NEW DIRECTIONS FOR STUDYING THE ROLE OF FREE RADICALS IN AGING

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ABSTRACT

Oxidative damage caused by free radicals *in vivo* is believed to play an important role in the etiology of aging and age-associated degenerative diseases. The most direct evidence supporting this theory is the recent finding that the transgenic *Drosophila* that overexpress the antioxidant enzymes catalase and superoxide dismutase exhibit an increase in life span. Although the increase in life span in *Drosophila* by these enzymes is certainly important, the next logical direction is to demonstrate whether increased antioxidant protection occurs similarly in mammals. Several transgenic mouse models that overexpress antioxidant enzymes are currently available. However, one major shortcoming in using these transgenic mice is the difficulty of producing antioxidant overexpression in more than a few tissues. Despite the potential shortcomings of using transgenic mice, these animals provide a unique system in which individual components of a complex system, such as the antioxidant defense system, can be modulated and examined independently. Transgenic mice are therefore potentially powerful tools to study the role of various components of the antioxidant system in the aging process.

A parallel direction in the study of free radical roles in aging is to investigate the modulation of transcription factors by oxidative stress. Among these, the transcription factors, NF- κ B and AP-1 are implicated in oxidative stress. The activities of these oxidative stress-response transcription factors are regulated by upstream signaling molecules, which involve a cascade of phosphorylation and dephosphorylation events leading to their activation. In this article, we review recent studies that use molecular approaches to investigate the biological role of oxidant stress. Each of these studies potentially provide new insights into the roles of free radicals and free radical damage in the aging process.

KEY WORDS

Oxidative stress, Transcription factors, Antioxidant defense, Transgenic mice, Overexpression and deletion of genes, Signal transduction

INTRODUCTION

Oxidative damage caused by free radicals *in vivo* is believed to play an important role in the etiology of aging (1-4). Because damage from free radicals can lead to deleterious effects on the body, several defense systems have evolved to provide protection against free radical damage. Antioxidant enzymes are capable of converting free radicals into stable compounds before they inflict major damage to cellular macromolecules. These antioxidant enzymes include Cu/Zn-superoxide dismutase (Cu/Zn-SOD), Mn-superoxide dismutase (Mn-SOD) (5), catalase (CAT) (6), and glutathione peroxidase (GPX) (7).

The antioxidant system is a complex, multicomponent system, making it difficult to directly test the role of oxidative stress in the aging process. One of the current approaches in free radical research is to use transgenic animal models that overexpress one or more antioxidant enzymes. The advent of DNA technology has made it possible to genetically engineer cells or animals that overexpress specific gene(s). The expression of a transgene can be targeted by using tissue-specific regulatory regions in the promoter region of the transgene, allowing the production of a stable cell/organism that can be genetically characterized and studied over a long period. One key advantage of these approaches is that they allow investigators to independently modulate individual components of a complex system, such as the antioxidant defense system. Thus, transgenic animals and transfected cells potentially provide excellent models for studying the role of oxidative stress in aging and disease processes.

Oxidative stress affects a variety of the biochemical processes involved in the regulation of the transcription of stress/defense-response genes. The transcription of a gene is influenced by various regulatory elements, referred to as promoters, enhancers, and silencers. These elements are composed of discrete DNA sequence motifs that constitute binding sites for sequence-specific DNA-binding proteins. The stress response genes are regulated by a small number of regulatory proteins (transcription factors). Of the various transcrip-

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tion factors involved in regulation of stress genes, transcription factors NF- κ B (nuclear factor of kappa-B) and AP-1 (activated protein-1) are shown to play a major role in oxidative stress, and are therefore referred to as oxidative stress-response transcription factors. Activation of these transcription factors can elicit a cascade of stress response gene activities to protect the organism against stress conditions. This is relevant to aging because the age-associated changes in these transcription factors may have a great impact on the stress genes that protect the organism against damaging intrinsic factors and environmental insults. Thus, one newly developing research direction for the study of free radicals in the aging process is to investigate the modulation of transcription factors by oxidative stress.

A number of review articles have been written over the past several years describing the role of pro- and antioxidants in aging (see ref. 1-4 for review). The focus of the present article is to describe new approaches to antioxidant studies, such as the use of transgenic models, and to present new information on oxidative stress-mediated activation of the transcription factors involved in the induction of the gene(s) that protect the organism against oxidative damage.

New Approaches to Study the Antioxidant System

Damage from free radicals produced as a result of normal metabolism or during oxidative stress are postulated to be involved in a variety of diseases and in the decline of the physiologic function that occurs during the aging process. Because of the importance of antioxidant enzymes in cellular protection against the damaging effects of free radicals, it is critical that the role of individual antioxidant enzymes in the antioxidant defense system be elucidated. Studying the antioxidant defense system is complicated by its complexity and redundancy. However, recent advances in technology have led to the production of transfected cells or transgenic animals that overexpress one or more of the antioxidant enzymes. These provide investigators with an excellent system for studying the role of individual enzymes in cellular defense against reactive oxygen species.

Cu/Zn-Superoxide Dismutase (Cu/Zn-SOD) and Catalase (CAT) Transfection Studies

The first study to transfect gene coding of a component of the antioxidant system into cells was conducted in 1986. In this study, stable transformants of mouse L-cells and human HeLa cells were produced using the human gene for Cu/Zn-SOD (8). These transfected cells expressed as much as 6-fold more Cu/Zn-SOD activity than the non-transfected cells, and were observed to be more resistant to cytotoxicity from exposure to paraquat (an intracellular generator of superoxide radicals). Protection from paraquat-induced toxicity has also been shown in several other transfected cell

lines in which the expression of Cu/Zn-SOD was increased from 1.6- to 7-fold (9-11). Recently, transfection of human Cu/Zn-SOD into bovine and porcine aortic endothelial cells was shown to lead to increased cell viability following cold-preservation-rewarming injury, a condition believed to be related to oxygen free radicals (12). Cu/Zn-SOD overexpression therefore has been shown to provide protection against several forms of oxidative stress.

However, Cu/Zn-SOD overexpression does not result in exclusively beneficial effects. For example, rat PC-12 cells transfected with Cu/Zn-SOD have been shown to have an impaired neurotransmitter uptake (13). Also, the transfection of Cu/Zn-SOD into bovine adrenocortical cells has been shown to have cytotoxic effects, including increased cell death, cell fusion, and nuclear fragmentation (14). Furthermore, although HeLa cells transfected with human Cu/Zn-SOD were found to have an increased resistance to paraquat, these cells also had higher levels of lipid peroxidation both in the presence and the absence of paraquat (8). The authors suggest that the increase in the Cu/Zn-SOD expression altered the balance between the rate of hydrogen peroxide (H_2O_2) formation by Cu/Zn-SOD and its removal by CAT and GPX, resulting in increased H_2O_2 production and increased lipid peroxidation. Further support for this suggestion is shown in mouse JB6 cells transfected with the human Cu/Zn-SOD gene (15). These cells had a 2- to 3-fold increase in Cu/Zn-SOD activity and were actually more sensitive to an oxidative burst produced by xanthine/xanthine oxidase, had decreased cell growth, and increased DNA damage compared to non-transfected cells. However, when the cells were transfected with both CAT and Cu/Zn-SOD and the activity of both enzymes was increased 2- to 3-fold, the cells no longer demonstrated an increased sensitivity to the oxidative stress seen in the cells transfected with Cu/Zn-SOD alone. Thus, the increase in CAT activity appears to protect the cells from the adverse effects of transfecting with Cu/Zn-SOD alone. These studies illustrate the complex, integrated nature of the antioxidant system, where components balance to work in concert.

