Total	Fetal/Adult	217
	Erythrocytes	Total	Bone Marrow Maturation	218
Rat	Blood	Total	Neonate/Adult	219
	Lung	Total	Fetus/Neonate	220
		Total	Fetal/Neonate/Adult	221, 222
		Total	Neonate/Adult	216, 217
		Cu/Zn	Fetus/Neonate	223
		Cu/Zn, MnSOD	Fetus/Neonate	224
		Cu/Zn, MnSOD	Birth/Neonate	225
		Cu/Zn, MnSOD protein	Fetus/Neonate	226
		MnSOD	Fetus/Adult	220, 227
	Liver	Total	Neonate/Adult	219
		Total	Fetal/Neonate/Adult	228
		Cu/Zn, MnSOD protein	Gestation	229
		Cu/Zn, MnSOD	Fetus/Neonate/Adult	230, 231
	Hepatocytes/Liver	MnSOD	Neonate/Adult	232-235
	Liver mitochondria	MnSOD	Fetus/Adult	236
	Brain	MnSOD	Gestation	232
		Total*	Neonate/Adult	219
		Total	Neonate/Adult	237
		Cu/Zn, MnSOD*	Neonate/Adult	231
		Cu/Zn, MnSOD	Neonate/Adult	238
		Cu/Zn, MnSOD	Fetus/Neonate/Adult	235
	Heart	Cu/Zn, MnSOD protein	Fetus/Neonate	239
	Kidney	Cu/Zn, MnSOD protein	Fetus/Neonate	226
	Kidney	Cu/Zn, MnSOD protein	Gestation	229, 239
	Pancreas	Cu/Zn, MnSOD protein	Gestation	229
	Thyroid	Cu/Zn, MnSOD protein	Gestation	229
	Gastrointestinal	Cu/Zn, MnSOD protein	Gestation	229
	Testicle	Cu/Zn (0.94 kb) mRNA	10-day to 60-days	240
Mouse	Erythroleukemia	Cu/Zn	Differentiation	241
	Lung	Total	Fetal/Neonate	215
		Total	Neonate/Adult	216
	Liver	Cu/Zn activity and mRNA	Gestation	242
		Total	Fetal/Neonate/Adult	243
	Liver	Total	Neonate/Adult	244
	Kidney	Total	Neonate/Adult	244
Hamster	Lung	Total	Fetal/Neonate	215
		Total	Neonate/Adult	216
Guinea Pig	Lung	Total	Fetal/Neonate	215
		Total	Neonate/Adult	216
	Choclea	Total	Fetal/Neonate/Adult	245
Sheep	Lung	Total	Gestation	246
	Kidney Cortex	MnSOD mRNA	Fetal/Neonate/Adult	247
Human	Erythrocytes	Cu/Zn	Gestation	248
	Monocytes	Total	Differentiation *in vitro*	198
		MnSOD	Differentiation *in vitro*	249
	Lung	Total	Neonate/Adult	217
		Total*	Fetus/Neonate	193
		Cu/Zn*	Gestation	248
	Cells of Airways	Cu/Zn, MnSOD protein	Fetus/Neonate	224
		Cu/Zn*, MnSOD* activity and protein	Neonate/Adult	250
	Liver	Cu/Zn, MnSOD*	Fetus/Neonate	193
	Trophoblast (culture)	MnSOD	Differentiatioon	251
	Fibroblast (culture)	Cu/Zn, MnSOD	Fetal/Adult	252, 253

-• determined but no change was observed

its close association with changes in the state of differentiation, normal development can proceed in the absence of SOD-2 expression (267). A long-standing hypothesis has been that developmental increases in total SOD activity are a preparatory change for the more oxygen-rich environment organisms must survive subsequent to their birth (214, 229). In this theory, the development-associated increase in total SOD activity is part of a programmed adaptive mechanism to enhance the survival of postnatal organisms. The increase in total SOD activity would presumably occur independently of the rate of oxidant generation in fetal cells and would be of a sufficient magnitude to compensate for the higher rate of $O_2^{\cdot}$ that is assumed to occur in a neonatal oxygen-rich environment. However, this view does not account for changes that occur early in development, inter-species differences or the fact that in many cases only one intracellular form of SOD changes (Table 2). For example, one human tissue reported to exhibit no perinatal increase in total SOD activity is lung (193, 250). Nevertheless, the fact that normal development can proceed through gestation in SOD-2 knockout mice, while newborns lacking SOD-2 succumb within a few days of birth (267) would seem to support the hypothesis of a preparatory change.

