Antioxidant and redox regulation of gene transcription

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ABSTRACT **Reactive oxygen species (ROS) are implicated in the pathogenesis of a wide variety of human diseases. Recent evidence suggests that at moderately high concentrations, certain forms of ROS such as H_2O_2 may act as signal transduction messengers. To develop a better understanding of the exact mechanisms that underlie ROS-dependent disorders in biological systems, recent studies have investigated the regulation of gene expression by oxidants, antioxidants, and other determinants of the intracellular reduction-oxidation (redox) state. At least two well-defined transcription factors, nuclear factor (NF) κB and activator protein (AP) -1 have been identified to be regulated by the intracellular redox state. The regulation of gene expression by oxidants, antioxidants, and the redox state has emerged as a novel subdiscipline in molecular biology that has promising therapeutic implications. Binding sites of the redox-regulated transcription factors NF-κB and AP-1 are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of diseases, e.g., AIDS, cancer, atherosclerosis and diabetic complications. Biochemical and clinical studies have indicated that antioxidant therapy may be useful in the treatment of disease. Critical steps in the signal transduction cascade are sensitive to oxidants and antioxidants. Many basic events of cell regulation such as protein phosphorylation and binding of transcription factors to consensus sites on DNA are driven by physiological oxidant-antioxidant homeostasis, especially by the thiol-disulfide balance. Endogenous glutathione and thioredoxin systems, and the exogenous lipoate-dihydrolipoate couple may therefore be considered to be effective regulators of redox-sensitive gene expression. The efficacy of different antioxidants to favorably influence the molecular mechanisms implicated in human disease should be a critical determinant of its selection for clinical studies.—Sen, C. K., Packer, L. Antioxidant and redox regulation of gene transcription. *FASEB J.* 10, 709–720 (1996)**

Key Words: reactive oxygen species · antioxidant therapy · human disease · thiols · nucleic acid · AIDS · atherosclerosis · NF-κB · AP-1

A DELICATE BALANCE BETWEEN intracellular oxidants and antioxidants likely influences health, aging, and longevity. Oxygen free radicals and their by-products that are capable of causing oxidative damage, collectively referred to as active or reactive oxygen species (ROS),[2] may be cytotoxic when produced in excess. However, moderate concentrations of intracellular ROS influence gene expression as well as posttranslational modification of proteins (1). To develop a better understanding of the exact mechanisms that underlie ROS-dependent disorders in biological systems, recent studies have focused on the regulation of gene expression by oxidants, antioxidants, and other determinants of the intracellular reduction-oxidation (redox) state.

At least two well-defined transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), are regulated by the intracellular redox state (**Fig. 1**). NF-κB and AP-1 are implicated in the inducible expression of a wide variety of genes involved in oxidative stress and cellular response mechanisms. Cellular redox state may modify more than just these transcription factors because oxidant challenge has been shown to perturb intracellular Ca^{2+} homeostasis (2). Such an effect may exaberate free radical reactions, activate endonuclease (or endonucleases), and contribute to apoptosis (3). This review selectively summarizes the various steps in NF-κB and AP-1 activation that are currently known to be regulated by oxidants, antioxidants, and other factors that influence intracellular redox status with the aim to further explore the potential of antioxidant therapy.

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[2]Abbreviations: AGE, advanced glycation end product; AIDS, acquired immunodeficiency syndrome; APEX nuclease, apurinin/apyrimidinic endonuclease; ARE, antioxidant response element; BSO, buthionine sulfoximine; DAG, diacylglycerol; EMSA, electrophoretic mobility shift assay; EpRE, electrophile response element; GSH, reduced glutathione; GSSG, glutathione disulfide; HIV, human immunodeficiency virus; HTLV1, human T cell leukemia virus 1; IL-2, interleukin-2; IL-2R, IL-2 receptor; JAK, Janus kinases; LA, α-lipoate; LDL, low-density lipoprotein; MAP, mitogen activated protein; NAC, N-acetylcysteine; PDTC, pyrrolidinedithiocarbamate; PP, protein phosphatase; Ref-1, redox factor-1; ROS, reactive oxygen species; RPTK, receptor-associated protein tyrosine kinase; sIg, surface immunoglobulin; SRE, serum response element; SRF, serum response factor; STAT, signal transducers and activators of transcription; TA, α-tocopherol acetate; TCF, ternary complex factor; TCR, T cell antigen receptor; TNF, tumor necrosis factor; TPA or PMA, 12-*O*-tetradecanoylphorbol 13-acetate; TRE, TPA response element; UV, ultraviolet.

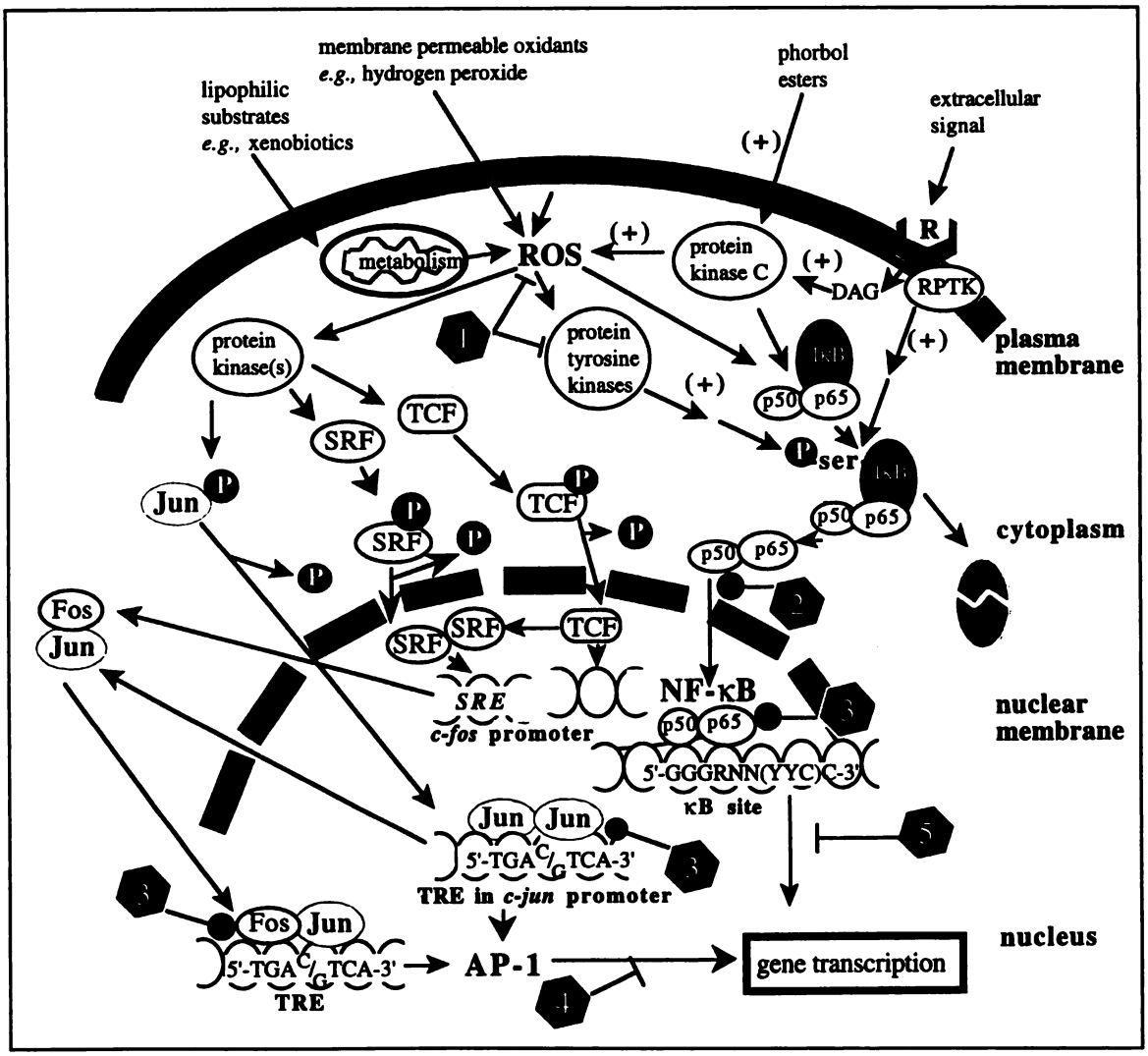

Figure 1. Hypothetical scheme illustrating the steps in cellular NF-κB and AP-1 activation that may be influenced by oxidants and antioxidants. Filled hexagons indicate the antioxidant-regulated steps. *1)* Antioxidants scavenge ROS and also may inhibit certain oxidant sensitive protein tyrosine kinases; *2)* reduced thioredoxin may reduce activated NF-κB proteins, thus facilitating nuclear translocation; *3)* reduced thioredoxin and perhaps also glutathione promote in vitro DNA binding of NF-κB and AP-1; *4)* certain antioxidants, especially phenolic ones, repress AP-1 transactivation; and *5)* as a net effect antioxidants inhibit NF-κB transactivation. DAG, diacylglycerol; RPTK, receptor-associated protein tyrosine kinases; ROS, reactive oxygen species; TCF, ternary complex factor; SRF, serum response factor; SRE, serum response element; TRE, tetradecanoylphorbolacetate (TPA) response element.