Although an increase in Cu/Zn-SOD activity, without proportional increases in CAT and/or GPX activity, appears to result in an increase in the amount of damage to the cell, some evidence supports a protective role for the overexpression of CAT alone. Endothelial cells infected with a replication-deficient, recombinant adenovirus containing CAT cDNA had an increase in CAT activity and were resistant to high intracellular levels of H_2O_2 (16). Also, transfection of acatalasemic mouse fibroblasts containing low levels of endogenous catalase with a human catalase cDNA clone resulted in a 2.8- to 4-fold increase in CAT activity and a significant resistance to oxidative stress generated by H_2O_2 and hyperoxia (17).

Manganese Superoxide Dismutase (Mn-SOD) Transfection Studies

Mn-SOD is a particularly important component of the antioxidant system due to its presence in the mitochondria (the major producer of reactive oxygen molecules and, consequently, an important target of free radical damage). Overexpression of Mn-SOD in transfected cells has been shown to have a protective effect against a variety of insults related to oxidative stress. For example, it has been shown to increase cell survival in response to the cytotoxic effects of the cytokines interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) and other cytotoxic drugs proposed to generate superoxide anions or other reactive oxygen species (18,19). Several cell lines overexpressing Mn-SOD have also been shown to have increased resistance to irradiation (19,20). Finally, increased expression of Mn-SOD has been shown to make cells more resistant to the effects of paraquat (21,22) and to hyperoxic challenge (23).

Studies of transfected cells have also provided evidence that Mn-SOD may act as a tumor suppressor gene. For example, increased expression of Mn-SOD has been shown to suppress radiation-induced neoplastic transformation (24) and enhance cell differentiation in mouse C3H10T1/2 cells (25). In human melanoma cells, its transfection was accompanied by a decrease in the expression of melanoma-associated and proliferating-cell nuclear antigens (26). These transfected cells lost the ability to develop tumors when introduced into athymic mice, while the non-transfected control cells developed tumors within 5 to 7 days. Recently, transfection of Mn-SOD into a breast cancer cell line (MCF-7) was shown to inhibit tumor growth when the cells were injected into nude mice, compared to the control, wild-type MCF-7 cells (27).

Glutathione Peroxidase (GPX) Transfection Studies

Overexpression of GPX has also been shown to have a protective effect against oxidative stress. T47D human breast cells exhibiting 10- to 100-fold increases in GPX activity were more resistant to the growth inhibition and DNA damage produced by incubating cells with hydrogen peroxide, cumene hydroperoxide, or menadione (28). Stable transfectants of mouse NIH/3T3 fibroblasts and human MCF-7 breast carcinoma cells with a human cytosolic GPX expression vector showed more resistance to both paraquat and peroxides (29).

Overexpression of Antioxidant Enzymes in Transgenic Drosophila

To determine whether the beneficial effects of increased levels of antioxidant enzymes could be extended to have a positive effect on life span, transgenic *Drosophila* that overexpress the antioxidant enzymes Cu/Zn-SOD and CAT were produced (30-32). Although the overexpression of either Cu/Zn-SOD (30 to 60%) or

CAT (70 to 80%), individually, did not affect the life span of these transgenic flies (30-32), they were more resistant to H_2O_2 .

The most convincing evidence for the involvement of free radicals in aging comes from a recent study that demonstrates life span increases (14 to 34%) in transgenic *Drosophila* that simultaneously overexpress both CAT and Cu/Zn-SOD (33). These transgenic lines show a 25 to 37% increase in Cu/Zn-SOD activity and a 45 to 70% increase in CAT activity. In addition, these transgenic flies have lower levels of protein oxidation, which strongly suggests that reduced free radical/oxidative damage is responsible for the increased survival of transgenic *Drosophila*. These data are consistent with the concept that SOD and CAT work as a team to protect against damage from reactive oxygen species.

Overexpression of Antioxidant Enzymes in Transgenic Mice

The study of *Drosophila* that overexpress both Cu/Zn-SOD and CAT provides the first direct evidence showing that free radicals and oxidative damage may be important factors in aging. However, no similar study examines the effect of increased antioxidant enzyme activity on the life span in mammals. In 1987, the first transgenic mouse to overexpress an antioxidant enzyme was produced by the laboratories of Epstein and Groner (34). These laboratories produced transgenic mice that overexpressed the human Cu/Zn-SOD gene, and characterized its effects using several biochemical and physiological parameters. The overexpression of Cu/Zn-SOD in these mice is greatest in the brain, where the activity of Cu/Zn-SOD is as much as 6-fold higher than in non-transgenic controls (34). Epstein's laboratory measured the activity of Cu/Zn-SOD in various regions of the brain of the transgenic mice (35), finding the overexpression of Cu/Zn-SOD highest in the cerebellum (3.4-fold) and lowest in the brain stem (2-fold). In addition to the increase in Cu/Zn-SOD activity, they also found a 1.7- to 2.3-fold increase in the activity of Mn-SOD in various brain regions of these animals. The activity of CAT was also higher (1.3- to 2.3-fold) in the brain regions of these transgenic mice. In contrast, the activity of GPX was not significantly altered in the brain regions of the transgenic mice. Epstein proposed that the increase in CAT activity is an adaptive response compensating for the increase in H_2O_2 produced by the increase in SOD. However, in lung tissue from transgenic mice that overexpress Cu/Zn-SOD, there was very little change in the activity of Mn-SOD, GPX, CAT, or glutathione reductase, even though the Cu/Zn-SOD activity was increased 2- to 3-fold (36).

Cu/Zn-SOD overexpression in transgenic mice affords several protective effects, including an increased resistance to pulmonary oxygen toxicity, decreased histopathologic lung damage, such as pulmonary edema, formation of hyaline membranes, and inflammation (36). In the brain, the increased expression of Cu/Zn-SOD

activity confers protection against ischemic injury (37), cold-induced injury (38), and neurotoxicity of glutamate (39) and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug that causes damage to the nigrostriatal pathway similar to the damage seen in Parkinson's disease (40). However, a number of defects have also been observed in the transgenic mice that overexpress Cu/Zn-SOD. For example, pathological changes have been reported in neuromuscular junctions in tongue and hindlimb (41,42), a decrease in serotonin uptake has been found in platelets (43), and a decrease in prostaglandin synthesis in the kidney and cerebellum of transgenic mice has been measured (44). In addition, these mice have been reported to undergo premature thymic involution starting at 3-4 months of age, compared to age 10-12 months in control mice (45).

Recently, a second transgenic mouse overexpressing Cu/Zn-SOD has been produced using the HMGCofA reductase promoter to drive the expression of the human SOD cDNA. Peritoneal macrophages in these mice have a 2-fold increase in Cu/Zn-SOD activity, resulting in a decrease in the immune function of these cells that is characterized by a decrease in both microbicidal and fungicidal activity (46).

Two laboratories have produced transgenic mice that overexpress human Mn-SOD (47,48). Animals carrying a human Mn-SOD gene under the control of the human SP-C (surfactant protein C) promoter have increased expression of Mn-SOD in the mitochondria of distal respiratory epithelial lung cells. These transgenic animals exhibit a 60% increase in the activity of lung Mn-SOD and show an increased resistance to oxidative stress when exposed to 95% oxygen, as demonstrated by an increase in survival time and a decrease in lung pathology (47). Another transgenic contains the human Mn-SOD gene, driven by the human B-actin promoter. Total lung Mn-SOD activity is increased 180% in these mice, but they do not show an increased resistance to exposure of >99% oxygen, compared to non-transgenic controls (48). More recently, transgenic mice that overexpress human GPX under the direction of the human metallothionein IIA promoter have also been produced (49). These mice exhibit an increase in the expression of GPX in several regions of the brain and show no signs of abnormal development or behavior. The usefulness of each of these transgenic models is limited by gene overexpression occurring in only a finite number of tissues. Global overexpression in nearly all tissues of the organism would be optimal in the examination of the effect of increased antioxidant protection on life span.