There are several lines of evidence to suggest that, when they occur, early developmental increases in SOD activity (particularly MnSOD) affect the subsequent course of developmental pathways (36). In simple organisms, such as the slime mold *Physarum polycephalum*, differentiation occurs as a diploid encystment. Microplasmodia, which have no cell walls, differentiate into microsclerotia that have cell walls (203, 205). This process is associated with a 46-fold increase in MnSOD activity (205). A non-differentiating strain fails to form microsclerotia under similar conditions and also fails to exhibit any change in SOD activity. The addition of SOD protein to the non-differentiating strain *via* liposomes was observed to stimulate differentiation (201). Liposomally augmented SOD protein also stimulates differentiation in Friend cell leukemia (268) and overexpression of the gene encoding the manganese-containing form of the enzyme stimulates differentiation of human melanoma (269) and C10HT1/2 cells (270). Furthermore, overexpression of the SOD-2 gene in fibrosarcoma has been found to limit metastasis (31).

Regardless of the evolutionary strategy that leads to increased SOD activity in later developmental stages, the effects of experimental SOD augmentation on differentiation are probably not the result of its antioxidant properties. If the increases observed in SOD activity occur without a correspondingly greater change in oxidant generation then the increase would be passive and exert no further effects. Alternatively, if the change in SOD activity exceeds any differentiation-associated increase in oxidant generation, antioxidation should stimulate differentiation. In fact, other antioxidants fail to stimulate differentiation, but some oxidants do (201, 268). Indeed, increased free radical generation and accumulation of oxidation reaction products have repeatedly been observed during the differentiation and development of a wide variety of cells and organisms (205, 243, 271-277). It was our observations in *Physarum* (201, 205) and Friend cell leukemia (268) that led us to postulate that an upsurge of oxidant production rather than increased antioxidant defenses were stimulatory to pathways involved in differentiation (36, 192, 201, 205, 268). Changes in antioxidant defense associated with differentiation may be little more than a response to increasing levels of oxidation. Others have since reached a similar conclusion using a variety of normal and transformed cell models (199, 272-274, 277-280).

If the changes in SOD activity associated with differentiation are responses to increases in cellular oxidant production, why does the addition of SOD to undifferentiated cells stimulate differentiation? This is probably true because, as just noted, it is frequently oxidation that stimulates differentiation and, at least under some conditions, SOD activity increases oxidation. A number of studies have demonstrated that increasing SOD-1 activity elevates H_2O_2 concentration (82, 178, 201, 281-283). Large increases in SOD activity, particularly SOD-1 may actually exacerbate the effects of oxidative stress (82, 281, 283-286). When mixed with H_2O_2, Cu/Zn SOD is inactivated *via* reduction of the Cu^{2+} to Cu^{1+}. This is followed by a Fenton type reaction involving additional H_2O_2 and Cu^{1+} that produces $\cdot OH$ radicals (287). Exposure of Cu/Zn SOD to H_2O_2 gives the appearance of catalyzing a peroxidative reaction primarily because it increases $\cdot OH$ radical formation (288). Cu/Zn SOD has also been reported to catalyze $\cdot OH$ radical formation in homogenates while MnSOD does not (178). Similarly, mixing protective amounts of Cu/Zn SOD and protective amounts of glutathione exacerbates reperfusion injury to renal epithelium while MnSOD and GSH mixtures afford greater protection than either component alone (289).

We previously reported that SOD-2 enzyme activity (252, 253), protein abundance, RNA abundance and rate of transcriptional initiation are all higher in human skin fibroblast cultures derived from adult donors than in those established from fetal skin (253). Further examination of this cell model revealed a corresponding difference in H_2O_2 concentration (Table 3). Only a minor change was observed in SOD-1 and is not presented here.

Table 3. Analysis of H_2O_2 Generation in Fibroblasts from People of Different Ages

	Group	Mean	Total ANOVA[1]	Groups Compared	LSD[2] p-value
SOD-2 Activity	Fetal	1.29		Fetal/Young	0.0029
	Young	8.79	0.000001	Fetal/Old	0.000006
	Old	19.73		Young/Old	0.14
H_2O_2	Fetal	1.09		Fetal/Young	0.00009
	Young	2.29	0.000043	Fetal/Old	0.00003
	Old	2.46		Young/Old	0.69

1. All effects
2. post hoc analysis; LSD=Least Significant Difference

Because the manganese-containing form of SOD is less prone to generate toxic oxidation effects (178, 289), we investigated whether increasing the activity of this form of SOD could actually account for the differences observed in H_2O_2. H_2O_2 concentration was determined in three clones of SV-40-transformed fibroblast clones that overexpress SOD-2 and two control lines transfected with vector only. The average of these results is presented in Figure 1.

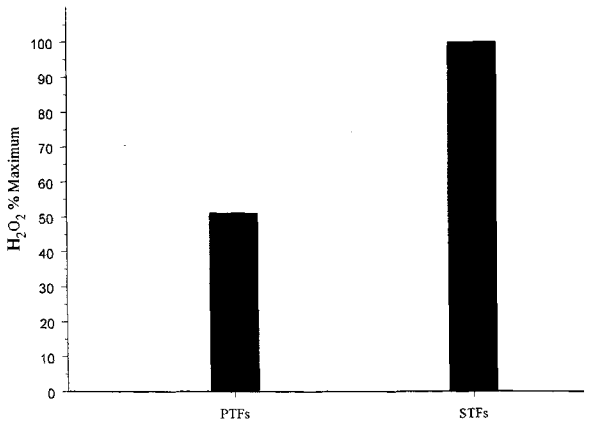

Figure 1. H_2O_2 generation in cell lines transfected with SOD-2. STF = SOD transfected fibroblasts. PTF = plasmid transfected fibroblasts.