NF-κB: A REL FAMILY TRANSCRIPTIONAL ACTIVATION

Rel proteins

The eukaryotic transcription factor NF-κB is a member of the Rel family of proteins that are present in the cytosol in an inactive form. The Rel protein family comprises two groups classified on the basis of their structure, funcion, and mode of synthesis. Group I includes p50 and p52, which are synthesized as precursor proteins of 105 (p105) and 100 (p100) kDa, respectively. Group II comprises p65, Rel or c-Rel, RelB, and the *Drosophila* proteins dorsal and Dif. Rel proteins undergo proteolytic processing to be transformed to mature proteins. Group I mature proteins are characterized by the presence of a Rel homology domain, which is about 300 amino acids in length, and includes a DNA binding domain and a nuclear localization signal. Group II mature Rel proteins possess the Rel homology domain as well as one or more transcriptional activator domain (or domains). Rel/NF-κB family proteins can form homo- or heterodimers. Dimerization is functionally important because it is required for DNA binding of the activated factor (4, 5).

IκB

In the dormant form, two types of Rel protein complexes are found in the cytosol: *1)* a Rel homo- or heterodimer (e.g., p50-p65) bound to a member of the IκB family of inhibitor proteins, and *2)* a complex between a mature Rel group II protein (e.g., p65) and a group I precursor

protein (e.g., p105). The IκB protein family is characterized by the presence of *1)* a partially conserved domain that docks between five to seven ankyrin repeat motifs 33 amino acid long that allow these proteins to interact with the Rel proteins, and *2)* a carboxy-terminal protein destabilizing sequence rich in the amino acids proline, glutamate/aspartate, and serine/threonine, also known as the PEST sequence. The presence of this PEST sequence facilitates proteolytic degradation of IκB proteins (4, 5).

NF-κB activation

Cytosolic NF-κB activation can be brought about by a variety of pathogenic or pathogen-elicited stimuli including cytokines, mitogens, bacteria and related products, virus and viral products, physical stress such as UV and ionizing radiation, oxidants such as H_2O_2, and a variety of chemical agents such as phorbol esters, certain phosphatase inhibitors, etc. These stimuli trigger reactions that lead to the degradation of the bound IκB protein. Loss of the inhibitory IκB protein bound to the Rel protein complex p50-p65 is followed by a rapid translocation of the p50-p65 heterodimer to the nucleus. Under favorable conditions, transcription is switched on after the binding of p50-p65 to *cis*-acting κB sites in the promoters and enhancers of genes (Fig. 1). NF-κB binding is implicated in the activation of several gene families encoding cytokines, cytokine-receptors, cell adhesion molecules, acute-phase proteins, and growth factors, and as a stimulator of viral replication (4, 5).

Clinical implications of NF-κB

One major clinical significance of NF-κB activation is that it is involved in human immunodeficiency virus (HIV) gene expression. The long terminal repeat of HIV-1 has been shown to contain two NF-κB binding sites that may be crucial in regulating AIDS latency (6). Baur et al. (7) reported a dose-dependent inhibitory effect of the natural thiol antioxidant α-lipoate on HIV-1 replication. We were interested in the possible mode of action of α-lipoate on HIV-1 and observed that α-lipoate inhibits NF-κB activation in response to a variety of stimuli in Jurkat human T cells (**Fig. 2**).

NF-κB may also play a role in atherogenesis, because minimally modified human low-density lipoproteins (LDL) result in activation of NF-κB (8). NF-κB can also be activated in response to an atherogenic diet (9) and by advanced glycation end products (AGE; 10). NF-κB binding sites have also been found in genes for various adhesion molecules (11). Furthermore, minimally modified LDL induces NF-κB-regulated genes that encode proteins essential for leukocyte margination and adhesion, events that may be firmly associated with the pathogenesis of the atherosclerotic plaque. AGE-induced NF-κB activation may also be a factor that mediates diabetic vascular complications, as many endothelial genes known to be activated in diabetes are regulated by the κB site (12). Some other target genes that are regulated by NF-κB activity include those that affect acute inflammation responses, cytokine production, and nitric oxide synthase expression (4). Effective strategies to inhibit NF-κB activation should be of therapeutic importance.

ROS AS INDUCERS OF NF-κB ACTIVATION

Activation of NF-κB by ROS

Phosphorylation of IκB is a prerequisite for the activation of NF-κB (4, 5). The possible existence of specific IκB kinases has been hypothesized, however, definite evidence in human cells is not yet available. Numerous studies indicate that ROS may serve as common intracellular agents that contribute to the process of NF-κB activation in response to a diverse range of stimuli (4). Baeuerle and colleagues (4) have developed a Wurzburg subclone of Jurkat T cells that are highly sensitive to micromolar concentrations of hydrogen peroxide. In our laboratory we have also observed that in certain cell types, e.g., Wurzburg T cells and L6 skeletal muscle myotubes, the ROS, hydrogen peroxide, is a sensitive inducer of NF-κB activation. We have also observed that sodium hypochlorite, another oxidant, is capable of weakly inducing NF-κB activation in L6 skeletal muscle myotubes (C. K. Sen, S. Roy, and L. Packer, unpublished observation). Hypochlorite is a physiological microbicidal oxidant produced by stimulated neutrophils during the

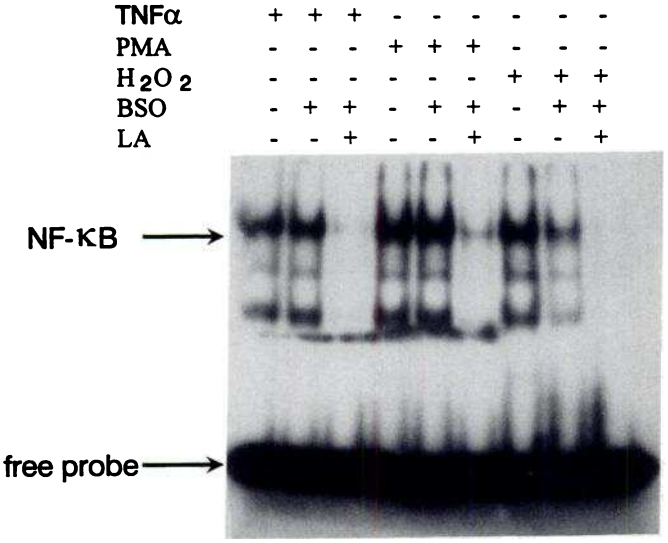

Figure 2. Inhibition of NF-κB activation by α-lipoate is independent of the ability of α-lipoate to enhance the intracellular glutathione level in human T cells. Wurzburg T cells were preincubated with the glutathione synthesis inhibitor buthionine sulfoximine (BSO, 0.2 mM) either in the presence or absence of 0.5 mM α-lipoate for 18 h. Such BSO treatment decreased intracellular GSH level by 80–90%. Although α-lipoate treatment can markedly increase intracellular glutathione levels, this effect is arrested in cells cotreated with BSO and α-lipoate. However, even in glutathione synthesis-arrested cells, α-lipoate inhibited NF-κB activation induced by PMA, TNF-α, or H_2O_2. Cells were harvested after 4 h for nuclear extraction and electrophoretic mobility shift assay (EMSA).

oxidative burst by myeloperoxidase. Interleukin-2 (IL-2) and IL-2 receptor (IL-2Rα) genes contain a κB *cis*-regulatory element in their enhancer regions. Los et al. (13) showed that in T lymphocytes, hydrogen peroxide induces NF-κB activation, IL-2 release, and IL-2R expression.

Los et al. (14) showed that CD-28-mediated activation of NF-κB requires the production of intracellular ROS by 5-lipoxygenase. NF-κB activation by the viral transactivator Tax from human T- cell leukemia virus 1 (HTLV1) has been suggested to be ROS mediated (15). Consistently, HIV-1 Tat protein-dependent potentiation of tumor necrosis factor α (TNF-α) -induced NF-κB activation has been observed to be mediated by changes in cellular redox status (16). Not only ROS themselves but biological derivatives such as oxidatively damaged DNA and advanced glycation end products have also been identified as inducers of NF-κB activation (17). Localization of the exact step (or steps) that are the loci of oxidant action in NF-κB activation is unresolved. The possibility of the presence of oxidant sensitive IκB kinase (or kinases) may not be ruled out, however, concrete evidence is lacking. Available information indicate that several tyrosine kinases are responsive to ROS.

Which form of ROS activates NF-κB?

Hydrogen peroxide has been found to be effective in activating DNA binding of NF-κB in vivo but not in vitro (18). Such an observation suggests that a by-product of hydrogen peroxide in an in vivo situation probably is responsible for the NF-κB binding. Cells overexpressing antioxidant enzymes with a well-defined activity for ROS removal are a valuable tool to test the specific form of ROS that are involved in triggering the activation of NF-κB. In our laboratory, transient catalase ($H_2O_2 \rightarrow H_2O$) overexpression studies with cos-1 cells have shown that hydrogen peroxide may not serve as a messenger for TNF-α or phorbol ester induced NF-κB activation (19). More recently, Schmidt et al. (20) stably overexpressed catalase and cytosolic superoxide dismutase ($O_2^{\cdot-} \rightarrow H_2O_2$) in mouse epidermal JB6 cells. Cells overexpressing catalase were unable to activate NF-κB in response to TNF-α or okadaic acid. The catalase inhibitor aminotriazole restored the NF-κB response. Overexpression of cytosolic superoxide dismutase, which causes cytosolic hydrogen peroxide accumulation, potentiated such NF-κB response. These results from different cells using a different overexpression systems support the hypothesis that hydrogen peroxide and/or its derivatives in certain cell types is involved in the activation of NF-κB.

ROS/ANTIOXIDANT-SENSITIVE PROTEIN PHOSPHORYLATION IN NF-κB ACTIVATION

Oxidant-induced changes in $[Ca^{2+}]_i$ involved in NF-κB activation?