An alternative strategy to producing transgenic animals in which a particular gene is overexpressed is to produce animals in which a gene has been targeted for deletion. It is possible to introduce mutations in individual genes by a methodology employing the use of pluripotent mouse embryonic stem (ES) cells. The transgene DNA is inserted into these cells by microinjection or electroporation, and following homologous

recombination between the transgene and the endogenous DNA, a gene-specific mutation results. The ES cells that contain the disrupted or mutated DNA are clonally isolated, microinjected into blastocytes, then transferred to surrogate mothers. These mice will produce chimeric progeny that contain DNA from the host mouse and the ES cells. The targeted gene is passed through the germline and can be eventually bred to homozygosity.

There are currently two mouse models in which the Mn-SOD has been altered. In the first model, exon 3, which codes for the active site of the gene, has been replaced with a portion of the neomycin gene (50). Mice that are homozygous for the deleted gene do not survive longer than 5 to 10 days. However, mice that are heterozygous for the mutated gene appear normal and exhibit normal breeding behavior out to at least 9 months of age. These animals have approximately 50% of the Mn-SOD activity present in control animals in liver, kidney, heart, and brain. Our laboratory is currently investigating whether the extent of free radical damage with age is altered in the knockout mice and whether the decrease in the activity of Mn-SOD has any effect on life span or the incidence of age-related pathology in these animals. The second Mn-SOD knockout mouse was produced by a disruption of exons 1 and 2. These homozygous knockout mice survive up to 3 weeks and have several pathological abnormalities including anemia, neural degeneration, motor disturbances, and mitochondrial ultrastructural damage (51).

Modulation of Transcription Factors by Oxidative Stress

Exposure of a cell or an organism to life-threatening conditions may induce the activation of gene(s) that play an important role in defending the organism. For example, a variety of agents such as UV light, gamma-rays, elevated temperature, oxygen radicals, bacterial and viral pathogens, inflammatory cytokines, and heavy metals are known to elicit an emergency response of gene expression in most cell types of the organism (52,53). Activating signals generally occur via the signal transduction system and finally reach various transcription factors in the nucleus where they activate the expression of stress response genes involved in the defense of the organism against adverse conditions.

A large number of proteins, such as heat shock proteins, metallothionein, DNA-repair proteins, acute phase proteins, etc., are produced when cells are exposed to biological, chemical, and physical insults (54-56). Some of these proteins are altered in aging due to changes in the DNA binding activity of their transactivating factors (57-62). For example, studies from our laboratory of rat hepatocytes and lymphocytes have shown that the heat shock gene (*hsp70*) is affected by aging and that the decline in *hsp70* transcription is correlated with an age-related decline in the DNA binding activity of heat shock transcription factors (61,62). Age-associated alterations in the activity of the stress-response transcription factors could greatly affect the

regulation of the stress-response genes that protect the organism from stress conditions. Moreover, the regulatory changes that affect the homeostatic levels of the products of these genes may be the underlying basis for the gradual decline in cellular function. Thus, oxidative stress and other factors are shown to target the signal pathway components that regulate the activation of these transcription factors.

Oxidative Stress-Response Transcription Factors

The induction of transcription factors mediated by oxidative stress in bacteria has been studied in detail. Two reactive oxygen intermediate-responsive transcription factor systems, called oxyR (63) and soxRS (64), have been identified. These transcription factors control the expression of multiple antioxidant enzymes in response to H_2O_2 and superoxide (O_2^-), respectively. However, the transcription factors that regulate oxidative stress-mediated antioxidant response in eukaryotic cells have not been fully characterized.

Several studies have suggested that the transcription factor NF- κ B, and to a lesser extent AP-1, would be equivalent to the bacteria transcription factor oxyR. Four lines of evidence suggest oxidative stress is involved in the activation of transcription factors NF- κ B and/or AP-1. First, in some cell lines, NF- κ B is directly activated upon treatment of cells with micromolar amounts of H_2O_2 in the culture medium (52,65). Second, stable overexpression of CAT has been shown to impair NF- κ B activation in response to the inflammatory cytokine (e.g., TNF- α and okadaic acid) (66). Third, reperfusion (re-exposure) of ischemic (hypoxic) cell/tissue to oxygen has been shown to trigger the activation of NF- κ B (52). Fourth, various free radical scavengers such as N-acetyl-L-cysteine (NAC), dithiocarbamates, and vitamin E have been shown to inhibit NF- κ B activation in response to a variety of oxidative stress-inducing agents (65).

NF- κ B is a multisubunit transcription factor found in many different cell types and tissues, but has been best characterized in immune system cells, such as B and T cells, macrophages, and monocytes (67). Various agents, such as inflammatory cytokines (e.g., TNF- α and IL-1), phorbol ester-12-myristate-13-acetate (PMA), lipopolysaccharide, UV light, gamma rays, viral and bacterial proteins, and double-stranded RNA that induce oxidative stress are also capable of activating NF- κ B (67). Activation of NF- κ B causes a rapid induction of the gene expression that plays an important role in the defense systems of organisms.

In most cells, NF- κ B is present in the cytoplasm as a non-DNA binding complex composed of three subunits: two DNA-binding proteins, p50 and p65 (also known as Rel A), and an inhibitory subunit (I κ B) bound to p65 (68). In resting (non-stressed) cells, NF- κ B is known to reside in an inactive form in the cytoplasm. This form is stabilized by the inhibitory subunit (I κ B), which is tightly bound to the DNA-binding subunits (p50 and p65) (69).

Figure 1 shows that upon activation of cells by agents that induce oxidative stress, I κ B is removed and the liberation of NF- κ B is able to translocate into the nucleus and activate target genes by binding to regulatory elements in enhancers and promoters. The mechanism by which antioxidants cause inhibition of NF- κ B is not well understood; however, a recent study has shown that antioxidants may have some effect on I κ B by delaying its dissociation and inhibiting the phosphorylation step required for its dissociation from NF- κ B protein complex. Because antioxidants may act upstream of the kinase/phosphatase system, I κ B has been suggested to be redox-controlled (52).

Figure 1. Schematic illustration of how free radicals produced by various agents can either be quenched by antioxidants or can activate the transcription factor NF- κ B. Free radicals may act as physiological second messengers, causing the dissociation of the inhibitory subunit (I κ B) of NF- κ B. The DNA binding subunits of NF- κ B translocate into the nucleus where they bind to the NF- κ B binding site and stimulate gene expression.

It has been shown that most, if not all, inducers of NF- κ B seem to rely on the production of free radicals, and that antioxidants inhibit the activation of NF- κ B. Schreck et al. (65) have shown that those agents (e.g., butyl peroxide) that generate H_2O_2 , activate NF- κ B; however, agents that lead to the generation of O_2^- are not effective in NF- κ B activation. It has been postulated that, with respect to oxygen-free radical specificity, NF- κ B activa-

tion is more closely related to bacterial oxyR than to the soxRS system. Because a variety of free radical scavengers, such as cystine derivatives (e.g., NAC), metal chelators, vitamin E, etc., suppress activation of NF- κ B, it has been suggested that, in eukaryotic cells, the NF- κ B acts as a "primary" oxidative stress-response transcription factor (52,65,66).

