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NUCLEAR FACTOR κB: AN OXIDATIVE STRESS-RESPONSIVE TRANSCRIPTION FACTOR OF EUKARYOTIC CELLS (A REVIEW)

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NF-κB is a multiprotein complex that can activate a great variety of genes involved in early defence reactions of higher organisms. In nonstimulated cells, NF-κB resides in the cytoplasm in an inactive complex with the inhibitor IκB. Pathogenic stimuli cause release of IκB and allow NF-κB to enter the nucleus, bind to DNA control elements and, thereby, induce the synthesis of mRNA. A puzzling feature of NF-κB is that its activation is triggered by a great variety of agents. These include the cytokines interleukin-1 and tumor necrosis factor, viruses, double-stranded RNA, endotoxins, phorbol esters, UV light and ionizing radiation. We recently found that also low concentrations of H_2O_2 activate NF-κB and that various antioxidants prevent the induction by H_2O_2. Subsequent analysis revealed that antioxidants not only suppress the activation of NF-κB by H_2O_2 but by all other inducers tested so far. In this review, we will discuss the evidences that NF-κB is an oxidative stress-responsive transcription factor of higher eukaryotic cells.

KEY WORDS: Gene expression, oxidative stress, NF-κB, cytokines, phorbol ester, taxol.

INTRODUCTION

Cells can respond to extracellular stimuli by the *de novo* synthesis of proteins. Stimuli extensively studied in this respect are stress reactions induced by heat shock, radiation (ultraviolet light and ionizing radiation) and chemicals.[1] A very frequent regulatory event in such responses is the upregulation of mRNA synthesis by transcriptional activator proteins. These sequence-specific DNA binding proteins interact with promoter and enhancer elements of target genes and thereby induce the assembly of a transcription initiation complex.[2] This allows RNA polymerase II to synthesize messenger RNA from the target gene. The activity of transcriptional activator proteins can be induced by a variety of mechanisms frequently involving the control of their DNA binding, nuclear uptake or the assembly or disassembly of protein subunits.

Important chemical inducers of gene expression are reactive oxygen intermediates (ROI).[3] Cells must therefore be able to sense an increase in ROI and to subsequently recruit inducible transcription factors for translation of the stress signal into an altered pattern of gene expression. In enteric bacteria, various transcription factors were identified and characterized that control gene expression in response to an increased concentration of ROI.[4] One factor is oxyR which mediates, specifically in response to H_2O_2, the expression of at least eight genes involved in protecting the bacterial

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cell from oxidative damage. A conformational change of oxyR upon direct oxidation of the protein appears to unleash its transcription activating potential.[5] A second bacterial system, called soxRS, is specifically induced by superoxide-generating agents like paraquat and quinones. The factor encoded by the soxR gene is thought to initiate transcription from the soxS gene in response to O_2^-. As in the case of oxyR, a redox-induced conformational change of the soxR protein seems to activate the transcription factor. The newly produced soxS protein, another transcription factor, activates then a series of defence proteins.[6] In contrast to the bacterial systems, very little is known about oxidative stress-responsive transcription factors of higher eukaryotic organisms. In this review, we summarize experimental evidences supporting a role for the well-characterized, inducible transcription factor NF-κB in oxidative stress-responsive gene regulation of higher eukaryotic cells.

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RESULTS AND DISCUSSION

Properties of NF-κB

NF-κB is a member of a novel family of transcription factors sharing a common structural motif for DNA binding and dimerization.[7] The DNA binding, nuclear form of NF-κB is composed of a heterodimer with one 50 kD (p50) and 65 kD (p65) polypeptide.[8] Both subunits can independently bind to DNA as homodimers but have highest affinity as heterodimer for the frequent binding motif 5′-GGGACTTTCC-3′ (consensus motif: 5′-GGGRNNYYCC-3′). The subunits share a region of homology of approximately 300 amino acids with sequences required for specific DNA binding and dimerization. Only the p65 subunit has strong potential to activate transcription.[9] NF-κB appears to be present in most cell types of higher vertebrates. The activity of NF-κB and related factors is controlled by inhibitory subunits, called IκB proteins.[10] IκB proteins show specific binding to the individual DNA binding subunits. A structural characteristic of the inhibitory proteins is a wide-spread repeat structure of 30 amino acids, called SWI6/ankyrin repeats.[11]