These results clearly demonstrate that increases in MnSOD are also capable of elevating H_2O_2 concentration. Although it generates H_2O_2, MnSOD is less likely to cause toxic oxidation effects than Cu/Zn SOD because its metal core is less likely to catalyze formation of ·OH radicals (178, 289). In fact the increases in MnSOD activity associated with differentiation may be of a sufficient magnitude to stimulate oxidation by generating H_2O_2 while at the same time actually limiting ·OH formation by removing $\cdot O_2^-$. Shifts in the redox environment resulting from the production of H_2O_2 by SOD may thus account for effects of the enzyme on differentiation.

OXIDATIVE STRESS AND GENE REGULATION

The effects of oxidative stress described above may be viewed as coarse adjustments in cellular regulatory controls; however, redox effects are not limited to this type of general influence. ROS and antioxidants are now believed to play specific roles in a number of signal transduction pathways. Unlike the very general effects that might be expected with the global changes in redox status associated with differentiation and aging, ROS effects in signal transduction tend to be localized and highly specific. Active oxygen species are reported to activate NF-κB, a multisubunit transcription factor that activates the expression of genes associated with immune responses (290). Conversely, antioxidants and reductants decrease NF-κB activity and translocation (291, 292). It has been demonstrated that oxidants activate NF-κB by causing the release of an inhibitory subunit (IκB) from the NF-κB complex (290). Interestingly, others have observed that strong oxidizing agents such as diamide, or sulfhydryl modifying reagents inhibit the DNA-binding activity of NF-κB (293, 294). These observations suggest a complex role for redox state in which oxidation promotes removal of IκB and translocation, while reduction promotes DNA-binding after IκB removal. This suggests a much higher level of compartmentalization of cellular redox active components than was previously suspected.

A number of other genes and pathways appear to be regulated, at least in part, by variations in cellular redox status. H_2O_2 is a second messenger in the signal transduction pathway from mitochondria to nuclei in *Petunia hybrida* cells stimulated to activate alternate oxidase gene expression (295) and for PDGF in stimulated mammalian cells (296). Indeed, changes in the cellular redox state can modulate the transcriptional activation of the collagen (297) and collagenase (298) genes, the post-transcriptional control of ferritin (299, 300), activation of Myb (301) and Egr-1 (302) proteins as well as the binding activity of the fos/jun (AP-1) protein conjugate (293, 303). A specific protein tyrosine-phosphatase has been isolated from H_2O_2-stimulated human cells (304). A summary of redox effects on different transcription factors as well as elements of signal transduction pathways is presented in Table 4.

It is important to bear in mind that different pathways can interact, which can lead to unexpected effects. For example, many antioxidants stimulate AP-1 DNA binding activity, but *t*-butylhydroquinone decreases AP-1 binding activity by increasing Fra and formation of Fra/Jun heterodimers; these dimers exhibit a lower binding affinity than Fos/Jun heterodimers (305). As discussed above antioxidants prevent NF-κB activation but increase binding activity of the active form. It is always possible that secondary effects of chemical treatments rather than their oxidant/antioxidant properties are responsible for the effects presented in Table 4. However, a number of studies have used oxidants to block the effects of antioxidant compounds (302, 306-308) and *vice versa* (21, 290, 293, 294, 309-321). Furthermore, structural analogs that lack antioxidant properties fail to induce these changes (322). Taken together, these observations suggest that the redox potential of these chemicals rather than other characteristics are, at least partly, if not totally responsible for their effects. While Table 4 presents an overview of effects and some differences that may occur between different cell models, a comprehensive presentation of all redox effects on these factors and their interactions is far beyond the scope of the present discussion. For a more detailed discussion of several of the effects listed in Table 4 the reader is referred to several excellent reviews (323-327).