In contrast to the strong NF- κ B binding activity responding to H_2O_2 , the AP-1 binding activity is weakly induced by H_2O_2 (70,71). The similarities between PMA-induced NF- κ B and AP-1 activation have been investigated in response to H_2O_2 and the antioxidant NAC. For example, it was found that NF- κ B was strongly activated in H_2O_2 -treated HeLa cells, and activation of NF- κ B by PMA was suppressed by the antioxidant NAC treatment of HeLa cells (52,72). This suggested that H_2O_2 synergizes the effect of PMA, supporting the idea that PMA and H_2O_2 signals converge into a common oxidative stress-dependent pathway. In contrast to NF- κ B, the DNA binding activity of AP-1 was strong when cells were stimulated with PMA alone. However, treatment of cells with H_2O_2 in the presence of PMA reduced the AP-1 binding activity in these cells, while the antioxidant treatment enhanced PMA effect. The effect of antioxidants on AP-1 binding activity was shown to be protein synthesis dependent and involved transcriptional induction of c-fos and c-jun genes. Because of the de novo synthesis requirement of Fos and Jun proteins (AP-1), AP-1 is suggested to be involved in eukaryotic oxidative stress responses as a "secondary" antioxidant-response transcription factor (52).

Most studies on the induction of AP-1 by various stimuli focus on studying the expression of c-fos and c-jun, which are assumed to reflect the capability of the AP-1 binding activity. The expression of c-fos and c-jun are also induced by a variety of agents that induce oxidative stress (71,73-75). The induction of AP-1 binding activity has been shown to be induced by antioxidants (66). Furthermore, it has also been shown that the induction of c-fos and c-jun mRNA by H_2O_2 and by the antioxidant NAC have similar kinetics of induction. This indicates that signals emerging from Fos and Jun proteins sense either pro-oxidant or antioxidant conditions in the cell following a similar pathway. It has been speculated that activation of c-fos and c-jun by oxidants and antioxidants occurs at the level of signal transduction molecules (discussed below). For example, Devary et al. (76) have shown that UV induction of c-jun expression requires activation of thyronine kinase and Raf-like serine/threonine kinases. Because UV light is known to induce free radicals (77), and UV effects are blocked with the free radical scavenger, NAC (76), it is believed that the UV-induced signals might converge into the same oxidative stress-dependent pathways as H_2O_2 and other inducers of AP-1. Consistent with this notion is the fact that tyrosine kinases are also activated upon treatment of cells with H_2O_2 and other free radical-generating agents (e.g., IL-1) (75).

The mechanism by which antioxidants induce activation of AP-1 is not clearly understood. However, it has been shown that Fos and Jun proteins contain conserved redox-sensitive cysteine residues in their DNA-binding domains (78,79). These cysteine residues are found partially oxidized, which causes a loss in DNA-binding activity; but treatment of protein with a reducing agent (e.g., dithiothreitol) restored DNA-binding activity of Fos/Jun (AP-1). Thus, the induction of AP-1 by antioxidants was based on the assumption that pre-existing c-Jun in the HeLa cells is in an oxidized form; and therefore, an antioxidant treatment of the cells could directly or indirectly result in reduction of cysteine residues, leading to increased DNA binding and transactivation of c-Jun, a constituent of the AP-1 transcription factor.

Oxidative Stress and Signal Transduction

The response of cells to their environment is mediated by a variety of signal transduction pathways culminating in genetic changes in their nuclei. In this manner, cells alter the expression of the genes and proteins that lead to changes in their functional capacity. In cells from old animals, such genetic changes may result in modulation of stress response genes (e.g., antioxidant, metallothionein, or heat shock genes) that have a great impact in defending the organism against intrinsic factors and environmental insults.

Most of the earliest nuclear changes that occur within minutes of cellular exposure to oxidative stress involve alterations in pre-existing proteins. The most common post-translational modification is protein phosphorylation (80). The proteins that ultimately transduce the changes in gene expression, the transcription factors, are often phosphoproteins, and their phosphorylation state is sensitive to stress or stimulation via the action of specific protein kinases and phosphatases. Examples include the oxidative stress-response transcription factors NF- κ B and AP-1 (82). These transcription factors transduce signals in response to agents that stimulate protein tyrosine kinases through the protein kinase C (PKC) signal transduction pathway (80). However, they can also be activated by other signaling systems including changes in redox potential, UV light, and other cellular stresses (83).

AP-1 is composed of dimers of proteins of the Fos and Jun proto-oncogene families. Members of the Jun family of proteins (e.g., c-Jun, JunB and JunD) can homodimerize or heterodimerize with members of the Fos family of proteins (e.g., c-Fos, FosB, Fra1, and Fra2). In unstimulated cells, AP-1 levels are very low. Following stimulation, most of the members of the Fos/Jun family are transcriptionally induced, but with different kinetics (84,85). Recent studies have shown that the transcriptional activity of c-fos and c-jun is regulated by the upstream signaling molecules (81,86-88). It appears that the upstream signaling molecules differentially regulate c-fos and c-jun transcription. For ex-

ample, it has been shown that phosphorylation of transcription factor TCF (ternary complex factor) by mitogen-activated protein kinases (MAPK) play a crucial role in c-fos expression. However, the induction of c-jun appears to be mediated by a different protein kinase, JNK (c-jun amino terminal kinase), which phosphorylates and augments the transcriptional activity of c-jun (89,90). The upstream signaling pathways involved a cascade of phosphorylation and dephosphorylation events that led to augmentation of c-fos and c-jun transcription.

The current model for the MAPK signaling events that lead to regulation of c-fos and c-jun transcription is shown in Figure 2. Agents that induce oxidative stress activate c-fos and c-jun transcription via a receptor-ligand complex to initiate a transmembrane signal to the cytoplasm (82). Tyrosine phosphorylation of phospholipase-C γ (PLC- γ) stimulates its activity, causing the generation of second messengers that stimulate PKC activation and trigger an elevation of intracellular calcium. These biochemical events lead to the stimulation of GTP-bound Ras. Ras is activated as a result of PKC-mediated inhibition of Ras-GTPase-activating proteins and activation of adaptor molecules known as Grb2 and the Ras Grb-exchange SOS (son of sevenless) (86,88). In the GTP-bound state, Ras stimulates the MAPK pathway, leading to activation of a number of MAPK isoforms such as ERKs (extracellular signal-regulated kinases) and JNKs. In the plasma membrane, active GTP-bound Ras directly binds and promotes the activation of the protein kinase Raf-1. Active Raf-1 phosphorylates and activates the MAPK/ERK kinases through the activation of MAPK kinase, termed MEK (ERK kinase). Active ERKs phosphorylate and regulate the activity of numerous additional proteins in both the cytosol and nucleus (86-88). Thus, one function of the Ras/Raf-1/MEK/ERK/JNK signal transduction pathway is to transmit a stimulatory signal received at the plasma membrane into the nucleus, where it affects the transcription factors involved in the regulation of stress-response gene(s).

Some agents act through a recently identified family of protein kinases that are potentially stimulated by stress signals and are termed SAPK (stress-activated protein) kinases (91). Three SAPK genes have been cloned, termed α , β , and γ . These kinases are widely expressed and are 90% related to each other and 45% related to the MAPK family (91). The 46 kDa form of SAPK-g has been independently cloned as JNK1 kinase (92). The cloning and function of the SAP kinase (SEK) has recently been reported (93). This protein kinase is, itself, directly regulated by another protein kinase termed MEKK (MEK kinase) (94), extending the known component of the stress-activated protein kinase pathway. Like the MAPKs, SAPKs require tyrosine and threonine phosphorylation for activity, as shown in Figure 2. However, these enzymes are not phosphorylated or activated by MEK, and therefore appear to be on a distinct signal transduction pathway. This view is sup-