In B cells, hydrogen peroxide increases tyrosine phosphorylation of the insulin receptor and other cellular proteins. This information is of particular relevance in this context because tyrosine kinase activity is known to be required for hydrogen peroxide-induced NF-κB activation. Protein tyrosine kinase inhibitors, e.g., genistein and herbimycin, block such activation (21). In this study, H_2O_2 treatment induced inositol-1,4,5-triphosphate (IP_3) production within 10 s of exposure. IP_3 binds to Ca^{2+} channels, opening the channels and resulting in intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilization. In a recent flow cytometric study with Jurkat T cells, we consistently observed a multifold increase in the $[Ca^{2+}]_i$ within 10–30 s of 0.25 mM hydrogen peroxide treatment (**Fig. 3**). This ROS-induced response was suppressed in antioxidant (α-lipoate pretreated cells (Fig. 3). High $[Ca^{2+}]_i$ is known to contribute to oxidative stress susceptibility by activating Ca^{2+}-dependent intracellular proteolytic processes, which may lead to the breakdown of intracellular proteins and release of protein bound metal ions. Calcium-activated proteases such as calmodulin and calpain play a crucial role in intracellular proteolysis. The thiol protease, m-calpain, is implicated in the specific degradation of a wide variety of transcription factors (22). Because proteolytic processing of IκB proteins is an important step in NF-κB activation, ROS-induced increase in $[Ca^{2+}]_i$ may be a significant step in its induction of NF-κB activation. Serine-threonine phosphorylation is another sensitive regulator of NF-κB activation because okadaic acid and calyculin A, serine/threonine protein phosphatase-1/-2A inhibitors, are strong inducers of NF-κB activation (23). Oxidant-induced $[Ca^{2+}]_i$ may also induce NF-κB activation by activating such phosphorylation processes (24). Recently, NF-κB activation could be inhibited by the intracellular Ca^{2+} antagonist 8-(diethylamino)octyl-3,4,5,-trimethoxybenzoate (20). Taken together, these data suggest that changes in $[Ca^{2+}]_i$ play a crucial role in NF-κB activation.

Src and syk protein tyrosine kinases are redox sensitive

The pattern of protein tyrosine phosphorylation after ROS treatment has striking similarilty with that following surface immunoglobulin (sIg) -dependent physiological stimulation (25). Src family protein tyrosine kinases—e.g., lck, fyn, and lyn—are activated after sIg stimulation. At least two members of the src family, $p56^{lck}$ and $p59^{fyn}$, have been found to be activated by hydrogen peroxide and also by the thiol oxidizing agent diamide (26–28). Another member of the protein tyrosine kinase family that has been observed to be highly responsive to treatment of B cells with hydrogen peroxide is syk (26). Syk was responsive to hydrogen peroxide, UV light, and sIg stimulations, suggesting a common pathway of signal transduction. The antioxidant N-acetylcysteine (NAC) inhibited antigen-mediated syk activation in mast cells (29). In macrophages phorbol ester, zymosan, and β-glucan induced tyrosine phosphorylation, and all were shown to be ROS mediated (30, 31). In fibroblasts, TNF and IL-1 rapidly induced the formation of ROS as well (32). In T

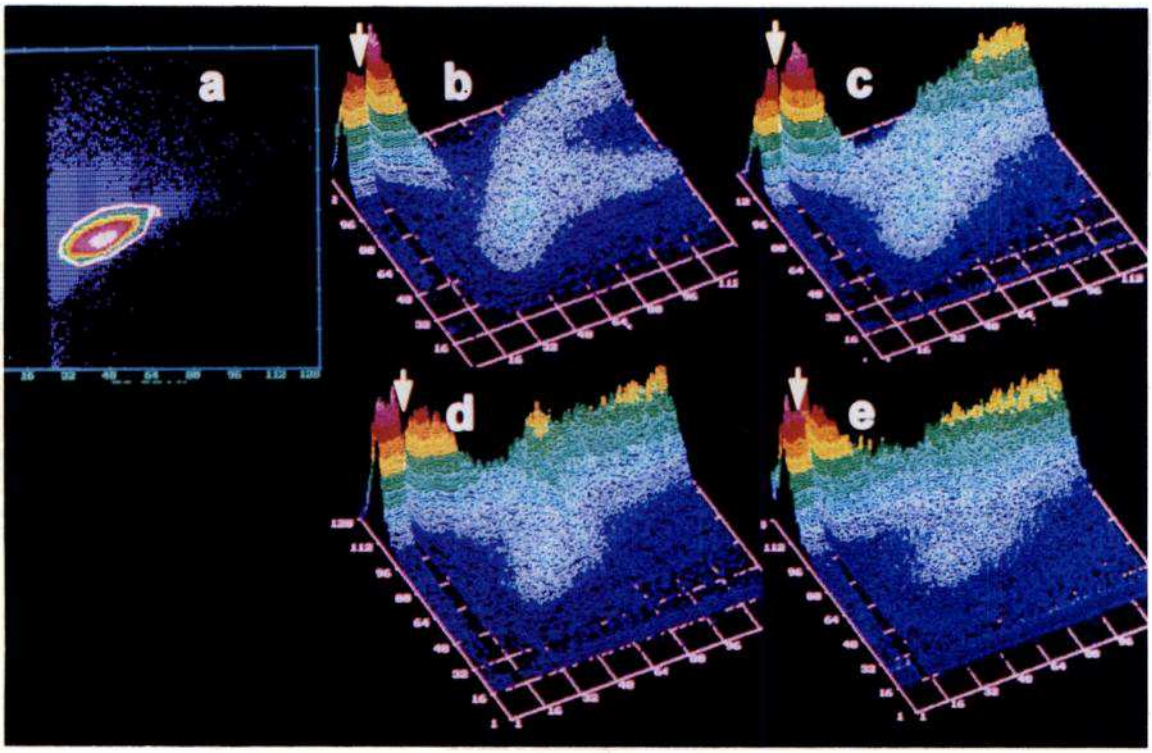

Fig. 3. Oxidant-induced transient increase in cytosolic Ca^{2+} is stabilized in cells pretreated with α-lipoate. A gated (*a*) homogenous group of human Jurkat T cells was studied. Cells were treated with either 0 (*b*), 1 mM (*c*), 2 mM (*d*), or 5 mM (*e*) α-lipoate for 18 h. Cells were then washed once and loaded with indo-1 (Molecular Probes). Real time changes in intracellular Ca^{2+} were followed using a fluorescence-activated cell sorter (EPICS ELITE XL, Coulter). The ratio of fluorescence intensities at 510 and 405 nm in response to a 350 nm UV laser excitation was continuously followed for 10 min at a flow rate of 250–300 cells/s. After 45 s of baseline measurement, cells were challenged with 0.25 mM H_2O_2 (indicated by an arrow). Experiments were carried out in the presence of 1 mM of the extracellular calcium chelator ethyleneglycoltetraacetic acid to follow oxidant induced mobilization of intracellular calcium reserves. In the contour plots *b–e*, the abscissa represents time. The entire X-axis represents 600 s split into 8 equal segments. The ordinate represents the unbound/bound indo-1 ratio. Thus, a downward deflection of the ratio illustrated in the figure represents an increase in cytosolic Ca^{2+}. Elevation of the contour from the plane of the paper (i.e., Z-axis) represents relative cell number. The colors (*a–e*) represent the relative cell number at any given point on the plot. From the violet to the red end of the visible spectrum, colors represent progressively increasing cell densities. Source: C. K. Sen, S. Roy, and L. Packer, unpublished results.

cells, a syk-related tyrosine kinase ZAP-70 associated with the antigen receptor (TCR) is highly responsive to hydrogen peroxide (33). Both syk and ZAP 70 contain SH2 domains and are therefore expected to participate in the complex SH2-mediated signaling cascade. Immunoprecipitated syk family kinases were not responsive to oxidants or antioxidants, indicating that these kinases may not be directly regulated by ROS (26). It may thus be hypothesized that only certain cellular syk family kinase regulatory components are sensitive to ROS. For example, ZAP 70 may be activated after phosphotyrosine-phosphatase inhibition (34). All protein-tyrosine phosphatases have reactive cysteine residues in their active site, a feature that makes some of these enzymes oxidant sensitive. In vitro studies (35–37) have shown that comparable to vanadate, hydrogen peroxide selectively inhibits phosphotyrosine phosphatase activity.

JAK-STAT pathways are involved in the regulation of src and syk tyrosine kinases

$p56^{lck}$ is associated with CD4-CD8 surface molecules and IL-2 receptor β-chains, and $p59^{fyn}$ is associated with the T cell receptor/CD3 complex. Surface receptor-associated protein tyrosine kinases, especially the Janus kinases or JAK, directly modify latent cytoplasmic forms of the STAT (signal transducers and activators of transcription) family of transcription factors. Tyrosine-phosphorylated STAT factors appear in the nucleus, bind to DNA, and activate transcription (38). The JAK-STAT system is known to activate the ROS-sensitive tyrosine kinases that are possibly involved in NF-κB activation. It has been shown that JAK1, JAK2, and JAK3, as well as STAT3 and STAT5, are involved in the cytokine receptor-mediated activation of lck, fyn, and syk tyrosine kinases (39).