The activation of NF-κB

IκB is required for the inducible activation of NF-κB. In non-stimulated cells, NF-κB is not in the nucleus (and therefore not activating genes) but in the cytoplasm due to its association with IκB.[10] In complex with IκB, NF-κB cannot detectably bind to DNA and seems to be excluded from regulated nuclear uptake. The latter comes from IκB masking positively charged nuclear location signal sequences in p65 and p50. Extracellular stimulation of cells causes release of IκB which restores nuclear uptake, DNA binding and the transcriptional activation potential of NF-κB. Thus, the release of IκB appears to be the central event required for activation of NF-κB and, ultimately, for gene induction and synthesis of new proteins in response to extracellular stimuli. The activation process of NF-κB is depicted in Figure 1.

In vitro experiments showed that several purified protein kinases can release IκB from NF-κB, presumably by direct phosphoryl transfer from ATP onto IκB.[12] There are however no data yet from intact cells supporting a direct action of the tested protein kinases on IκB. It can be expected that any reaction selectively altering the structure of IκB or p65 can potentially perturb the interaction of the proteins and result in activation of NF-κB.

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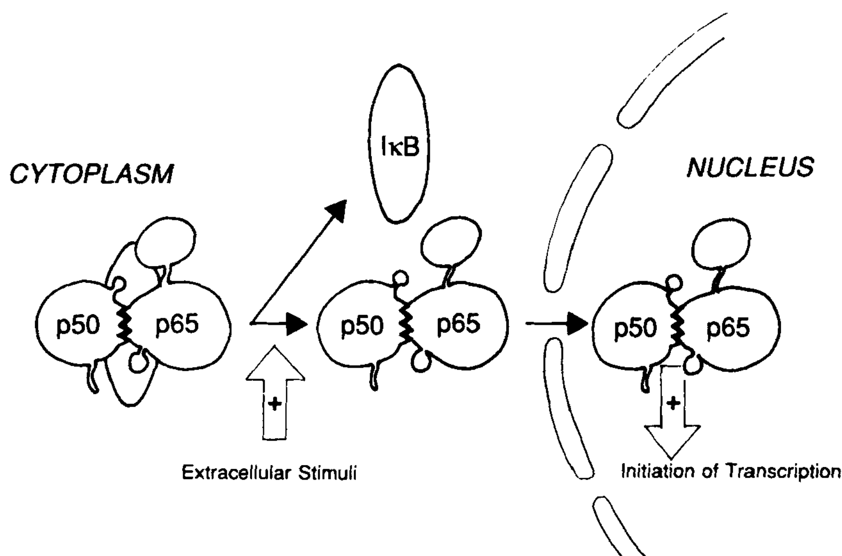

FIGURE 1 The activation of NF-κB.

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In contrast to many other inducible transcription factors a multitude of conditions can activate NF-κB in intact cells.[7,13] For instance, the inflammatory cytokines tumor necrosis factor α (TNF) and interleukin-1 (IL-1), double-stranded RNA (dsRNA), bacterial lipopolysaccharide (LPS), phorbol esters, UV light, protein synthesis inhibitors and viruses were reported to induce NF-κB. Common to all these inducers is that they either represent pathogenic stimuli (viruses, LPS, UV) or are a consequence from pathogenic stimulation (reduced protein synthesis, production of inflammatory cytokines). Genes activated by NF-κB upon such stimuli encode cytokines, immune receptors and acute phase proteins. It has therefore been proposed that NF-κB has specialized as signal transducer and mediator in the immediate early phases of antiviral, immune and acute phase response.[13]

Clearly, some if not all inducers of NF-κB cause the activation of protein kinases within cells. For instance, TNF, LPS, IL-1 and phorbol esters are known to transiently activate protein kinase C (PKC). However, as shown in the case of TNF and IL-1, inhibitors of PKC did not affect activation of NF-κB.[14,15] The variety of inducers could simply mean that there are parallel mechanisms to dissociate the NF-κB-IκB complex in the cytoplasm. Alternatively, primary signals elicited by the various inducers within the cell could funnel into a common pathway that uses only one mechanism to release IκB. Because induction of NF-κB by the different inducers can occur independently of protein synthesis, it is unlikely that, for instance, a newly synthesized cytokine acts as common secondary signal.