One of the most striking aspects of the comparisons presented in Table 4 is that the effect of oxidants and reductants on any given pathway can be highly specific to cell type. Nowhere was this more evident than in the elegant studies of Collart *et al.* (390) who showed that the effects of H_2O_2 and ionizing radiation on induction of

Table 4. Redox-Sensitive Genes and Regulatory Factors

Gene or Protein	Organism or Cell Type	Stimulus	Effect	Reference
Protein Tyrosine Phosphatase	Rat Hepatoma (Fao)	H_2O_2	Inhibition	321
		H_2O_2 + Vanadate	Inhibition	321
	Human Fibroblasts (EK4)	H_2O_2	Increased mRNA	304
Ltk	Transfected COS Cells	Diamide	Activated Protein	328
$p56^{lck}$	Human T cells	Diamide or H_2O_2	Stimulated Activity	329
Tyrosine Kinase				
Syk	Mast Cells (RBL-2H3)	NAC[1]	Inhibited activation	330
	Chicken B Cells	H_2O_2	Activated	331
Lyn	Mast Cells (RBL-2H3)	NAC	No Effect	330
ZAP-70	T Lymphocytes	UV-radiation, H_2O_2	Activated	332
Hypoxia-Inducible Factor-1	Hep3B	Diamide, NEM, or H_2O_2	Impaired DNA Binding	333
		Dithiothreitol or H_2O_2	Inhibited Hypoxia Signaling	333
EGF Receptor	Vascular Smooth Muscle	H_2O_2	Tyrosine Phosphorylation	334
		H_2O_2	SHC-Grb2-SOS Complex	334
	HeLa, Rat-1/HER	UV-radiation, H_2O_2	Tyrosine Phosphorylation	335
Catalase	Human RPE[4]	H_2O_2	Increased Activity/mRNA	320
Metallothionein	Human RPE[4]	H_2O_2	Increased mRNA/Protein	320
	HeLa tk^-	UV-radiation	Increased mRNA	336
Metal-Responsive Transcription Factor-1	Hepa Cells	*t*-Butyl-OOH, H_2O_2	Increased DNA Binding	337
Thyroid Transcription Factor I	HeLa Cells	GSSG, Diamide	Decreased DNA Binding	338
Protein Kinase C	Human Jurkat T Cells	H_2O_2	Increased Activity	339
SOK-1 (Ste-Like Kinase)	COS-7 Cells	H_2O_2	Activated	340
Erg-1	Mouse MC3T3-E1	H_2O_2	Stimulated Transcription	341, 342
Erg-1 protein	Mouse MC3T3-E1	H_2O_2	Acumulation/Activation	343
	Baculovirus Expression Model	DDT	Increased DNA Binding	302
Leukocyte Adhesion Molecules				
CD11b	Human PMN	Activated PMNs	Increased Protein	344
CD18	Human PMN	Activated PMNs	Increased Protein	344
L-Selectin	Human PMN	Activated PMNs	Decreased Protein	344
Sp-1	Rat liver	Aging or H_2O_2	Decreased DNA Binding	325
	K562	H_2O_2, NEM, GSSG	Decreased DNA Binding	345
Adapt15/gadd7	Hamster HA-1 Cells	H_2O_2	Increased mRNA	346
Adapt33	Hamster HA-1 Cells	H_2O_2	Increased mRNA	347
Adapt78	Hamster HA-1 Cells	H_2O_2	Increased mRNA	348
***MafG* Homolog (*Adapt 66*)**	Hamster HA-1 Cells	H_2O_2	Increased mRNA	349
A170 Stress Protein	Mouse Peritoneal Macrophages	H_2O_2	Increased Protein	350
JE Gene	Mouse MC3T3-E1	Catalase Overexpression	Increased mRNA	351
		H_2O_2	Increased mRNA	342
Collagenase (MMP-1)	Human Foreskin Fibroblasts	Paraquat, H_2O_2	Increased mRNA	298
Collagenase	HeLa tk^-	UV-radiation	Increased Transcription	336
Heme Oxygenase I	Human Fibroblasts (FEK_4)	Ultraviolet A, H_2O_2	Increased mRNA/Protein	352-354
	Mouse M1 Myeloleukemia	H_2O_2	Increased mRNA	312, 313
Ferratin	Human Fibroblasts (FEK_4)	Ultraviolet A, H_2O_2	Increased Protein	353
Heparin-Binding EGF-like Growth Factor	Rat Gastric Epithelial Cells	H_2O_2	Increased mRNA	319
Amphiregulin	Rat Gastric Epithelial Cells	H_2O_2	Increased mRNA	319
NF-AT	Jurkat T Cells	H_2O_2	Decreased Transcriptional Activation by NF-AT	355
C/EBP β	Rat Embryo Fibroblasts	Anoxia	Increased Transcription, Protein	356
ATF-4	Rat Embryo Fibroblasts	Anoxia	Increased Transcription, Protein	356
IL-2	Jurkat T Cells	H_2O_2	Decreased mRNA	355
IL-8	HepG2	H_2O_2	Stimulated Production	309
	Pulmonary epithelium (A549)	H_2O_2	Stimulated Production	309
	Human Skin Fibroblasts	H_2O_2	Stimulated Production	309
Cytosolic Phospholipase A	Rat Asterocytes	H_2O_2	Stimulates Phosphorylation	357
Thymidine Incorporation	Rat Asterocytes	H_2O_2	Inhibited	357
JE/MCP-1 and CSF-1	Mouse MC3T3-E1	XanthineOxidase + Hypoxanthine	Increased mRNA	358