ANTIOXIDANTS INHIBIT NF-κB ACTIVATION

Phenol and catechol antioxidants

Inhibition of induced NF-κB activation by antioxidants confirms the involvement of ROS in the activation process. Antioxidants alone are not effective enough to influence NF-κB function (40). However, studies in our

laboratory and in others reveal that a variety of structurally diverse antioxidants are capable of inhibiting NF-κB activation induced by a wide range of stimuli ranging from phorbol esters to TNF-α, to hydrogen peroxide. This evidence lends further support to the hypothesis that ROS are involved in a common pathway of NF-κB activation. Studies from our laboratory have shown that vitamin E derivatives inhibit TNF-α-induced NF-κB activation in human Jurkat T cells. Incubation of the cells with varying concentrations (0.01–1 mM) of α-tocopherol acetate or α-tocopherol succinate resulted in a concentration-dependent inhibition of NF-κB activation. Only 10 μM of pentamethylhydroxychromane, a vitamin E derivative lacking the phytyl tail, was sufficient to obtain a complete inhibition of such NF-κB activation (41). α-Tocopheryl succinate not only inhibited activation and translocation of NF-κB to the nucleus, but was also capable of inhibiting the in vitro binding of the activated proteins to the κB DNA site (42). Catechol derivatives with characterized antioxidant properties, e.g., nitecapone and OR-1246, were also able to inhibit TNF-α-induced NF-κB activation in Jurkat cells (43).

Thiol antioxidants

Intracellular thiol redox status appears to be a critical determinant of NF-κB activation. At low levels of cytosolic glutathione disulfide (GSSG), T cells fail to activate NF-κB in response to appropriate stimuli, whereas high GSSG concentration inhibits the binding of activated NF-κB to its cognate DNA site. Thus, it appears that an intermediate optimal level of intracellular GSSG is required for effective NF-κB activation (44). Droge et al. (44) have found that glutathione (GSH) deficiency of T cells is associated with a suppression of NF-κB function. This effect is perhaps related to very low levels of GSSG in GSH-deficient cells.

Another physiologically relevant thiol that plays a crucial role in the regulation of NF-κB function is reduced thioredoxin, an important cellular protein oxidoreductase with antioxidant functions. Transient expression and exogenous addition of thioredoxin cause a dose-dependent inhibition of phorbol ester-induced NF-κB activation in human cervical carcinoma HeLa cells (45). However, we observed that in L6 skeletal muscle myotubes exogenous thioredoxin potentiates TNF-α or hydrogen peroxide-induced NF-κB activation (unpublished observation). Consistent with our observation, it has been found that thioredoxin up-regulates DNA binding of activated NF-κB in vitro and augments gene expression from NF-κB-regulated, HIV long terminal repeat in intact cells (27, 46, 47). Thioredoxin, also known as adult T cell leukemia-derived factor in humans, is overproduced in HTLV-1 transformed T cell lines in which NF-κB is constitutively activated. After the loss of IκB, the Rel proteins of NF-κB require further reduction to acquire its full DNA binding activity. Intracellular production of thioredoxin may be increased after transient oxidant challenge by H_2O_2, TNF, or other agents. Thioredoxin may be implicated in reducing activated cytosolic NF-κB, and thus potentially contribute to its nuclear translocation and DNA binding (27).

In recent years, α-lipoate has been identified as a therapeutically important biological antioxidant (48). We observed that pretreatment of Wurzburg T cells for 18 h with α-lipoate suppresses NF-κB activation induced by either hydrogen peroxide, phorbol ester, or TNF. Previous results from our laboratory have shown that α-lipoate pretreatment of T cells results in a 100% increase in intracellular glutathione reserves (48). Using glutathione synthesis-arrested Wurzburg T cells, we observed that the effect of α-lipoate on NF-κB was not the result of its proglutathione effect (Fig. 2). Other reducing thiol agents are also known to influence NF-κB activation. For example, pyrrolidinedithiocarbamate (PDTC) inhibits the induced degradation of IκB-α, as shown with TNF-α and phorbol ester stimulation of Jurkat T cells (49–51). Micromolar concentrations of PDTC can also inhibit NF-κB activation induced by the viral transactivator Tax from HTLV1 (15). N-acetylcysteine, a proglutathione antioxidant, inhibited activation of NF-κB induced by UV light (52). Dithiolthiones, thiol antioxidants with established anticarcinogenic and proglutathione properties, also inhibit NF-κB activation in response to oxidant and other stimuli (53).

Okadaic acid-induced NF-κB activation appears to be cell specific

The antioxidants N-acetylcysteine and dihydrolipoate have been shown to inhibit NF-κB activation in Jurkat cells induced by TNF-α, phorbol ester, and lymphotoxin. However, these antioxidants failed to inhibit NF-κB activation in human Jurkat T cells and U937 monocytic cells treated with okadaic acid (75 ng/ml) or calyculin A (25 ng/ml). Thus, okadaic acid and calyculin A, which are serine/threonine protein phosphatase-1/-2A inhibitors, apparently activated NF-κB in an antioxidant-insensitive protease-sensitive manner (23). It was therefore proposed that inhibition of PP-1/PP-2A by ROS in Jurkat or U937 cells is not the mechanism of NF-κB activation by TNF-α, phorbol ester or lymphotoxin. Further studies in our laboratory indicate that the mode of activation of NF-κB by okadaic acid may be cell specific. In the Wurzburg subclone of Jurkat T cells, okadaic acid-induced NF-κB activation was observed to be partially inhibited by 30 mM N-acetylcysteine. However, 1 mM α-tocopherol acetate revealed no such inhibitory effect (**Fig. 4**). A recent study by Schmidt et al. (20) demonstrated that in HeLa cells, induction of NF-κB activation by 0.7 μM (i.e., 575 ng/ml) okadaic acid could be inhibited by 0.1 mM of the antioxidant PDTC as well as by 20 mM N-acetylcysteine. The authors showed that treatment of HeLa cells with 575 ng/ml okadaic acid increased intracellular ROS level by 2- to 2.5-fold. Investigations using HeLa and F26 human fibroblast primary cells showed that okadaic acid

treatment increased the level of intracellular ROS in a dose-dependent manner, however, the concentration of okadaic acid required to obtain maximal effect was high, about 0.9 μM or 740 ng/ml (20).

BINDING OF ACTIVATED NF-κB TO ITS COGNATE DNA SITE IS REDOX SENSITIVE

Cysteine residue in a short amino acid stretch of Rel proteins regulates NF-κB DNA binding

Evidence discussed in the previous section indicates that NF-κB activation, i.e., dissociation of IκB and translocation of the activated Rel protein dimer to the nucleus, are antioxidant inhibitable in response to almost all types of stimuli. Antioxidants, e.g., reduced thiols, however, have a contrasting effect on the binding of the Rel protein complex to the cognate DNA site. Reduced thiols, including dithiothreitol, cysteine, dihydroplipoate, and reduced thioredoxin, enhance the DNA binding of activated NF-κB (27, 46, 47, 54, 55). The highly conserved Rel homology domain is responsible for DNA binding of NF-κB. A short stretch of amino acids (the RXXRXRXXC motif, R = arginine, C = cysteine, X = other amino acid) at the beginning of the domain is essential to contact DNA directly (56–58). The cysteine residue in the motif is critical and must be maintained in a reduced state to allow DNA binding because oxidation of this cysteine residue interferes with DNA binding of NF-κB (27, 47, 56–58). Perhaps reduced thiols enhance DNA binding of NF-κB by maintaining this critical cysteine residue in a reduced state (47).

DNA repair enzyme modulates NF-κB DNA binding

Another cellular redox mechanism that may independently or in synergism with the thioredoxin system help to enhance NF-κB DNA binding is the apurinin/apyrimidinic endonuclease system or APEX nuclease (59), also known as redox factor-1 or Ref-1 (60). Apurininc/apyrimidinic sites are generated in DNA as result of spontaneous hydrolysis or oxidative damage and the subsequent action of DNA glycosylases removing the modified DNA bases. Such sites are the most frequent lesion found in cellular DNA, and amount to more than 10^4 residues per mammalian cell per day. The nuclease activity of Ref-1 is specific for the initial repair of DNA templates damaged by various noxious stimuli: ROS, UV light, and ionizing radiation nucleases.

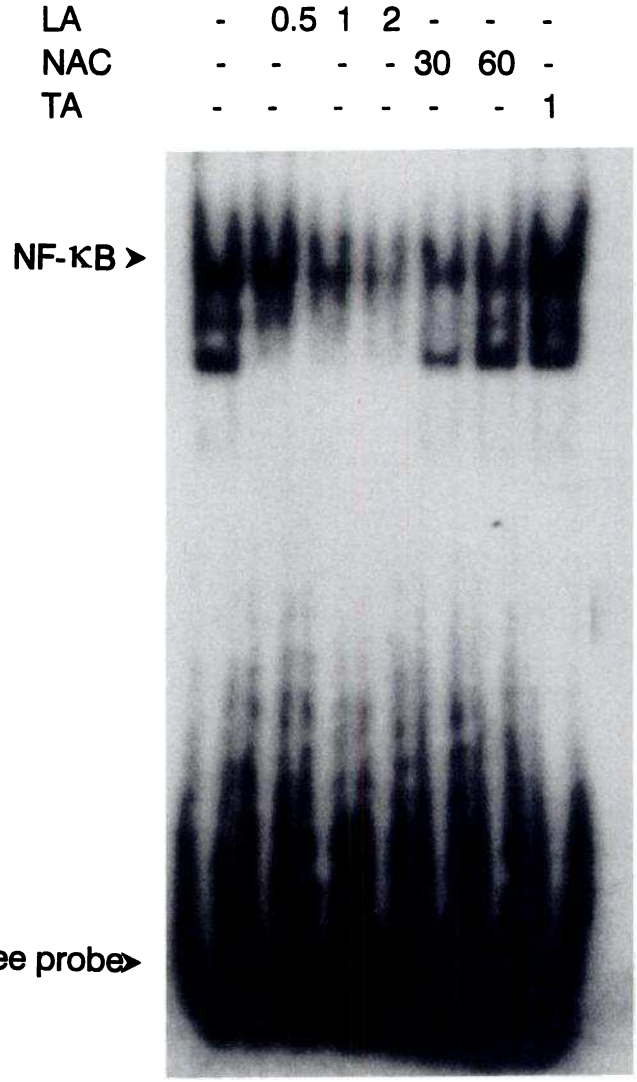

Figure 4. Okadaic acid induced NF-κB activation in Wurzburg T cells is antioxidant sensitive. Cells were pretreated with α-lipoate (LA, 18 h), N-acetyl-L-cysteine (NAC, 2 h), or α-tocopherol acetate (TA, 2 h) and then treated with 50 ng/ml okadaic acid for 2 h, after which they were harvested for nuclear extraction and then subjected to EMSA.