Induction of NF-κB by hydrogen peroxide

TNF is known to induce oxidative stress in cells.[16] Some cell types are killed by the cytokine. The addition of antioxidants, reduction of pO_2 or overexpression of Mn-dependent superoxide dismutase (SOD) increases the survival of such cell types

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suggesting that oxygen radicals are involved in the cytotoxic effects of TNF.[17,18] Because TNF is a strong inducer of NF-κB, we asked the question whether oxidative stress, as induced by the addition of hydrogen peroxide to the culture medium, can activate NF-κB. In the T lymphoma cell line Jurkat, 30 to 150 μM H_2O_2 induced the appearance of active NF-κB in nuclei.[19] The activation was fast, independent on new protein synthesis and the treatment did not affect other inducible transcription factors. H_2O_2 could not activate NF-κB when catalase was added to the culture medium. The actual intracellular concentration of H_2O_2 responsible for the activation of NF-κB is not known but, given the instability of H_2O_2 in biological systems, appears to be in a low micromolar range. The activation of NF-κB in response to H_2O_2 was not restricted to lymphoid cells. In the human epithelial carcinoma cell line HeLa, NF-κB was activated at 100 μM H_2O_2 (M. Meyer, R. Schreck, P.H. Hofschneider and P. Baeuerle, manuscript in preparation). The activation in HeLa cells was optimal at 250 μM H_2O_2. Another oxidizing agent activating NF-κB in Jurkat cells and the mouse pre-B cell line 70Z/3 was butylperoxide at a concentration of 300 μM[20] (Table I).

We noted that various subclones of Jurkat cells differed in their responsiveness to H_2O_2 (Figure 2, compare lanes 3 and 7). This could come from varying levels of catalases or peroxidases produced (and released) by the cells. Interestingly, H_2O_2 strongly enhanced the activation of NF-κB by phorbol 12-myristate 13-acetate (PMA) in a low-responder (subclone J6; Figure 2, lane 4), and to a lesser extent in a high-responder (subclone JR; lane 8) indicating that the two inducers use a common pathway.

TABLE I

Stress reactions that activate and fail to activate NF-κB. In all experiments, the strongly H_2O_2-responding Jurkat T cell subclone JR was used. The listed substances were added to the culture medium at the indicated concentration range and incubation periods. Nuclear extracts were prepared from treated and control cells and analyzed by electrophoretic mobility shift assays for the appearance of NF-κB DNA binding activity. As discussed in the text, Ara-C gave a very slight induction of NF-κB in the tested cell line.

Condition	Class	Concentration range used	Incubation period
Activating:			
Hydrogen peroxide	Peroxide	30–150 μM	1–2 h
Butyl peroxide	Peroxide	300 μM	1 h
Not Activating:			
Paraquat	O_2^- generating	0.1 μM–5 mM	3.5 h, 18 h
Doxorubicin	O_2^- generating	10–50 μM	2 h
Mitomycin C	O_2^- generating	1–50 μg/ml	2 h
Menadion	O_2^- generating	1–100 μM	2 h
4-Nitroquinolineoxide	DNA damaging	1–50 μM	2 h
1-β-D-arabinofuranosylcytosin (Ara-C)	DNA damaging	0.1–100 μM	2 h
3,3′-(1,4-naphtylidene) dipropionate	Singlett oxygen generating	0.1–8 mM	1 h
Sodium nitroprusside	NO-generating	1–250 μM	1 h
Clofibrate	Peroxisome proliferator	1–1000 μM	18 h
$CuSO_4$	Chemical stress	100–750 μM	4 h
$CdSO_4$	Chemical stress	50 μM	4 h
Sodium arsenite	Chemical stress	50 μM	4 h
42°C	Heat shock		1 h