Figure 2. A current model for the intracellular signaling network involving MAP kinase and SAP kinase pathways acting on c-fos and c-jun transcription. External (e.g., mitogenic or stress factors) and internal (e.g., free radicals produced by oxidative metabolism) signals are transduced via activation of protein tyrosine kinases, which lead to the phosphorylation and activation of downstream signaling molecules such as MAP and SAP kinases. Activation of these kinases lead to their nuclear translocation where they regulate the transcription of c-fos and c-Jun (AP-1). MAPK, mitogen-activated protein kinase; SAPK, stress-activated protein kinase; SEK, SAP kinase; MEK, MAP kinase; PLC, phospholipase-C; PKC, protein kinase C; IP3, phosphatidylinositol-3-phosphate; DAG, diacylglycerol; GAP, GTPase activating protein.

ported by the observation that many SAPK agonists poorly activate MAPKs and vice versa (91). SAPKs are potentially activated by a variety of cellular stresses including heat shock, UV, and so on (91,95). One of the many targets of MAPKs and SAPKs is the phosphorylation of the Fos and Jun protein in response to several stress signals (80,81,91,92). Stimulation of the signal transduction pathway results in phosphorylation of the Jun protein at two residues proximal to the major transactivation domain of the protein (96,97). Phosphorylation is stimulated by a variety of agents such as mitogen, cytokine, PMA, UV, as well as free radicals and heat shock (80,96-98). These studies suggest that activation of Fos and Jun is an important and common response not just to mitogens but to many other stress signals. Elucidation of the pathways culminating in phosphorylation and transcriptional activation of c-fos and c-jun is therefore an area of intense interest.

Metallothionein Transcription Factors Targeted by Oxidative Stress

A number of studies (99,100) have shown the agents that induce oxidative stress (e.g., LPS, cytokines, PMA, chloroform, turpentine, carbon tetrachloride, and

paraquat) can also induce the expression of a cysteine-rich heavy metal binding protein, metallothionein (MT). Overexpression of MT (i.e., MT-1 and MT-II) genes have been shown to protect cells against heavy metal toxicity, whereas underexpression in cells or in mice with null mutations of genes leads to a highest sensitivity to heavy metal, (i.e., Cd) (53). Several studies have indicated that, in addition to its role in metal homeostasis, MT is also an efficient free radical scavenger (101,102). For example, it has been shown that yeast and mammalian MT can be functionally substituted for SOD in protecting an organism from oxidative stress (102). These studies suggest that MT plays a very important role in protecting an organism from oxidative stress. A recent study has shown that treatment of a mouse hepatoma cell line (Hepa cells) with H_2O_2 resulted in rapid transcription of the MT-1 gene. The oxidative stress-mediated activation of the MT-I gene involves multiple cis-acting promoter composite elements consisting of an overlapping of either major late transcription factor (MLTF) binding site and an antioxidant response element (ARE) and/or a metal response element (MRE) binding site (103). This study suggested that oxidative stress-mediated activation of transcription factors involved in MT-I gene expression is different from that of heavy metal-induced activation of transcription factor and MT gene expression. The ARE can be bound by a factor that can be activated by oxidative stress; however, the mechanism of this activation is not known. The ARE core sequence (GTGACCCGC) bears homology with an AP-1 binding site and the MLTF binding site (CCGTGAC) overlapped with the ARE sequence (104). It has been postulated that oxidative stress mediates activation of both MLTF and the transcription factors that bind to the ARE binding site in the promoter region of MT gene. A recent study has shown that H_2O_2 mediated the activation of a transcription factor that binds to MRE. A zinc-finger transcription factor, MTF-1 (metallothionein transcription factor-1), that binds to MRE of the MT-I promoter has recently been cloned (105). MTF-1 is believed to be controlled by a metal-sensitive inhibitor (106). It has been suggested that when cells are exposed to oxidative stress (e.g., H_2O_2), the MTF-1 dissociates from the inhibitor either directly by oxidizing thiols and releasing zinc or by oxidation of the inhibitor (106). This is analogous to the oxidative stress-mediated dissociation of I κ B from the NF- κ B DNA binding proteins (p50 and p65), which leads to activation of the NF- κ B transcription factor.

Concluding Remarks and Future Perspectives

The present article describes newly developed molecular approaches to study the biological role of oxidative stress. One currently developing area of research is the use of transgenic animal models that overexpress or underexpress specific antioxidant enzymes. These animals allow investigators to study the impact of alter-

ations on individual components of the antioxidant system in the processes of free radical damage and aging. Secondly, we have described current directions in the study of the signal transduction-redox pathways that modulate the oxidative stress-response transcription factors involved in the regulation of antioxidant enzymes. Each of these new directions is relevant to elucidating the molecular mechanisms involved in the aging process.

Eukaryotic organisms have an elaborate oxidative defense mechanism that is triggered by free radicals and other stress factors. The response to many oxidative agents is mediated by the activation of signal transduction pathways, which in turn leads to the activation of a cascade of kinases and phosphatases. The kinases may act in a pathway that senses damage and thereby initiates repair processes. On the other hand, this pathway may target the expression of genes that induce programmed cell death in an effort to eliminate a potentially mutated cell. Measuring the status of the activation of the pathway should provide important new insights into the effects of diverse agents on cells, and identify whether these effects are caused by the intrinsic or extrinsic factors that generate free radicals. The upstream signaling molecules involved in the regulation of the activity of oxidative stress-response transcription factors NF- κ B and AP-1 in turn regulate the expression of families of stress response genes such as antioxidant enzymes, metallothionein, and heat shock proteins. The functions of some of these genes are altered by aging. The oxidative stress-response transcription factors are the key regulatory proteins that respond to changes in both intrinsic and extrinsic factors that generate free radicals. Thus, the agents that induce oxidative damage act as second messengers to target the same signal transduction-redox pathways that regulate the activity of transcription factors that activate antioxidant genes.

Age-associated declines in cellular function could occur because of changes in the structure and function of regulatory proteins. Alterations in the interaction of these regulatory proteins with their respective cis-acting DNA sequences may be a contributing factor in the age-related decline of the antioxidant defense system. This hypothesis also raises the question of whether age-associated declines in cellular function could result from changes in the activity and/or expression of the upstream signaling molecules that regulate the downstream oxidative stress-response transcription factors. Although these questions need further investigation, it appears that alterations in the regulation of oxidative stress-response transcription factors might contribute to age-related changes in the oxidative defense system. The accumulation of free radicals due to intrinsic factors or environmental insults would modulate the antioxidant enzymes because they target the same signal transduction-redox pathways that lead to the activation of the oxidative stress-response transcription factors. The increase in free radicals as a result of aerobic metabo-

lism and environmental insult may result in a chronic failure of the oxidative response gene(s) that are properly regulated by free radicals and may be the basis of the gradual decline in cellular function and increased susceptibility to disease during aging. Future research will determine the potential application of these biochemical and molecular approaches in understanding the molecular mechanism of the age-related decrease in antioxidant gene expression, as well as in evaluating new therapeutic interventions of aging and age-associated disease.