TRANSCRIPTIONAL ACTIVATION BY AP-1

Fos and Jun proteins, and their interaction

AP-1 is an important mediator of tumor promotion (see refs 61 and 62), and thus is a focal point in cancer research. Interaction between cJun and cFos proteins, products of c-*jun* and c-*fos* protooncogenes, via a "leucine-zipper" domain has been observed to have crucial regulatory implications on the expression of a wide variety of genes, especially growth factor-inducible genes (61). cJun allows cFos to regulate gene expression by serving as an anchor that allows the Fos-Jun heterodimer to bind to a cognate DNA site. In addition, cJun contains three short regions in its amino-terminal half that are important for transcriptional activation in vivo. Dimerization of Fos-Jun or Jun-Jun is a prerequisite for DNA binding; however, such interaction is not limited only to cFos and cJun. Other members of the Fos and Jun family of proteins are also known to interact with each other. Members of the Fos family identified so far include vFos, cFos, FosB, Fra-1 (Fra, Fos-related antigen), and possibly Fra-2. At present, the Jun family is comprised of vJun, cJun, JunB, and JunD. All the Jun family proteins are capable of forming homo- and heterodimers, which are capable of binding to

a cognate DNA site. Fos proteins do not associate with each other but are capable of associating with any member of the Jun family to form stable dimers that have higher DNA binding acitivity than Jun dimers. Despite the high degree of sequence conservation of Fos and Jun proteins, certain Fos-Jun combinations appear to be more active in DNA binding than others (61). Although cFos containing heterodimers activate AP-1 target genes, Fra-containing heterodimers do not (63).

AP-1 DNA binding

DNA binding of Fos-Jun or Jun-Jun complexes, or AP-1, is governed by a "basic region" found immediately upstream of the "leucine-zipper" domain. Binding of the dimers to a 12-*O*- tetradecanoylphorbol 13-acetate (TPA) response element (TRE) of several cellular and viral genes is a prerequisite for AP-1-induced transcriptional regulation. TPA is a remarkable activator of protein kinase C and other agents that lead to protein kinase C activation such as serum and growth hormones. cFos proteins and mRNA have shorter half-lives than cJun proteins and mRNA. As a result, before induction most of the AP-1 complex is present in the form of Jun homo- and heterodimers. After induction, Fos proteins are expressed and most of the induced AP-1 are Fos-Jun heterodimers. The differential regulation of the c-fos and c-*jun* genes supports the contention that the function of cFos induction is to generate an initial spike of transcriptional activation, whereas cJun is responsible for a steady sustained AP-1 activity. The *cis*-acting element that mediates cFos expression in response to activation by serum, certain growth factors, UV irradiation, and TPA is a 20 bp dyad symmetry or serum response element (SRE) located in the c-*fos* promoter. A 67 kDa transcription factor serum response factor (SRF) is phosphorylated and binds to the SRE as a homodimer. This DNA binding of SRF is enhanced by the physical interaction of a phosphorylated 62 kDa ternary complex factor (TCF) protein that binds to a second DNA binding site adjacent to SRE. cJun expression is driven by the binding of preexisting AP-1 proteins to a TRE in the c-*jun* promoter (Fig. 1). For a description of AP-1 and its mode of action, see ref 61.

Clinical implications of AP-1

A collapse of the regulatory mechanisms that govern normal growth and development is manifested as neoplasia. Insights into the molecular basis of such events have been gained through the biochemical and genetic study of protooncogenes, the progenitors of retroviral transforming genes. Apart from being implicated in tumor promotion, AP-1 activation is involved in disorders of the nervous system. Most neuronal tissues express basal levels of AP-1, which increase dramatically in response to certain stimuli. Electric stimulation or drug-induced seizure induces AP-1 activation in the hippocampus, which in turn enhances growth factor mRNA expression. Stimulation or blocking of dopamine receptors is another signal that activates AP-1 DNA binding activity depending on which type of dopamine receptor is expressed in the responding cell. Strategies to pharmacologically manipulate AP-1 transcriptional activity are expected to be of therapeutic importance (64).

INDUCTION OF AP-1 ACTIVATION BY ROS

Direct evidence suggesting the involvement of ROS in AP-1 activation

Evidence suggesting the direct involvement of ROS in AP-1 activation have been obtained mostly by using defined oxidative stress generating systems to challenge cultured cells. Studies from a number of laboratories have demonstrated that superoxide produced by a xanthine/xanthine oxidase system and hydrogen peroxide induce the expression of several early response genes including c-*fos* and c-*jun*, the major components of AP-1 (65-69).

Involvement of ROS in AP-1 activation induced by various other stimuli

Recently it was shown that growth factor-induced AP-1 activation is also ROS dependent. TNF-α and basic fibroblast growth factor induced ROS production, which acted as a common signal to stimulate c-*fos* gene expression (70). Angiotensin II-induced AP-1 DNA binding and proliferative hypertrophic responses in skeletal muscle-derived cells are ROS mediated (71). Mitogenic stimulation is also known to induce oxidative stress. After 30–60 min of such stimulation, a buildup of intracellular ROS has been detected (72). Scavengers of the most dangerous form of ROS, the $^{\cdot}OH$, inhibited lymphocyte mitogenesis (73). Mitogen-induced AP-1 activation has been observed to be antioxidant inhibitable and has thus been suggested to be ROS mediated (74). ROS is also involved in the activation of AP-1 by ionizing radiation (75). Oxidative stress caused by ionizing radiation and hydrogen peroxide challenge was a potent inducer of c-*jun* expression. This effect was observed to be protein kinase C dependent and was evident in both normal and tumor human cells (69).

How do ROS activate AP-1?

Not much is known about the exact mechanisms underlying ROS-dependent AP-1 activation. Perturbation of cellular thiol redox status has been suggested to be a signal that may be implicated in the induction of c-*fos* and c-*jun* expression caused by asbestos-induced oxidative stress (76). In support of this, high intracellular glutathione disulfide has been shown to be implicated in AP-1 activation (54). It has been also suggested that AP-1 activation under oxidative conditions may be, at least in part, mediated by phosphorylation of Jun proteins (67, 77–79). In vascular smooth muscle cells, micromolar concentrations

of hydrogen peroxide has been shown to enhance protein kinase C activity by more than 100% in Go cells, and by more than 200% in G1 cells. Especially in the Go cells, hydrogen peroxide and TPA had an additive effect on protein kinase C stimulation (80). Despite its remarkable effect on protein kinase C activity, hydrogen peroxide did not activate AP-1 binding to DNA. In contrast, hydrogen peroxide suppressed TPA-induced AP-1 activation. Hydrogen peroxide was able to trigger AP-1 activation in cells that were treated to down-regulate protein kinase C activity. These results suggest that hydrogen peroxide influences AP-1 activation in a manner distinctly unique compared to the mode of TPA-induced AP-1 activation (80).

Information available regarding the pathway (or pathways) involved in ROS-induced AP-1 activation suggests that SRF binding to SRE, and TCF phosphorylation are involved in such processes. Both SRE and AP-1 sites in c-*fos* promoter have been shown to be implicated in upregulating cFos expression in response to hydrogen peroxide challenge (81). Consistent with the notion of the presence of ROS sensitive kinase cascade (or cascades), oxidative stress-sensitive mitogen-activated protein (MAP) kinase, and MAP kinase phosphatase have been evident (82).

AP-1 ACTIVITY IS ANTIOXIDANT SENSITIVE

Phenolic antioxidants alone enhance AP-1 DNA binding but repress transactivation

Unlike the regulation of NF-κB activation, certain antioxidant compounds alone are capable of influencing AP-1 activation. Phenolic antioxidants exhibit antiinflammatory, antiatherosclerotic, and anticarcinogenic activities (83). Paradoxically, phenolic antioxidants, e.g., butylated hydroxytoluene and butylated hydroxyanisole alone, have been observed to substantially increase the expression of the protooncogenes c-*fos* and c-*jun* mRNA and to induce AP-1 DNA binding (84). That phenolic compounds may induce AP-1 DNA binding has been supported by another independent observation that d-α-tocopherol alone induces such activation in a protein kinase C-independent manner in vascular smooth muscle cells (80). Such an effect of the phenol was not observed with d-β-tocopherol, suggesting that the effect may not be dependent on the antioxidant property of d-α-tocopherol.

A role for c-*fos* and c-*jun* in the specific activation of gene expression, mediated by a complex site called the electrophile response element (EpRE) found in the mouse glutathione S-transferase Ya-subunit gene, has been suggested (85). This EpRE includes a smaller site called the antioxidant response element (ARE), which was originally identified in the rat homolog of the same gene and in the NADP(H) quinone reductase gene. The ARE consensus sequence contains a part that is similar to the AP-1 consensus. Moreover, EpRE and ARE are known to be bound by cFos-cJun and cJun, respectively, indicating that AP-1 may be implicated in the electrophile and antioxidant responses.