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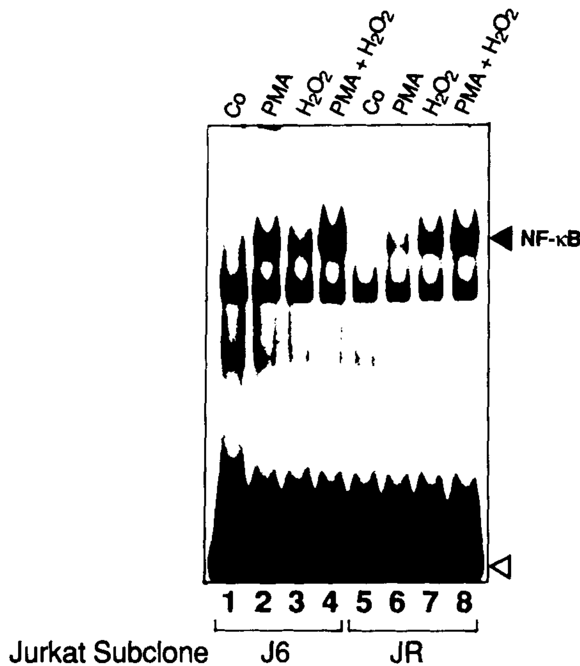

FIGURE 2 Activation of NF-κB in two Jurkat T cell subclones in response to hydrogen peroxide and phorbol ester treatments. The Jurkat subclones J6 (lanes 1–4) and JR (lanes 5–8) were treated with 100 μM H_2O_2 (lanes 3 and 7), 50 ng/ml PMA (lanes 2 and 6) or a combination of both stimuli (lanes 4 and 8). High-salt nuclear extracts were prepared from cells and analyzed for NF-κB DNA binding activity by electrophoretic mobility shift assays using a ^{32}P-labelled double-stranded oligonucleotide with an optimal binding motif for NF-κB (described in detail in Ref. 19). A fluorogram of a native 4% polyacrylamide gel is shown. The position of the NF-κB-DNA complex is indicated by a filled arrowhead. The open arrowhead indicates the position of unbound DNA probe.

Taken together, these findings indicate that NF-κB is a transcription factor which is preferentially induced by peroxides. In that respect, NF-κB would be more similar to the bacterial oxyR system than the soxRS system. It seems that also in eukaryotic cells peroxide and superoxide signals can be distinguished.

Table I lists a variety of agents that failed to activate NF-κB in cultured Jurkat T cells. Some of the compounds were reported to increase the intracellular concentration of superoxide anions but none could induce significant amounts of NF-κB activity. This suggests that superoxide does not play a direct role. Consistent with this idea is the observation that human breast cancer cells constitutively overexpressing a stably transfected Mn-dependent superoxide dismutase (MnSOD) gene are not impaired in TNF-induced activation of NF-κB when compared to the parental cell line.[21] Rather, a slightly enhanced mobilization of NF-κB was observed in the MnSOD-overexpressing cell line. Also, the endoperoxide 3,3′-(1,4-naphtylidene)dipropionate ($NDPO_2$),[22] a substance that releases singlett oxygen, caused no significant induction of NF-κB when compared to stimulation with H_2O_2 or TNF. Nitric oxide radicals (NO·) have a well-established biological role in signalling processes.[23] Neither could Nω-methyl-L-arginine, an inhibitor of NO·

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synthesis, nor the NO· generating agent sodium nitroprusside prevent or support NF-κB activation in cell culture experiments, respectively (see Table II). Also, cellular stress reactions induced by heavy metal ions, heat shock or a peroxisome proliferator could not significantly support the activation of NF-κB (Table I).