Vascular Endothelial Growth Factor (VEGF)	Human RPE, Melanoma, Rat Glioblastoma	XanthineOxidase + Hypoxanthine or H_2O_2	Increased mRNA stability	359
P4501A1 (CYP1A1)	Rat Hepatocytes	H_2O_2	Decreased mRNA	318
P4501A2 (CYP1A2)	Rat Hepatocytes	H_2O_2	Decreased mRNA	318
Basic Fibroblast Growth Factor (bFGF)	Human Smooth Muscle	H_2O_2	Increased Receptor Binding Affinity for bFGF	360
ADF/Trx (Thioredoxin)	Human Jurkat Cells	H_2O_2, Menidione, Diamide	Increased Transcription	361
	HeLa Cells	H_2O_2	Increased mRNA/Protein	362
Gadd45	Human Cells	Ionizing Radiation	Increased mRNA	363
Gadd153	CHO cells	H_2O_2, UV-radiation	Increased mRNA	364
HoxB5 (Hox-2.1)	Human Cells	DTT	Inhibited DNA Binding	308
HIV-1	HeLa tk$^-$	UV-radiation	Increased Transcription	336
USF	HeLa	DTT	Increased DNA Binding	365
NF-κB	Lymphocytes (ACH-2, U1)	Proflavin + light	Activated	366
	Human T-lymphocytes (J.Jhan) And Monocytes (U937)	BHA, NGA, Tocopherol	Blocked PMA Activation	292
			Blocked TNF Activation	292
	Human Jurkat T Cells	H_2O_2	Activated	290
		NAC	Prevented H_2O_2 Activation	290
	Human Neuroblastoma	H_2O_2	Activated	367
	Human Lung Adenocarinoma	NAC, DTT, 2-ME	Activated	315
	Human Astrocytoma	Buthionine Sulfoximine	Enhanced LPS Activation	368
		NAC	Blocked LPS Activation	368
	HeLa	Thioredoxin Overexpression	Decreased DNA Binding	369
		PDTC	Decreased DNA Binding	291, 369, 370
		NAC	Decreased DNA Binding	291, 370
	HeLa (S3)	UV-radiation	Activated	371
	HeLa tk$^-$	UV-radiation	Increased DNA Binding	336
	Jurkat T Cells	NEM, Diamide	Blocked DNA Binding	293
	PC12	H_2O_2	Increased DNA Binding	372
	Human Astrocytoma	Buthionine Sulfoximine	Enhanced LPS Activation	368
	Rat Hepatocytes	Antimycin A	Activated	373
	JB-6	SOD-Overexpression	Enhanced Activation by TNF	374
		Catalase-Overexpression	Blocked Activation by TNF	374
		Aminotriazole	Removed Catalase Block	374
	COS-1	Catalase-Overexpression	No Effect	375
	Mouse M1 Myeloleukemia	H_2O_2	Increased DNA Binding	312, 313
Activation by Tax	J6 Subclone Jurkat T	PDTC	Inhibited	376
κB-Binding Proteins	Human T Cells	Diamide	Blocked DNA binding	294
Myb Protein	Purified protein	Diamide	Blocked DNA binding	301
Hsp70	Traumatically Injured Mouse Brain, After Focal Ischemia, or Kainic Acid-Induced Seizure	Transgenic Animals Overexpressing SOD-1	Altered profile of mRNA induction	377-379
c-Ha-*ras*	Rabbit Articular Chondrocytes	H_2O_2	Decreased mRNA	380
c-myc	Rat Vasular Smooth Muscle	H_2O_2	Increased mRNA	381
	Rat Proximal Tubule Epithelim	Xanthine/Xanthine Oxidase	Increased mRNA	311
	Rabbit Articular Chondrocytes	H_2O_2	Decreased mRNA	380
	Mouse Epidermal Cells (JB6)	Xanthine/Xanthine Oxidase	Increased mRNA	382
c-fos	Mouse MC3T3-E1	H_2O_2	Increased Transcription	342
	NIH3T3	UV-radiation	Increased mRNA	383
	Mouse M1 Myloleukemia	H_2O_2	Increased Transcription	312, 313
	Mouse Epidermal Cells (JB6)	Xanthine/Xanthine Oxidase	Increased mRNA	382
	Traumatically Injured Mouse Brain, After Focal Ischemia, or Kainic Acid-Induced Seizure	Transgenic Animals Overexpressing SOD-1	Altered profile of mRNA induction	55, 378, 379
	HeLa	UV-radiation, H_2O_2	Increased mRNA	384
		PDTC	Increased Transcription	370
		NAC, BHA, PDTC, H_2O_2	Increased mRNA	385
	?	H_2O_2	Increased mRNA	317
	HeLa tk$^-$	UV-radiation	Increased Transcription	336
	Rat Vascular Smooth Muscle	H_2O_2	Increased mRNA	381, 386
		H_2O_2, Arachidonic Acid	Increased mRNA	386
		NGA	Blocked H_2O_2, Arachidonic Acid	386
	Rat Proximal Tubule Epithelim	Xanthine/Xanthine Oxidase	Increased mRNA	311
		SOD	Blocked X/XO Effect	311
	Rat Lens	H_2O_2	Increased mRNA	387, 388
		NAC	Increased RNA	388
			Blocked H_2O_2	388
	HepG2	Phenolic Antioxidants	Increased mRNA	322
	L929	Thioredoxin, PDTC	Increased mRNA	369
	HL-525 (PKC-deficient HL60)	Ionizing Radiation	Increased mRNA	310
Fos Protein	Rat Vasular Smooth Muscle	H_2O_2, Arachidonic Acid	Increased Protein	314