A recent finding confirms that although AP-1 may bind to and activate ARE containing promoters, it is not the major ARE binding protein (86). The yet uncharacterized ARE binding major protein-ARE complex had increased 5% nondenaturing gel mobility compared with the AP-1-ARE complex. Although as observed in previous studies, the phenolic antioxidant *tert*-butyl hydroquinone alone activated AP-1 DNA binding, it did not affect AP-1 transcriptional activity. *Tert*-butylhydroquinone induced c-*jun*, *junB*, fra-1, and *fra*-2, but was a poor inducer of c-*fos* and had no effect of *fosB*. As a result of such an effect, AP-1 dimers formed were deficient in cFos and FosB and were mainly comprised of Fra proteins heterodimerized with Jun protein. Such Fra-Jun complexes are stable and bind to TRE but have very low AP-1 transcriptional activation effects (63). Butylhydroquinone-induced AP-1 DNA binding thus did not have significant effects on the transcriptional responses. In addition, Fra-1 protein was observed to repress AP-1 transcriptional activity induced by TPA or expression of cFos and cJun (86). TPA-induced AP-1 transcriptional activity was repressed in a dose-dependent manner by *tert*-butylhydroquinone. Such a finding was consistent with a previous report that although d-α-tocopherol alone activates AP-1 DNA binding, it strongly inhibits AP-1 activation caused by TPA (80). The suppressive effect of *tert*-butylhydroquinone was specific for AP-1-dependent promoters activated by TPA and was not due to a general inhibition of translation or transcription. Because AP-1 is an important mediator of tumor promotion, it appears that phenolic antioxidants may interfere with tumor promotion by triggering the expression of Jun-Fra heterodimers that repress AP-1 transcriptional activity (86).

AP-1 function is responsive to thiol antioxidants

Inconsistent results have been obtained from different laboratories investigating the effect of thiol antioxidants on AP-1 activation. N-acetylcysteine inhibited a tyrosine-kinase-dependent induction of cJun expression, indicating that the loci of action of different forms of antioxidants are specific to their chemical class (87). Inhibition of AP-1 activation by NAC has also been observed in cells challenged with a carcinogen, phenobarbital (88). More recently, NAC has been observed to ameliorate the induction of c-*fos* and c-*jun* mRNAs caused by asbestos-induced oxidative stress (76). NAC also prevented c-*jun* mRNA expression induced by hydrogen peroxide or radiation (69). Regulation of Fos and Jun expression via direct interaction of oxidants and antioxidants with specific cysteine groups of these polypeptides had been evident previously (89, 90). One or more proteins that possibly interact with SRE (91) during AP-1 activation may be sensitive to intracellular changes in thiol redox milieu caused by antioxidants. It

has been consistently found that AP-1 transactivation is influenced by intracellular thioredoxin and glutathione status (40, 45). However, some results available from different experimental systems indicate that the mode of AP-1 regulation by reduced thiols is opposite from the regulation of NF-κB. Overexpression of graded amounts of thioredoxin in cells increased TPA-induced AP-1 transcriptional activity in a dose-dependent manner. The effect was shown to be specific to the antioxidant property of thioredoxin (40, 45). These studies also showed that other reducing thiol agents (PDTC and NAC) enhance DNA binding activity and transactivation of AP-1. PDTC did not influence protein kinase C activity indicating that the antioxidant probably acted downstream of and independently from protein kinase C (40). Elevation of intracellular levels of GSSG before stimulation increases AP-1 transactivation (54).

AP-1 DNA BINDING ACTIVITY IN VITRO IS REDOX REGULATED

Cys in Lys-Cys-Arg of Fos and Jun proteins regulate AP-1 DNA binding

Fos and Jun DNA binding in vitro is regulated by the reduction-oxidation of a single conserved cysteine residue (Lys-Cys-Arg) in the DNA binding domains of the two proteins. The requirement of a single cysteine residue and the sensitivity of Fos and Jun to the -SH alkylating agent N-ethylmaleimide excludes the possibility that oxidation of the cysteine residue involves intra- and intermolecular disulfide bond formation. It was suggested that conversion of the cysteine to reversible oxidation products such as sulfenic (RSOH) or sulfinic (RSO_2H) acids could contribute to the regulation of DNA binding (92).

Regulation of AP-1 DNA binding by thiols and the DNA repair enzyme Ref-1

A hepatic nuclear protein has been recognized to reduce Fos and Jun and stimulate AP-1 DNA binding. This effect of the nuclear protein could be considerably stimulated by reduced thioredoxin (92). Further studies with HeLa nuclear extracts revealed the existence of a ubiquitous nuclear redox factor Ref-1 (60), also known as APEX nuclease (59). Ref-1, the protein product of the *ref-1* gene, and other chemical reducing agents stimulate AP-1 DNA binding in vitro by acting on the regulatory cysteine residue of Lys-Cys-Arg. Replacement of the critical cysteine residue of a truncated Fos protein by serine resulted in a threefold increase in AP-1 DNA binding activity that was no longer redox regulated. Such observations indicate that redox regulation of AP-1 DNA binding limits the total level of Fos-Jun in vivo and that escape from this control enhances transforming activity (93). Oxidized thioredoxin and GSSG inhibit AP-1 DNA binding in vitro, the effect being most pronounced with the former.

SUMMARY AND CONCLUSIONS

The regulation of gene expression by oxidants, antioxidants, and the redox state has emerged as a novel subdiscipline in molecular biology that has promising therapeutic implications. Binding sites of the redox-regulated transcription factors NF-κB and AP-1 are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of diseases: AIDS, cancer, atherosclerosis, and diabetic complications. The role of ROS as signal transducing molecules has been convincingly supported by many observations. Very recently H_2O_2 has been shown to be required in platelet-derived growth factor signal transduction further supporting the contention that antioxidants may be effective cardioprotective agents (94). Biochemical and clinical studies have indicated that antioxidant therapy may be useful in the treatment of a wide variety of diseases. Such indications provide a strong rationale for subjecting antioxidant compounds to further scrutiny in order to unveil the molecular basis of oxidant/antioxidant action. Information obtained thus far from such studies clearly indicates that critical steps in the signal transduction cascade are sensitive to oxidants and antioxidants. Perhaps many of the basic events of cell regulation such as protein phosphorylation and binding of transcription factors to consensus sites on the DNA, are driven by the physiological oxidant-antioxidant homeostasis, especially by the thiol-disulfide balance. Endogenous glutathione and thioredoxin systems, and the exogenous lipoate-dihydrolipoate couple may therefore be considered to be effective regulators and investigative tools in the elucidation of redox sensitive gene expression. The efficacy of different antioxidants to favorably influence the molecular mechanisms known to be implicated in the etiology of human disease should be a critical determinant of their selection for clinical studies. FJ

Research supported by NIH grants CA 47597-07 and GM 27345-15. We are thankful to Dr. Takashi Okamoto of the Nagoya City University Medical School, Japan, and to Dr. Maret G. Traber for review comments. Because of space restriction, many studies were only indirectly cited and details not related to antioxidant/redox regulation were not covered.

REFERENCES

1. Sies, H. (1991) *Oxidative Stress. Oxidants and Antioxidants*, pp. 650, Academic Press, London.
2. Trump, B. F., and Berezesky, I. K. (1992) The role of cytosolic Ca^{2+} in cell injury, necrosis and apoptosis. *Curr. Opin. Cell Biol.* **4**, 227–232
3. Aagaard-Tillery, K. M., and Jelinek, D. F. (1995) Differential activation of a calcium-dependent endonuclease in human B lymphocytes. Role in ionomycin induced apoptosis. *J. Immunol.* **155**, 3297–3307
4. Baeuerle, P. A., and Henkel, T. (1994) Function and activation of NF-κB in the immune system. *Annu. Rev. Immunol.* **12**, 141–179
5. Thanos, D., and Maniatis, T. (1995) NF-κB: a lesson in family values. *Cell* **80**, 529–532
6. Griffin, G. E., Leung, K., Folks, T. M., Kunkel, S., and Nabel, G. J. (1989) Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. *Nature (London)* **339**, 70–73
7. Baur, A., Harrer, T., Peukert, M., Jahn, G., Kalden, J. R., and Fleckenstein, B. (1991) Alpha-lipoic acid is an effective inhibitor of human immuno-deficiency virus (HIV-1) replication. *Klin. Wochenschr.* **69**, 722–724
8. Parhami, F., Fang, Z. T., Fogelman, A. M., Andalibi, A., Territo, M. C., Berliner, J. A. (1993) Minimally modified low density lipoprotein-induced

inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. *J. Clin. Invest.* **92**, 471–478
9. Liao, F., Andalibi, A., deBeer, F. C., Fogelman, A. M., and Susis, A. J. (1993) Genetic control of inflammatory gene induction and NF-κB-like transcription factor activation in response to an atherogenic diet in mice. *J. Clin. Invest.* **91**, 2572–2579
10. Yan, S. D., Schmidt, A. M., Anderson, G. M., Zhang, J., Brett, J., Zou, Y. S., Pinsky, D., and Stern, D. (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J. Biol. Chem.* **269**, 9889–9897
11. Chen, C. C., Rosenbloom, C. L., Anderson, D. C., and Manning, A. M. (1995). Selection inhibition of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by inhibitors of IκB-α phosphorylation. *J. Immunol.* **155**, 3538–3545
12. Collins, T. (1993) Endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. *Lab. Invest.* **68**, 499–508
13. Los, M., Droge, W., Stricker, K., Baeuerle, P. A., and Sculze-Osthoff, K. (1995) Hydrogen peroxide as a potent activator of T lymphocyte functions. *Eur. J. Immunol.* **25**, 159–165
14. Los, M., Schenck, H., Hexel, K., Baeuerle, P. A., Droge, W., and Schulze-Osthoff, K. (1995) IL-2 gene expression and NF-κB activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J.* **14**, 3731–3740
15. Schreck, R., Grassman, R., Fleckenstein, B., and Baeuerle, P. A. (1992) Antioxidants selectively suppress activation of NF-κB by huuman T-cell leukemia virus type I Tax protein. *J. Virol.* **66**, 6288–6293
16. Westendorp, M. O., Shatrov, V.A., Schulze-Osthoff, K., Frank, R., Kraft, M., Los, M., Krammer, P. H., Droge, W., and Lehmann, V. (1995) HIV-1 Tat potentiates TNF-induced NF-κB activation and cytotoxicity by altering the cellular redox state. *EMBO J.* **14**, 546–554
17. Legrand-Poels, S., Bours, V., Piret, B., Pflaum, M., Epe, B., Rentier, B., and Piette, J. (1995) Transcription factor NF-κB is activated by photosensitization generating oxidative DNA damage. *J. Biol. Chem.* **270**, 6925–6934
18. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of NF-κB transcription factor and HIV-1. *EMBO J.* **10**, 2247–2258
19. Suzuki, Y. J., Mizuno, M., and Packer, L. (1995) Transient overexpression of catalase does not inhibit TNF- or PMA-induced NF-κB activation. *Biochem. Biophys. Res. Commun.* **210**, 537–541
20. Schmidt, K. N., Traencker, E. B.-M., Meier, B., and Baeruerle, P. A. (1995) Induction of oxidative stress by okadaic acid is required for activation of transcription factor NF-κB. *J. Biol. Chem.* In press
21. Schieven, G. L., Kirihara, J. M., Burg, D. L., Geahler, R. L., and Ledbetter, J. A. (1993) $p72^{syk}$ tyrosine kinase is activated by oxidizing conditions which induce lymphocyte tyrosine phosphorylation and Ca^{2+} signals. *J. Biol. Chem.* **268**, 16688–16692
22. Watt, F., and Molloy, P. L. (1993) Specific cleavage of transcription factors by the thiol protease, m-calpain. *Nucleic Acid Res.* **21**, 5092–5100
23. Suzuki, Y. J., Mizuno, M., and Packer, L. (1994) Signal transduction for NF-κB activation: proposed location of antioxidant-inhibitable step. *J. Immunol.* **153**, 5008–5015
24. Gopalakrishna, R., and Anderson, W. B. (1991) Reversible oxidative activation and inactivation of protein kinase C by the mitogen/tomor promoter periodate. *Arch. Biochem. Biophys.* **285**, 382–387
25. Schieven, G. L., and Ledbetter, J. A. (1993) Ultraviolet radiation induces differential calcium signals in human peripheral blood lymphocytes subsets. *J. Immunother.* **14**, 221–225
26. Schieven, G. L., Kirihara, J. M., Myers, D. E., Ledbetter, J. A., and Uckun, F. M. (1993) Reactive oxygen intermediates activate NF-κB in a tyrosine kinase dependent mechanism and in combination with vanadate activate the $p56^{lck}$ and $p59^{fyn}$ tyrosine kinases in human lymphocytes. *Blood* **82**, 1212–1220
27. Hayashi, T., Ueno, Y., and Okamoto, T. (1993) Oxidoreductive regulation of nuclear factor κB. Involvement of a cellular reducing catalyst thioredoxin. *J. Biol. Chem.* **268**, 11380–11388
28. Nakamura, K., Hori, T., Sato, N., Sugie, K., Kawakami, T., and Yodoi, J. (1993) Redox regulation of a src family protein tyrosine kinase p56lck in T cells. *Oncogene* **8**, 3133–3139
29. Valle, A., and Kinet, J.-P. (1995) N-Acetyl-L-cysteine inhibits antigen-mediated Syk, but not Lyn tyrosine kinase activation in mast cells. *FEBS Lett.* **357**, 41–44
30. Zor, U., Ferber, E., Gergely, P., Szucs, K., Dombradi, V., and Goldman, R. (1993) Reactive oxygen species mediate phorbol ester-regulated tyrosine phosphorylation and phospholipase A2 activation: potentiation by vanadate. *Biochem. J.* **295**, 879–888
31. Goldman, R., Ferber, E., Meller, R., and Zor, U. (1994) A role for reactive oxygen species in zymosan and β-glucan induced protein tyrosine phosphorylation and phospholipase A2 activation in murine macrophages. *Biochim. Biophys. Acta* **1222**, 265–276
32. Meier, B., Radeke, H. H., Selle, S., Younes, M., Sies, H., Resch, K., and Habermehl, G. G. (1989) Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-alpha. *Biochem. J.* **263**, 539–545
33. Schieven, G. L., Mittler, R. S., Nadler, S. G., Kirihara, J. M., Bolen, J. B., Kanner, S. B., and Ledbetter, J. A. (1994) ZAP-70 tyrosine kinase, CD45 and T-cell receptor involvement in UV and H_2O_2 induced T cell signal transduction. *J. Biol. Chem.* **269**, 20718–20726
34. Weiss, A., and Littman, D. R. (1994) Signal transduction by lymphocyte antigen receptors. *Cell* **76**, 263–274
35. Hecht, D., and Zick, Y. (1992) Selective inhibition of protein tyrosine phosphatase activities by H_2O_2 and vanadate in vitro. *Biochem. Biophys. Res. Commun.* **188**, 773–779
36. Hadari, Y. R., Geiger, B., Nadiv, O., Sabanay, I., Roberts, C. T., Jr., LeRoith, D., and Zick, Y. (1993) Hepatic tyrosine phosphorylated proteins identified annd localized following in vivo inhibition of protein tyrosine phosphatases: effects of H_2O_2 and vanadate administrations into rat livers. *Mol. Cell. Endocrinol.* **97**, 9–17
37. Sullivan, S. G., Chiu, D. T. Y., Errasfa, M., Wang, J. M., Qi, J. S., and Stern, A. (1994) Effects of H_2O_2 on protein tyrosine phosphatase activity in HER 14 cells. *Free Rad. Biol. Med.* **16**, 399–403
38. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., and Silvennoinen, O. (1994) Signaling by the cytokine receptor superfamily: JAKs and STATs. *Trends Biochem. Sci.* **19**, 222–227
39. Taniguchi, T. (1995) Cytokine signalling through nonreceptor protein tyrosine kinases. *Science* **268**, 251–255
40. Meyer, M., Schreck, R., and Baeuerle, P. A. (1993) H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary-responsive factor. *EMBO J.* **12**, 2005–2015
41. Suzuki, Y. J., and Packer, L. (1993) Inhibition of NF-κB activation by vitamin E derivatives. *Biochem. Biophys. Res. Commun.* **193**, 277–283
42. Suzuki, Y. J., and Packer, L. (1993) Inhibition of NF-κB DNA binding activity by α-tocpheryl succinate. *Biochem. Mol. Biol. Int.* **31**, 693–700
43. Suzuki, Y. J., and Packer, L. (1994) Inhibition of NF-κB transcription factor by catechol derivatives. *Biochem. Mol. Biol. Int.* **32**, 299–305
44. Droge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenck, H., Eck, H.-P., Roth, S., and Gmunder, H. (1994) Functions of glutathione and glutathione disulfide in immunology and immunopathology. *FASEB J.* **8**, 1131–1138
45. Schenk, H., Klein, M., Erdbrugger, W., Droge, W., and Schulze-Osthoff, K. (1994) Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-κB and AP-1. *Proc. Natl. Acad. Sci. USA* **91**, 1672–1676
46. Okamoto, T., Ogiwara, H., Hayashi, T., Mitsui, A., Kawabe, T., and Yodoi, J. (1992) Human thioredoxin/adult T cell leukemia-derived factor activates the enhancer binding protein of human immunodeficiency virus type I by thiol redox control mechanism. *Int. Immunol.* **4**, 811–819
47. Mathews, J. R., Wakasugi, N., Virelizier, J.-L., Yodoi, J., and Hay, R. T. (1992) Thioredoxin regulates the DNA binding activity of NF-κB by reduction of a disulfide bond involving cysteine 62. *Nucleic Acids Res.* **20**, 3821–3830
48. Packer, L., Witt, E. H., and Tritschler, H. J. (1995) Alpha-lipoic acid as a biological antioxidant. *Free Rad. Biol. Med.* **19**, 227–250
49. Beg, A. A., Finco, T. S., Nantermet, P. V., and Baldwin, A. S. (1993) Tumor necrosis factor and interleukin 1 lead to phosphorylation and loss of IκBα: a mechanism for NF-κB activation. *Mol. Cell. Biol.* **13**, 3301–3310
50. Henkel, T., Alkalay, I., Machleidt, T., Kronke, M., Ben-Neriah, Y., and Baeuerle, P. A. (1993) Rapid proteolytic degradation of IκBα induced by stimulation of cells with phorbol ester, cytokines and lipopolysaccharide is a necessary step in the activation of NF-κB. *Nature (London)* **365**, 182–185
51. Sun, S.-C., Ganchi, P. A., Ballard, D. W., and Greene, W. C. (1993) NF-κB controls expression of inhibitor IκBα: evidence for an inducible autoregulatory pathway. *Science* **259**, 1912–1915
52. Devary, Y., Rosette, C., DiDonato, J. A., and Karin, M. (1993) NF-κB activation by ultraviolet light not dependent on a nuclear signal. *Science* **261**, 1442–1445
53. Sen, C. K., Traber, K., and Packer, L. (1996) Inhibition of NF-κB activation in human T-cell lines by anetholdithiolthione. *Biochem. Biophys. Res. Commun.* **218**, 148–153
54. Galter, D., Mihm, S., and Droge, W. (1994) Distinct effects of glutathione disulphide on the nuclear transcription factors κB and the activator protein-1. *Eur. J. Biochem.* **221**, 639–648
55. Suzuki, Y. J., Mizuno, M., Tritschler, H. J., and Packer, L. (1995) Redox regulation of NF-κB DNA binding activity by dihydrolipoate. *Biochem. Mol. Biol. Int.* **36**, 241–246
56. Kumar, S., Rabson, A. B., and Gelinas, C. (1992) The RXXRXRXXC motif conserved in all Rel/κB proteins is essential for the DNA-binding activity and redox regulation of the v-Rel oncoprotein. *Mol. Cell. Biol.* **12**, 3094–3106
57. Bressler, P., Brown, K., Timmer, W., Bours, V., Siebenlist, U., and Fauci, A. S. (1993) Mutational analysis of the p50 subunit of NF-κB and inhibition of NF-κB activity by trans-dominant p50 mutants. *J. Virol.* **67**, 288–293
58. Toledano, M. B., Ghosh, D., Trinh, F., and Leonard, W. J. (1993) N-terminal DNA-binding domains contribute to differential DNA-binding specificities of NF-κB p50 and p65. *Mol. Cell. Biol.* **13**, 852–860
59. Mitomo, K., Nakayama, K., Fujimoto, K., Sun, X., Seki, S., and Yamamoto, K. (1994) Two different cellular redox systems regulate the DNA-binding activity of the p50 subunit of NF-κB in vitro. *Gene* **145**, 197–203
60. Xanthodukakis, S., Mioao, G., Wang, F., Pan, Y.-C., and Curran, T. (1992) Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO J.* **11**, 3323–3335
61. Angel, P., and Karin, M. (1991) The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation. *Biochim. Biophys. Acta* **1072**, 129–157