Oxidative stress is known to cause DNA damage. Earlier reports by Stein *et al.*[24,25] proposed that the DNA damaging agents 4-nitroquinolineoxide and mitomycin C can induce NF-κB activity via nuclear signals. Here, we tested 4-nitroquinolineoxide and 1-β-D-arabinofuranosylcytosin (Ara-C) in Jurkat T cells (Table I). Only Ara-C induced a slight activation of NF-κB. In order to test more rigorously whether DNA damage is a prerequisite for activation of NF-κB, we eliminated nuclear DNA through an enucleation procedure giving rise to viable cytoblasts. As shown in Figure 3, Jurkat T cells treated with cytochalasin B and subsequently enucleated by centrifugation through a Ficoll gradient did not become deficient for activation of NF-κB following a treatment with TNF (compare lanes 5, 6 with 1 to 4). Production

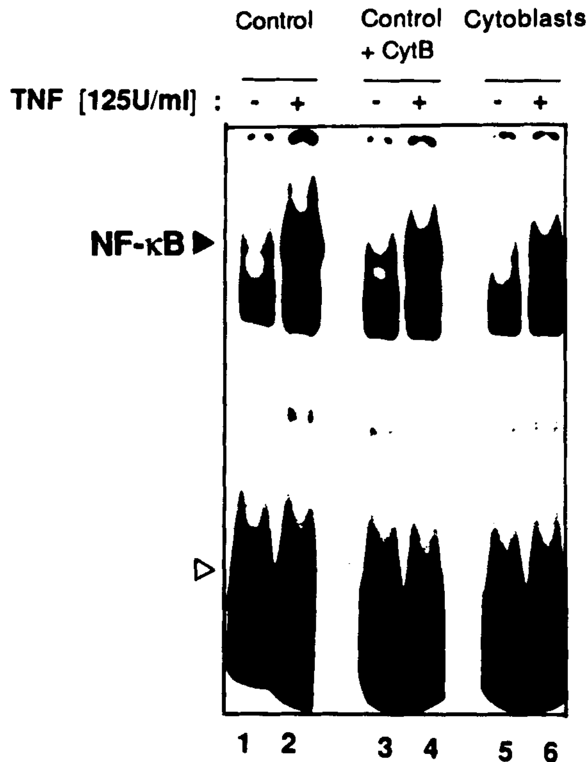

FIGURE 3 Activation of NF-κB in enucleated cells by TNF. Jurkat T cells were left untreated (lanes 1 and 2), incubated in 30 μM cytochalasin B and 10% Ficoll for 2.5 h (lanes 3 and 4), or subjected, in the presence of cytochalasin B, to centrifugation for 2 h at 107,000 × g and 37°C through a Ficoll step gradient which results in the removal of nuclei from cells.[57,58] Enucleated cells were collected at the 15–16% Ficoll interface. The enucleation efficiency was determined by staining nuclei with DAPI and found to be >95%. NF-κB was induced by treatment with 125 U/ml human recombinant TNF for 1 h (lanes 2, 4 and 6). Total cell extracts were prepared from cells and cytoblasts and analyzed by electrophoretic mobility shift assays. For details of illustration see legend to Figure 2.

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of nuclear signals through DNA damage seems therefore not to be a major pathway of NF-κB activation.

While in intact cells, oxidants activate the DNA binding of NF-κB (presumably by release of IκB), they inactivate the DNA binding *in vitro*.[26,27] Addition of high concentrations of dithiothreitol can reverse this inhibiting effect, which seems to come from oxidation of cysteine residues that are crucial for DNA binding of p50 and p65. It is at present unclear whether the direct oxidation of p50 and p65 NF-κB represents a physiological means of regulation or is an artifact of protein purification. Also the subunits Jun and Fos of the inducible transcription factor AP-1 have cysteine residues within their DNA binding domains that can undergo reversible oxidation and potentially allow regulation of DNA binding activity.[28]