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REFERENCES

1. Harman, D.: Aging: A theory based on free radical and radiation chemistry. *J. Gerontol.*, 11: 298-300, 1956.
2. Sohal, R.S.: The free radical hypothesis of aging: an appraisal of the current status. *Aging Clin. Exp. Res.*, 5: 3-17, 1993.
3. Nohl, H.: Involvement of free radicals in aging: a consequence or cause of senescence. *Brit. Med. Bull.*, 40: 653-667, 1993.
4. Harman, D.: Free radical theory of aging: the free radical diseases. *Age*, 7: 111-131, 1984.
5. McCord, J.M., and Fridovich, I.: Superoxide dismutase: An enzymic function for erythrocyte (neurocyte). *J. Biol. Chem.*, 244: 6049-6055, 1969.
6. Chance, B., Sies, H., and Boveris, A.: Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.*, 62: 527-605, 1979.
7. Maiorino, M., Chu, F.F., Ursini, F., Davies, K., Dorshaw, J.H., and Esworthy, R.S.: Phospholipid hydroperoxide glutathione peroxidase is the 18 kDa selenoprotein expressed in human tumor cell lines. *J. Biol. Chem.*, 255: 7728-7732, 1991.
8. Elroy-Stein, O., Bernstein, Y., and Groner, Y.: Overproduction of human Cu/Zn-superoxide dismutase in transfected cells: extenuation of paraquat-mediated cytotoxicity and enhancement of lipid peroxidation. *EMBO J.*, 5: 615-622, 1986.
9. Huang, T.T., Carlson, E.J., Leadon, S.A., and Epstein, C.J.: Relationship of resistance to oxygen free radicals to Cu/Zn-superoxide dismutase activity in transgenic, transfected, and trisomic cells. *FASEB J.*, 6: 03-910, 1992.
10. Krall, J., Bagley, A.C., Mullenbach, G.T., Hallewell, R.A., and Lynch, R.E.: Superoxide mediates the toxicity of paraquat for cultured mammalian cells. *J. Biol. Chem.*, 263: 1910-1914, 1988.
11. Kelner, M.J., Bagnell, R., Montoya, M., Estes, L., Ugluk, S.F., and Cerutti P.: Transfection with human copper-zinc superoxide dismutase induces bidirectional alterations in other antioxidant enzymes, proteins, growth factor response, and paraquat resistance. *Free Rad. Biol. Med.*, 18: 497-506, 1995.
12. Negita, M., Hayashi, S., Yokoyama, I., Emi, N., Nagasaka, T., and Takagi, H.: Human superoxide dismutase cDNA transfection and its *in vitro* effect on cold preservation. *Biophys. Res. Biochem. Comm.*, 218: 653-657, 1996.
13. Elroy-Stein, O., and Groner, Y.: Impaired neurotransmitter uptake in PC-12 cells overexpressing human Cu/Zn-superoxide dismutase-implication for gene dosage effects in Down Syndrome. *Cell*, 52: 259-267, 1988.
14. Norris, K.H., and Hornsby, P.J.: Cytotoxic effects of expression of human superoxide dismutase in bovine adrenocortical cells. *Mutat. Res.*, 237: 95-106, 1990.
15. Amstad, P., Peskin, A., Shah, G., Mirault, M.-E., Moret, R., Zbinden, I., and Cerutti, P.: The balance between Cu/Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress. *Biochemistry*, 30: 9305-9313, 1991.
16. Erzurum, S.C., Lemarchand, P., Rosenfeld, M.A., Yoo, J.-H., Crystal, R.G.: Protection of human endothelial cells from oxidant injury by adenovirus-mediated transfer of the human catalase cDNA. *Nucleic Acids Res.*, 21: 1607-1612, 1993.
17. Lindau-Shepard, B.A., and Shaffer, J.B.: Expression of human catalase in acatalasemic murine SV-B₂ cells confers protection from oxidative damage. *Free Rad. Biol. Med.*, 15: 581-588, 1993.
18. Wong, G.H.W., Elwell, J.H., Oberley, L.W., and Goeddel, D.V.: Manganese superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell*, 58: 923-931, 1989.
19. Hirose, K., Longo, D.L., Oppenheim, J.J., and Matsushima, K.: Overexpression of mitochondrial manganese superoxide dismutase promotes the survival of tumor cells exposed to interleukin-1, tumor necrosis factor, selected anticancer drugs, and ionizing radiation. *FASEB J.*, 7: 361-368, 1993.
20. Suresh, A., Tung, F., Moreb, J., and Zucali, J.R.: Role of manganese superoxide dismutase in radioprotection using gene transfer studies. *Cancer Gene Therapy* 1: 85-90, 1994.
21. St.Clair, D.K., Oberley, T.D., and Ho, Y.-S.: Overproduction of human Mn-superoxide dismutase modulates paraquat-mediated toxicity in mammalian cells. *FEBS Lett.*, 293: 199-203, 1991.

22. Warner, B., Papes, R., Heile, M., Spitz, D., and Wispe, J.: Expression of human Mn SOD in Chinese hamster ovary cells confers protection from oxidant injury. *Am. J. Physiol.*, 264: L598-L605, 1993.
23. Lindau-Shepard, B., Shaffer, J.B., and Del Vecchio, P.J.: Overexpression of manganous superoxide dismutase (MnSOD) in pulmonary endothelial cells confers resistance to hyperoxia. *J. Cell. Physiol.*, 161: 237-242, 1994.
24. St. Clair, D.K., Wan, X.S., Oberley, T.D., Muse, K.E., and St. Clair, W.H.: Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. *Mol. Carcinog.*, 6: 238-242, 1992.
25. St. Clair, D.K., Oberley, T.D., Muse, K.E., and St. Clair, W.H.: Expression of manganese superoxide dismutase promotes cellular differentiation. *Free Radic. Biol. Med.*, 16: 275-282, 1994.
26. Church, S.L., Grant, J.W., Ridnour, L.A., Oberley, L.W., Swanson, P.E., Meltzer, P.S., and Trent, J.M.: Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc. Natl. Acad. Sci. USA*, 90: 3113-3117, 1993.
27. Li, J.J., Oberley, L.W., St. Clair, D.K., Ridnour, L.A., and Oberley, T.D.: Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. *Oncogene*, 10: 1989-2000, 1995.
28. Mirault, M.E., Tremblay, A., Beaudoin, N., and Tremblay, M.: Overexpression of selenogluthathione peroxidase by gene transfer enhances the resistance of T47D human breast cells to clastogenic oxidants. *J. Biol. Chem.*, 266: 20752-20760, 1991.
29. Taylor, S.D., Davenport, L.D., Speranza, M.J., Mullenbach, G.T., and Lynch, R.E.: Glutathione peroxidase protects cultured mammalian cells from the toxicity of adriamycin and paraquat. *Arch. Biochem. Biophys.*, 305: 600-605, 1993.
30. Seto, N.O., Hayashi, S., and Tener, G.M.: Overexpression of Cu-Zn superoxide dismutase in *Drosophila* does not affect life-span. *Proc. Natl. Acad. Sci. USA*, 87: 4270-4274, 1990.
31. Orr, W.C., and Sohal, R.S.: Effects of Cu-Zn superoxide dismutase overexpression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster*. *Arch. Biochem. Biophys.*, 301: 4-40, 1993.
32. Reveillaud, I., Niedzwiecki, A., Bensch, K.G., and Fleming, J.E.: Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. *Mol. Biol. Cell*, 11: 632-640, 1991.
33. Durusoy, M., Diril, N., and Bozcuk, A.: Age-related activity of catalase in different genotypes of *Drosophila melanogaster*. *Exp. Gerontol.*, 30: 77-86, 1995.
34. Epstein, C.J., Avraham, K.B., Lovett, M., Smith, S., Elroy-Stein, O., Rotman, G., Bry, C., and Groner, Y.: Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in down syndrome. *Proc. Natl. Acad. Sci. USA*, 84: 8044-8048, 1987.
35. Przedborski, S., Jackson-Lewis, V., Kostic, V., Carlson, E., Epstein, C.J., and Cadet, J.L.: Superoxide dismutase, catalase, and glutathione peroxidase activities in copper/zinc-superoxide dismutase transgenic mice. *J. Neurochem.*, 58: 1760-1767, 1992.
36. White, C.W., Avraham, K.B., Shanley, P.F., and Groner, Y.: Transgenic mice with expression of elevated levels of copper-zinc superoxide dismutase in the lungs are resistant to pulmonary oxygen toxicity. *J. Clin. Invest.*, 87: 2162-2168, 1991.
37. Kinouchi, H., Epstein, C.J., Mizui, T., Carlson, E., Chen, S.F., and Chan, P.H.: Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. *Proc. Natl. Acad. Sci. USA*, 88: 11158-11162, 1991.
38. Chan, P.H., Yang, G.Y., Chen, S.F., Carlson, E., and Epstein, C.J.: Cold-induced brain edema and infarction are reduced in transgenic mice overexpressing CuZn-superoxide dismutase. *Ann. Neurol.*, 29: 482-486, 1991.
39. Chan, P.H., Chu, L., Chen, S.F., Carlson, E.J., and Epstein, C.J.: Reduced neurotoxicity in transgenic mice overexpressing human copper-zinc-superoxide dismutase. *Stroke*, 21: 80-82, 1990.
40. Przedborski, S., Kostic, V., Jackson-Lewis, V., Naini, A.B., Simonetti, S., Fahn, S., Carlson, E., Epstein, C.J., and Cadet, J.L.: Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- induced neurotoxicity. *J. Neurosci.*, 12: 1658-1667, 1992.
41. Avraham, K.B., Schickler, M., Sapoznikov, D., Yarom, R., and Groner, Y.: Down's syndrome: abnormal neuromuscular junction in tongue of transgenic mice with elevated levels of human Cu/Zn-superoxide dismutase. *Cell*, 54: 823-829, 1988.
42. Avraham, K.B., Sugarman, H., Rotshenker, S., and Groner, Y.: Down's syndrome: morphological remodeling and increased complexity in the neuromuscular junction of transgenic CuZn-superoxide dismutase mice. *J. Neurocytol.*, 20: 208-215, 1991.