62. Karin, M. (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J. Biol. Chem.* **270**, 16483–16486
63. Suzuki, T., Okuno, H., Yoshida, T., Endo, T., Nishina, H., and Iba, H. (1991) Difference in transcriptional regulatory function between c-Fos and Fra-2. *Nucleic Acids Res.* **19**, 5537–5542
64. Pennypacker, K. R., Hong, J-S., and McMillian, M. K. (1994) Pharmacological regulation of AP-1 transcription factor DNA binding activity. *FASEB J.* **8**, 475–478
65. Crawford, D., Zbinden, I., Amstad, P., and Cerutti, P. (1988) Oxidant stress induces the proto-oncogenes c-fos and c-myc in mouse epidermal cells. *Oncogene* **3**, 27–32
66. Shibanuma, M., Kuroki, T., and Nose, K. (1988) Induction of DNA replication and expression of proto-oncogenes c-myc and c-fos in quiescent Balb/3T3 cells by xanthine/xanthine oxidase. *Oncogene* **3**, 17–21
67. Devary, Y., Gottilieb, R. A., Lau, L. F., and Karin, M. (1991) Rapid and preferential activation of the c-jun gene during the mammalian UV response. *Mol. Cell. Biol.* **11**, 2804–2811
68. Nose, K., Shibanuma, M., Kikuchi, K., Kageyama, H., Sakiyama, S., and Kuroki, T. (1991) Transcriptional activation of early-response genes by hydrogen peroxide in a mouse osteoblastic cell line. *Eur. J. Biochem.* **201**, 99–106
69. Collart, F. R., Horio, M., and Huberman, E. (1995) Heterogeneity in c-jun gene expression in normal and malignant cells exposed to either ionizing radiation or hydrogen peroxide. *Radiat. Res.* **142**, 188–196
70. Lo, Y. Y. C., and Cruz, T. F. (1995) Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondriocytes. *J. Biol. Chem.* **270**, 11727–11730
71. Puri, P. L., Avantaggiati, M. L., Burgio, V. L., Chirillo, P., Collepardo, D., Natoli, G., Balsano, C., and Levrero, M. (1995) Reactive oxygen intermediates mediate angiotensin II-induced c-Jun/c-Fos heterodimer DNA binding activity and proliferative hypertrophic responses in myogenic cells. *J. Biol. Chem.* **270**, 22129–22134
72. Hunt, N. H., Goldstone, S. D., Van Reyk, D. M., Jeitner, T. M., and Fragonas, J.-C. (1994) *Oxidative Stress, Cell Activation and Viral Infection* (Pasquier, C., Olivier, R. Y., Auclair, C., Packer, L., eds) pp. 237–251, Birkhauser Verlag, Basel
73. Novogrodsky, A., Ravid, A., Rubin, A. L., and Stenzel, K. H. (1982) Hydroxyl radical scavengers inhibit lymphocyte mitogenesis. *Proc. Natl. Acad. Sci. USA* **79**, 1171–1174
74. Goldstone, S. D., Fragonas, J.-C., Jeitner, T. M., and Hunt, N.H. (1995) Transcription factors as targets for oxidative signalling during lymphocyte activation. *Biochim. Biophys. Acta* **1263**, 114–122
75. Datta, R., Hallahan, D. E., Kharbanda, S. M., Rubin, E., Sherman, M. L., Huberman, E., Weichselbaum, R. R., and Kufe, D. W. (1992) Involvement of reactive oxygen intermediates in the induction of c-jun gene transcription by ionizing radiation. *Biochemistry* **31**, 8300–8306
76. Janssen, Y. M. W., Heintz, N. H., and Mossman, B. T. (1995) Induction of c-fos and c-jun proto- oncogene expression by asbestos is ameliorated by N-acetyl-L-cysteine in mesothelial cells. *Cancer Res.* **55**, 2085–2089
77. Cerutti, P. A., and Trump, B. F. (1991) Inflammation and oxidative stress in carcinogenesis. *Cancer Cells* **3**, 1–7
78. Bauskin, A. R., Alkalay, I., Ben-Neriah, Y. (1991) Redox regulation of a protein tyrosine kinase in the endoplasmic reticulum. *Cell* **66**, 685–696
79. Radler-Pohl, A., Sachsenmaier, C., Gebel, S., Auer, H. P., Bruder, J. T., Rapp, U., Angel, P., Rahmsdorf, H. J., and Herrlich, P. (1993) UV-induced activation of AP-1 involves obligatory extranuclear steps including Raf-1 kinase. *EMBO J.* **12**, 1005–1012
80. Stauble, B., Boscoboinik, D., Tasinato, A., and Azzi, A. (1994) Modulation of activator protein-1 (AP-1) transcription factor and protein kinase C by hydrogen peroxide and D-α-tocopherol in vascular smooth muscle cells. *Eur. J. Biochem.* **226**, 393–402
81. Amstad, P. A., Krupitza, G., and Cerutti, P. A. (1992) Mechanisms of c-fos induction by active oxygen. *Cancer Res.* **52**, 3952–3960
82. Pahl, H. L., and Baeuerle, P. A. (1994) Oxygen and the control of gene expression. *BioEssays* **16, 497–502**
83. Kahl, R. (1991) *Oxidative Stress: Oxidants and Antioxidants* (Sies, H., ed) pp. 245–273, Academic Press, London
84. Choi, H.-S., and Moore, D. D. (1993) Induction of c-fos and c-jun gene expression by phenolic antioxidants. *Mol. Endocrinol.* **7**, 1596–1602
85. Friling, R. S., Bergelson, S., and Daniel, V. (1992) Two adjacent AP-1 like binding sites from the electrophile-responsive element of the murine glutathione S-transferase Ya subunit gene. *Proc. Natl. Acad. Sci. USA* **89**, 668–672
86. Yoshioka, K., Deng, T., Cavigelli, M., and Karin, M. (1995) Antitumour promotion by phenolic antioxidants: Inhibition of AP-1 activity through induction of Fra expression. *Proc. Natl. Acad. Sci. USA* **92**, 4972–4976
87. Devary, Y., Gottilieb, R. A., Smeal, T., and Karin, M. (1992) The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* **71**, 1081–1091
88. Pinkus, R., Bergelson, S., and Daniel, V. (1993) Phenobarbital induction of AP-1 binding activity mediates activation of glutathione S-transferase and quinone reductase gene expression. *Biochem. J.* **290**, 637–640
89. Bannister, A. J., Cook, A., and Kouzarides, T. (1991) In vitro DNA binding of Fos/Jun and BZLF1 but not C/EBP is affected by redox changes. *Oncogene* **6**, 1243–1250
90. Frame, M. C., Wilkie, N. M., Darling, A. J., Chudleigh, A., Pintzas, A., Lang, J. C., and Gillespie, D. A. (1991) Regulation of AP-1/DNA complex formation in vitro. *Oncogene* **6, 205-209**
91. Treisman, R. (1990) The SRE: a growth factor responsive transcriptional regulator. *Semin. Cancer Biol.* **1**, 47–58
92. Abate, C., Patel, L., Rauscher, F. J., III, and Curran, T. (1990) Redox regulation of Fos and Jun DNA-binding activity in vitro. *Science* **249**, 1157–1162
93. Okuno, H., Akahori, A., Sato, H., Xanthodukakis, S., Curran, T., and Iba, H. (1993) Escape from redox regulation enhances the transforming activity of Fos. *Oncogene* **8**, 695–701
94. Sundaresan, M., Yu, Z.-X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Requirement for generation of H_2O_2 for platelete-derived growth factor signal transduction. *Science* **270**, 296–299