		NGA	Blocked H_2O_2, Arachidonic Acid	314
fos-B	HL-525 (PKC-deficient HL60)	Ionizing Radiation	Increased mRNA	310
fra-1	HeLa	*t*-Butylhydroquinone	Increased mRNA	305
fra-2	HeLa	*t*-Butylhydroquinone	Increased mRNA	305
Fra	HeLa	*t*-Butylhydroquinone	Increased Protein	305
c-jun	Mouse MC3T3-E1	H_2O_2	Increased Transcription	342
	Mouse 3T3 Cells	H_2O_2	Increased mRNA	389
	Mouse M1 Myloleukemia	H_2O_2	Increased Transcription	312, 313
	Mouse Brain After Kainic Acid-Induced Seizure	Transgenic Animals Overexpressing SOD-1	Altered profile of mRNA Induction	379
	HeLa	UV-radiation, H_2O_2	Increased mRNA	384
	HeLa	*t*-Butylhydroquinone	Increased mRNA	305
	?	H_2O_2	Increased mRNA	317
	Human Leukemias			
	Myeloid (ML-2)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	Promyelocytic (HL-205)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	T-Lymphoblast (CEM)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	T-Lymphoblast (MOLT-4)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	B-Lymphblastoid (CCL-155)	Ionizing Radiation	Increased mRNA	390
	B-Lymphblastoid (Raji)	Ionizing Radiation	Increased mRNA	390
	Breast Carcinoma (MCF-7)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	Breast Carcinoma (MB231)	H_2O_2 or Ionizing Radiation	Increased mRNA	390
	Melanoma (HO)	H_2O_2* or Ionizing Radiation*	No Effect	390
	Melanoma (SK-MEL)	H_2O_2* or Ionizing Radiation*	No Effect	390
	Fibrosarcoma (Hs913t)	Ionizing Radiation	Increased mRNA	390
	Fibrosarcoma (HT1080)	Ionizing Radiation	Increased mRNA	390
	Human Fibroblasts (DET-551)	H_2O_2* or Ionizing Radiation	Radiation Increased mRNA	390
	Human Fibroblasts (IMR-90)	H_2O_2* or Ionizing Radiation	Radiation Increased mRNA	390
	Human Fibroblasts (WI-38)	H_2O_2* or Ionizing Radiation	Radiation Increased mRNA	390
	Prostate Carcinoma (LNCaP)	H_2O_2 or Ionizing Radiation	Increased mRNA (slight)	390
	Prostate Carcinoma (DU145)	H_2O_2 or Ionizing Radiation	Increased mRNA (slight)	390
	Teratocarcinoma (P3)	H_2O_2* or Ionizing Radiation	Radiation Increased mRNA	390
	Colon Carcinoma (HT-29)	Ionizing Radiation	Increased mRNA	390
	Jurkat T Cells	H_2O_2	Increased mRNA	355
	Rat Proximal Tubule Epithelim	Xanthine/Xanthine Oxidase	Increased mRNA	311
	Rat Vasular Smooth Muscle	H_2O_2	Increased mRNA	391
	Rat Lens	H_2O_2	Increased mRNA	388
		NAC	Increased RNA	388
			Blocked H_2O_2	388
	U937 Cells	Ionizing Radiation	Increased mRNA	392
	HepG2	Phenolic Antioxidants	Increased mRNA	322
	L929	Thioredoxin, PDTC	Increased mRNA	369
	HL-525 (PKC-deficient HL60)	Ionizing Radiation	Increased Transcription	310
Jun Protein	Rat Vasular Smooth Muscle	H_2O_2, Arachidonic Acid	Increased Protein	314
		NGA	Blocked H_2O_2, Arachidonic Acid Effects	314
jun-B	HL-525 (PKC-deficient HL60)	Ionizing Radiation	Increased mRNA	310
	HeLa	*t*-Butylhydroquinone	Increased mRNA	305
jun-D	L929	Ionizing Radiation	Increased mRNA	310
AP-1	Human Lung Adenocarinoma	H_2O_2	Activated	315
	Human Astrocytoma	Buthionine Sulfoximine	Enhanced LPS Activation	368
		NAC	Blocked LPS Activation	368
	Human Neuroblastoma	H_2O_2	Activated	367
	HeLa	Ref-1 (redox protein)	Increased DNA Binding	393, 394
		Thioredoxin Overexpression	Increased DNA Binding	369
		PDTC	Increased DNA Binding	369, 370
		NAC	Increased DNA Binding	370
		UV-radiation, H_2O_2	Increased DNA Binding	336, 384
		H_2O_2	Decreased DNA Binding	322
	HeLa	*t*-Butylhydroquinone	Decreased DNA Binding	305
	PC12	H_2O_2	Increased DNA Binding	372
	HepG2	Phenolic Antioxidants	Increased DNA Binding	322
	3T3-A4	UV-radiation	Activated	307
	3T3-A4	H_2O_2	Activated	307
	Cell Free System	NEM, Diamide	Decreased DNA Binding	303
	Liver Extracts	Thioredoxin	Increased DNA Binding	303
	Bacterially Expressed	DTT	Increased DNA Binding	306
MAP Kinase	HeLa Cells	NAC, BHA, PDTC, H_2O_2	Phosphorylation of Elk-1	385
		H_2O_2	Activated ERK-2	317
	Human Mesanglial Cells	IL-1β	Activated ERK-2	395
		NAC or Dithiothreitol	Blocked IL-1β	395
		H_2O_2 or Diamide	Activated MEK, ERK-2	395
	Rat1	H_2O_2	Activated ERK-2	317
	A431	NAC	Inhibited UV, H_2O_2 Effects on ERK-1 and 2	335
	NIH3T3	H_2O_2	Activated ERK-2	316, 317
	NIH3T3	Ionizing Radiation	Activated ERK-1, ERK-2	316
	Rat Vascular Smooth Muscle	Arachidonic Acid	Activated	396