43. Schickler, M., Knobler, H., Avraham, K.B., Elroy-Stein, O., and Groner, Y.: Diminished serotonin uptake in platelets of transgenic mice with increased Cu/Zn-superoxide dismutase activity. *EMBO J.*, 8: 1385-1392, 1989.
44. Minc-Golomb, D., Knobler, H., and Groner, Y.: Gene dosage of CuZnSOD and Down's syndrome: diminished prostaglandin synthesis in human trisomy 21, transfected cells and transgenic mice. *EMBO J.*, 10: 119-2124, 1991.
45. Nabarra, B., Casanova, M., Paris, D., Nicole, A., Toyama, K., Sinet, P., Ceballos, I., and London, J.: Transgenic mice overexpressing the human CuZn-SOD gene: ultrastructural studies of a premature thymic involution model of Down's Syndrome (Trisomy 21). *Laboratory Investigation*, 74: 617-626, 1996.
46. Mirochnitchenko, O., and Inouye, M.: Effect of overexpression of human Cu,Zn superoxide dismutase in transgenic mice on macrophage functions. *J. Immunol.*, 156: 1578-1586, 1996.
47. Wispe, J.R., Warner, B.B., Clark, J.C., Dey, C.R., Neuman, J., Glasser, S.W., Crapo, J.D., Chang, L.Y., and Whitsett, J.A.: Human Mn-superoxide dismutase in pulmonary epithelial cells of transgenic mice confers protection from oxygen toxicity. *J. Biol. Chem.*, 267: 23937-23941, 1992.
48. Ho, Y.S.: Transgenic models for the study of lung biology and disease. *Am. J. Physiol.*, 266: L319-L353, 1994.
49. Mirault, M., Tremblay, A., Furling, D., Trepanier, G., Dugre, F., Puymirat, J., and Pothier, F.: Transgenic glutathione peroxidase mouse models for neuroprotection studies. *Ann. N. Y. Acad. Sci.*, 738: 104-115, 1994.
50. Li, Y., Huang, T., Carlson, E., Yoshimura, M., Berger, C., Chan, P., Wallace, D., and Epstein, C.: Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.*, 11: 376-381, 1995.
51. Lebovitz, R.M., Zhang, H., Vogel, H., Cartwright, J., Dionne, L., Lu, N., Huang, S., and Matzuk, M.: Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. USA*, 93: 9782-9787, 1996.
52. Meyer, M., Schreck, R., and Baeuerle, P.A.: H₂O₂ and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J.*, 12: 2005-2015, 1993.
53. Dalton, T., Palmer, R.D., and Andrews, G.K.: Transcriptional induction of the mouse metallothionein-1 gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. *Nucleic Acid Res.*, 22: 5016-5023, 1994.
54. Hellmund, D.: *Heat Shock: Response of Eucaryotic Cells*. New York, Springer-Verlag, 1994.
55. Oh, S. H., Deagen, J.T., Whanger, P.D., and Wervung, P.H.: Biological function of metallothionein V. Its induction in rats by various stresses. *Am. J. Physiol.*, 234: E282-E305, 1978.
56. Koj, A.: Acute Phase Reactants. In: *Structure and Function of Plasma Proteins*. A.C. Allison, (ed.), New York, Plenum Publishing Corp., 1985, 73-125.
57. Baeuerle, P.A., and Baltimore, D.: I κ B: A specific inhibitor of the NF-kappa B transcription factor. *Science*, 242: 540-546, 1988.
58. Carter, K.C., Post, D.G., and Papaconstantinou, J.: Differential expression of the mouse alpha 1-acid glycoprotein genes during inflammation. *Biochem. Biophys. Acta.*, 1089: 197-205, 1991.
59. Liu, A., Lin, Y.C., Chio, H.S., Sarhage, F., and Li, B.: Attenuated induction of heat shock gene expression in aging fibroblast. *J. Biol. Chem.*, 264: 12037-12045, 1989.
60. Post, D.J., Carter, K.C., and Papaconstantinou, J.: The effect of aging on constitutive mRNA levels and lipopolysaccharide inducibility of acute phase genes. *N. Y. Acad. Sci.*, 621: 66-77, 1991.
61. Heydari, A. R., Wu, B., Takahashi, R., Strong, R., and Richardson, A.: Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol. Cell. Biol.*, 13: 2909-2918, 1993.
62. Pahlavani, M. A., Denny, M., Moore, S. A., Weindrich, R., and Richardson, A.: The expression of heat shock protein 70 decreases with age in lymphocytes from rats and rhesus monkeys. *Exp. Cell Res.*, 218: 310-318, 1995.
63. Storz, G., Tartaglia, L.A., Farr, S.B., and Ames, B.N.: Bacterial defenses against oxidative stress. *Trends Genet.*, 6: 363-368, 1990.
64. Dimple, B.: Regulation of bacterial oxidative stress genes. *Annu. Rev. Genet.*, 25: 315-337, 1991.
65. Schreck, R., Meier, B., Mannel, D., Droge, W., and Baeuerle, P. A.: Dithiocarbamates as potent inhibitors of NF- κ B activation in intact cells. *J. Exp. Med.*, 175: 1181-1194, 1992.
66. Schreck, R., Rieber, P., and Baeuerle, P.A.: Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kB transcription factor and HIV-1. *EMBO J.*, 10: 2247-2258, 1991.