		NGA	Blocked Arachidonic Acid	396
	PC12	H_2O_2	Activated ERK-2	317
	Rat Astrocytes	H_2O_2	Activated ERK	357
BMK-1 (ERK-5)	Rat and Human Vascular Smooth Muscle, and Human Umbilical Vein, Fibroblasts	H_2O_2	Activated	397
JNK1/SAPK	PC12	H_2O_2	Activated	317
	Rat Asterocytes	H_2O_2	Activated	357
	Rabbit Kidney Epithelium	UV-radiation	Activated	398
		H_2O_2	Activated	398
		Arachidonic Acid	Activated	398
		NAC	Blocked Arachiodonic Acid	398
	Chicken B Cells	H_2O_2	Activated	331
	Bovine Chondrocytes	H_2O_2 or Nitric Oxide	Activated	399
	Human Fibroblasts	UV-radiation or H_2O_2	Activated	400
	Human Mesanglial Cells	IL-1β	Activated ERK-2	395
		NAC or Dithiothreitol	Blocked IL-1β	395
	3T3-A4	UV-radiation	Activated	307
		NAC	Blocked UV-radiation	307
		H_2O_2	Blocked NAC Inhibition of UV	307

'No effect

c-jun in various cell types ranged from dramatic increases to no effect at all. Similarly, H_2O_2 is a second messenger leading to NF-κB induction in JB-6 cells. Overexpression of the catalase gene blocks induction of NF-κB in these cells following stimulation by TNF (374). Interestingly, catalase overexpression failed to block TNF stimulation of NF-κB in COS-1 cells (375). There is strong evidence that the lipoxygenase pathway may be involved in the induction of *c-fos* in some cells because induction is blocked by nordihydroguaiaretic acid (NGA), a known lipoxygenase inhibitor (292, 314, 386, 396). Yet, our studies with NGA reveal that it strongly induces *c-fos* transcription in human fibroblasts (see discussion below). Thus while Table 4 provides a summary of several known effects of redox active treatments, it must not be assumed that all treatments will produce identical effects in all cells.

The *c-fos* gene is one of the best-studied early response genes. It is induced by the activation of numerous protein kinase dependent pathways including cAMP dependent protein kinase, diacylglycerol/calcium dependent protein kinases (PKC) and the PDGF receptor (383, 401, 402). *c-fos* is also stimulated by environmental stresses such as UV-radiation and as a response to cytokines (336, 384, 402). Because many of the pathways leading to the induction of *c-fos* are known, an analysis of its induction by reductants and oxidants has been useful in elucidating the mechanisms by which perturbation of redox state alters gene expression (see Figure 2). Several extensive analyses reveal that the effect of H_2O_2 on *c-fos* and other early response genes such as *c-jun* and *c-myc* is mediated by protein kinase C (310, 342, 381, 384, 386, 391, 403). Interestingly, Raf-1 is also phosphorylated by PKC (404), while the oxidative activation of NF-κB is independent of PKC activity (291). Choi and Moore (322) demonstrated that an isolated *c-fos* SRE site responds to treatment with the phenolic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Phosphorylation of both $p67^{srf}$ (405) and $p62^{tcf}$ (406, 407) and their subsequent binding to the promoter element is required for activation of the SRE. Since phosphorylation of the $p62^{tcf}$ (Elk-1) is mediated by MAP-kinase (408), these observations clearly implicated MAP kinases in mediating the effects of reducing agents on *c-fos* transcription. Several studies have confirmed this hypothesis (see Table 4).