67. Baeuerle, P.A., and Baltimore, D.: The physiology of NF- κ B transcription factor. In: *Molecular aspects of cellular regulation, hormonal control regulation of gene transcription*. Cohen, P., and J.G. Foulkes (ed), Amsterdam, Elsevier Science Publishers B.V., 1991, 409-432.
68. Baeuerle, P.A.: The inducible transcription activator NF- κ B: Regulation by distinct protein subunits. *Biochem. Biophys. Acta*, 1072: 63-80, 1991.
69. Baeuerle, P.A.: The inducible transcription activator NF- κ B: Regulation by distinct protein subunits. *Biochem. Biophys. Acta*, 1072: 63-80, 1991.
70. Nose, K., Shibamura, M., Kikuchi, K., Kageyama, H., Sakiyama, S., and Kuroki, T.: Transcriptional activation of early-response genes by hydrogen peroxide in a mouse osteoblastic cell line. *Eur. J. Biochem.*, 201: 99-106, 1991.
71. Amstad, P.A., Krupitza, G., and Cerutti, P.A.: Mechanisms of c-fos induction by active oxygen. *Cancer Res.*, 52: 3952-3960, 1992.
72. Schreck, R., Albermann, K., and Baeuerle, P.A.: Nuclear factor κ B: an oxidative stress-response transcription factor of eukaryotic cells. *Free Rad. Res. Commun.*, 17: 221-237, 1992.
73. Stankova, J., and Pleszczynski, M.: Leukotriene B4 stimulates c-fos and c-jun transcription and AP-1 binding activity in human monocytes. *Biochem. J.*, 282: 625-629, 1992.
74. Datta, R., Hallahan, D.E., Kharbanda, S.M., Rubin, E., Sherman, M.L., Huberman, E., Weichselbaum, R.R., and Kufe, D.W.: Involvement of reactive oxygen intermediates in the induction of c-fos gene transcription by ionizing radiation. *Biochemistry*, 31: 8300-8306, 1992.
75. Munoz, E., Zubiaga, A.M., Huang, C., and Huber, B.T.: IL-1 induces protein tyrosine phosphorylation in T cells. *Eur. J. Immunol.*, 22: 1391-1396, 1992.
76. Devary, Y., Gottlieb, R.A., Smeal, T., and Karin, M.: The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell*, 71: 1081-1092, 1992.
77. Tyrrell, R.M.: Oxidative stress. In: *Oxidative stress. Oxidants and antioxidants*, Sies, H. (ed.). London, Academic Press, 1991, 57-84.
78. Abate, C., Patel, L., Rauscher, F.J., and Curran, T.: Redox regulation of fos and jun DNA binding activity in vitro. *Science*, 249: 1157-1161, 1990.
79. Frame, M.G., Wilkie, N.M., Darling, A.J., Chudleigh, A., Pintzas, A., Lang, J.C., and Gillespie, D.A.: Regulation of AP-1 DNA complex formation in vitro. *Oncogene*, 6: 205-209, 1991.
80. Hunter, T. and Karin, M.: The regulation of transcription by phosphorylation. *Cell*, 70: 375-387, 1992.
81. Pelech, S.L. and Sanghera, J.S.: Mitogen-activated protein kinases: Versatile transducers for cell signaling. *Trends in Biochem. Sci.*, 17: 233-238, 1992.
82. Papaconstantinou, J.: Unifying model of the programmed and stochastic theories of aging. *Stress response genes, signal transduction-redox pathways and aging*. *Ann. New York Acad. Sci.*, 719: 195-211, 1994.
83. Herrlich, P., Ponta, H., and Rahmsdorf, H.J.: DNA damage inducing gene expression: Signal transduction and relation to growth factor signaling. *Rev. Physiol. Biochem. Pharmacol.*, 119: 187-223, 1992.
84. Woodgett, J.R.: Fos and Jun: Two into one will go. *Seminars in Cancer Biology*, 4: 389-397, 1990.
85. Angel, P. and Karin, M.: The role of Jun, Fos, and the AP-1 complex in cell proliferation and transformation. *Biochem. Biophys. Acta.*, 1072: 129-157, 1991.
86. Davis, R.J.: Transcriptional Regulation by MAP kinases. *Mol. Reprod. Dev.* 42: 459-567, 1995.
87. Conrad, A. C., and Steck, P.A.: Protein tyrosine in signal transduction. *The Cancer Bulletin*, 47: 125-133, 1995.
88. Bernstein, L.R., Ferris, D.K., Colburn, N.H., and Sobel, M.E.: A family of mitogen-activated protein kinase-related proteins interacts in vivo with activator protein-1 transcription factor. *J. Biol. Chem.*, 269: 9401-411, 1994.
89. Seger, R., and Krebs, E.C.: The MAPK signaling cascade. *FASEB J.*, 9: 726-732, 1995.
90. Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M., and Neriha, Y.: JNK is involved in signal integration during costimulation of T lymphocytes. *Cell*, 77: 726-735, 1994.
91. Kyriakis, J. M., Banerjee, P., and Nikolakaki, E.: The stress-activated protein kinase subfamily of c-jun kinases. *Nature*, 369: 156-160, 1994.
92. D'eriard, B., Hibi, M., and Wu, I.H.: JNK1: A protein kinase stimulated by UV light and Ha-Ras taht binds and phosphorylates the c-jun activation domain. *Cell*, 76: 1025-1037, 1994.
93. Sanchez, I., Hughes, R., and Mayer, B.: SAP/ERK kinase-1 (SEK1) defines the SAP pathway regulating c-Jun N-terminal phosphorylation. *Nature*, 372: 794-798, 1994.
94. Yan, M., Dai, T., Dek, J., Kyriakis, J., Zon, L., Woodgett, J., and Templeton, D.: MEKK1 activates the stress activated protein kinase (SAPK) in vivo, not MAP kinase, via direct phosphorylation of the SAPK activator SEK1. *Nature*, 372: 798-800, 1994.

95. Pombo, C.M., Bonventre, J.V., Woodgett, J.R., Kyriakis, J. M., and Force, T.: The stress-activated protein kinases (SAPKs) are major c-Jun amino terminal kinases actiated by ischemia and reperfusion. *J. Biol. Chem.*, 269: 26546-26550, 1994.
96. Pulverer, B., Kyriakis, J., Avruch, J., Nikolakaki, H., and Woodgett, J.R.: Phosphorylation of c-Jun by MAP kinases. *Nature*, 353: 670-674, 1991.
97. Pulverer, B., Hughes, K., Franklin, C., Kraft, A., Leever, S., and Woodgett, J.: Co-purification of mitogen-activated protein kinases with phorbol ester-induced c-Jun kinase in U937 leukaemic cells. *Oncogene*, 8: 407-415, 1993.
98. Devary, Y., Gottlieb, R., Smeal, T., and Karin, M.: The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell*, 71: 1081-1091, 1992.
99. De, S.K., McMaster, M.T., and Andrews, G.K.: Endotoxin induction of murine metallothionein gene expression. *J. Biol. Chem.*, 265: 15267-15274, 1990.
100. Durnam, D.M., Hoffman, J.S., Quaife, C.J., Benditt, E.P., Chen, H.Y., Brinster, R.L, and Palmiter, R.D.: MT-III, a brain specific member of the metallothionein gene family. *Proc. Natl. Acad. Sci. USA*, 81: 1053-1056, 1984.
101. Abel, J., and de Ruiter, N.: Inhibition of hydroxyl-radical generated DNA degradation by metallothionein. *Toxicol. Lett.*, 47: 191-196, 1989.
102. Thornalley, P.J., and Vasak, M.: Possible role of metallothionein in protection against radiation-induced oxidative stress. *Biochem. Biophys. Acta*, 827: 36-44, 1985.
103. Rushmore, T.H., Morton, M.R., and Pickett, C.B.: The antioxidant responsive element. Activation by oxidative stress and identification of DNA consensus sequence required for functional activity. *J. Biol. Chem.*, 266: 11632-11639, 1991.
104. Nguyen, T, Rushmore, T.H., and Pickett, C.B.: Transcriptional regulation of a rat liver glutathione-S-transferase Ya subunit gene. *J. Biol. Chem.*, 269: 13656-13662, 1994.
105. Radtke, F., Heuchel, R., Georgiev, O., Hergersberg, M., Gariglio, M., Dembic, Z., and Schaffner, W.: The transcription factor MTF-I is essential for basal and heavy metal-induced metallothionein gene expression. *EMBO J.*, 12: 1355-1362, 1993.
106. Palmiter, R.D.: Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor MTF-I. *Proc. Natl. Acad. Sci. USA*, 91: 1219-1223, 1994.