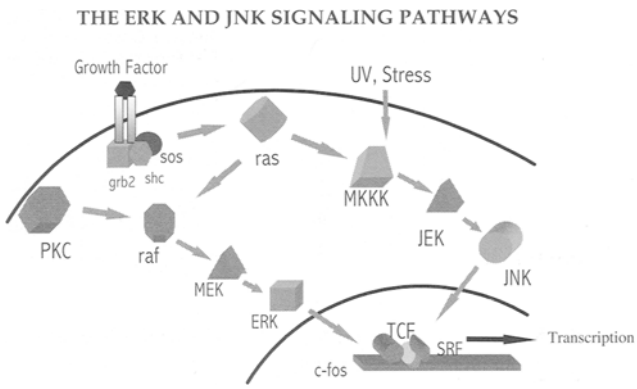

Figure 2. ERK and JNK signal transduction pathways leading to *c-fos* induction. Shc = src homology containing protein; Grp2 = Growth factor receptor-bound protein 2; ERK = extracellular signal-regulated kinase; JNK = Jun-NH_2-terminal kinase; MAP = mitogen regulated kinase; MEK = MAP kinase kinase; JEK = JNK kinase; MKKK = MAP Kinase Kinase Kinase; TCF = Ternary Complex Factor; SRF = Serum Response Factor.

The effects of the redox environment in the regulation of DNA binding activities appear, in many cases, to be mediated through cysteine residues in proteins. Conserved cysteine residues have also been implicated in the regulation AP-1 binding activity (326, 409) phosphofructokinase (410), 3-hydroxy-3-methylglutaryl-coA reductase (411) and tyrosine protein phosphatases (321).

Our recent studies have shown that chemical antioxidants can also induce transcription factors over different time courses, presumably through different pathways. We have examined the effects of two antioxidants on the induction of *c-fos* in young and senescent human fetal lung fibroblasts (WI-38). *N*-acetylcysteine (NAC) induces *c-fos* transcription in both proliferatively young

and senescent cells, while nordihydroguaiaretic acid (NGA) induces *c-fos* transcription in young cells but fails to stimulate it in senescent cells (412). We later found that the tocopherol derivative Trolox C can also stimulate *c-fos* in senescent fibroblasts. Down regulation of protein kinase C (PKC) by 24 hour pretreatment with 500 nM phorbol 12-myristate 13-acetate (PMA) prevents induction by subsequent stimulation with either PMA or NGA. This is consistent with the hypothesis that NGA induces *c-fos* transcription *via* a PKC-dependent mechanism. NAC induction of *c-fos* is unaffected by PMA pretreatment, while Trolox C super-induced *c-fos* following PMA pretreatment. However, none of these compounds stimulated translocation of PKC-α from the cytosol to the membrane in proliferatively young cells. We also observed that the magnitude of stimulation of the activities of MAP Kinases $p44^{mapk}$(ERK1) and $p42^{mapk}$ (ERK2) with serum or NGA decreases as a function of proliferative age. This decrease indicates that the response of senescent cells to signaling events that utilize the ras/MEK/MAP kinase-signaling pathway is impaired. We interpret these results to indicate that increasing the intracellular reducing potential stimulates *c-fos* expression through multiple pathways and that some, but not all, of these pathways are impaired in senescent cells (Tresini, M, Allen, R.G., and Cristofalo, V.J. unpublished).

SUMMARY

This review was intended to provide a brief overview of advances in free radical biology in recent years. The studies discussed here illustrate the potential for redox balance to influence or cause some aspects of aging and degenerative diseases, as well as development and differentiation. The effects of oxidants/antioxidants on cells may be partly due to damage or may arise from in their normal role as subcellular second messengers. Of particular importance in understanding the role of oxidants in cellular processes is the enzyme superoxide dismutase. The discovery of its activity not only proved that free radicals were generated in cellular metabolic pathways, but also made possible much of the subsequent research in this area by providing, for the first time, an antioxidant specific to a free radical.

ABBREVIATIONS

NAC = *N*-Acetylcysteine
NEM = *N*-Ethylmaleimide
DTT = Dithiothreitol
RPE = Retinal Pigment Epithelial Cells
2-ME = 2-Mercaptoethanol
BHA = Butylated Hydroxyanisole
NGA = Nordihydroguiaretic Acid
PMA = Phorbol 12-Myristate 13-Acetate
PDTC = Pyrrolodine Dithiocarbamate
AP-1 = Activator Protein 1
GSSG = Oxidized Glutathione
LPS = Lipopolysacchride

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