The effect of antioxidants on the activation of NF-κB

If the activation of NF-κB in response to H_2O_2 requires decomposition of H_2O_2 into more reactive oxygen species (such as hydroxyl radicals and other peroxides), several antioxidative substances should inhibit mobilization of the factor. It was indeed found that 30 mM N-acetyl-L-cysteine (NAC), 100 μM pyrrolidine dithiocarbamate (PDTC) or diethyl dithiocarbamate (DDTC), 14 mM 2-mercaptoethanol or 100 μM of the metal chelators orthophenanthroline and desferrioxamine potently suppressed the activation of NF-κB when added to cells prior to a treatment with H_2O_2 (Ref. 21) (listed in Table II). While the sulfur-containing agents might act as radical traps, the non-sulfur metal chelators might absorb iron and copper ions required for reactions of the Fenton and Haber-Weiss type.

The pharmacological tools allowed then to indirectly test whether the activation of NF-κB by other inducers also relies on a prooxidant state of the cell. For these studies, mainly the well-characterized pharmaceutic NAC,[19,29] and PDTC, which was found to be the most potent inhibitor in cell cultures,[19–21] were used. All inducers tested so far were blocked in their NF-κB-activating potential by the antioxidants (listed in Table III). This suggests that, although the inducers have definitely different effects on cells and can use distinct signalling molecules within cells, they also funnel into a common pathway when it comes to the activation of the NF-κB-IκB complex in the cytoplasm. So far, no exception was found. Even three virally encoded transactivator proteins known to induce NF-κB (HBx and MHBs^t from hepatitis B virus and Tax from the human T cell leukemia virus type 1) were blocked by the antioxidants while others of their pleiotropic effects were unaffected.[30,31]

The effects of the antioxidant PDTC were studied in detail.[20] PDTC showed a biphasic dose response curve. When the extracellular dose was increased to mM concentrations, the antioxidant lost its inhibiting activity towards the activation of NF-κB in response to TNF. PDTC was also inactive when cells were treated for more than 15 h with an otherwise inhibiting concentration. Early removal of the drug could restore sensitivity. A possible explanation for the desensitization effects is that cells adapt to the hypoxic state induced by the antioxidant by downregulating scavenging enzymes. As consequence, inducers of oxidative stress would have a more permissive effect. An alternative explanation is that high concentrations of antioxidants can stabilize rather than eliminate radicals in the cell.

In intact cells, the antioxidant PDTC suppressed the activation of NF-κB by PMA but did not interfere with the activation of the inducible factor AP-1, arguing

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against an effect of PDTC on the PMA-induced PKC.[21] This idea was supported by the finding that PDTC does not affect activity and membrane distribution of PKC upon PMA treatment of cells.[20] It thus appears that, in intact cells, PKC does not directly act on IκB. Rather, PKC uses ROI for signalling to NF-κB, which explains why antioxidants block activation of NF-κB. This mechanism is however not used for the activation of AP-1 by PKC.

Other types of antioxidants which block in T cells NF-κB activation in response to TNF and PMA are butylated hydroxyanisol at a concentration of 0.4 mM (N.

TABLE II

Agents tested for their suppressing effect on the activation of NF-κB. Cells of the Jurkat subclone JR were preincubated for the indicated periods with the indicated concentrations of the listed substances. TNF, as the most potent inducer of NF-κB in Jurkat cells, was used for stimulation of cells for 1 h. Nuclear extracts were prepared from control cells (+TNF) and cells pretreated with the substances before TNF addition and NF-κB DNA binding activity analyzed by electrophoretic mobility shift assays. ++++, close to 100% inhibition; +++, approximately 75% inhibition; ++, approximately 50% inhibition. The most effective concentration is given in parentheses. The effect of cysteine was reported by Mihm *et al.*[59] The substances seemed to have no severe toxic effect as judged from phase contrast microscopy of treated cells and assays detecting unrelated DNA binding activities in nuclear extracts

Agent	Class	Concentration range tested	Preincubation period	Inhibiting effect on NF-κB activation
N-Acetyl-L-cysteine	Scavenger (-SH)	0.1–30 mM	2.5 h	++++ (30 mM)
L-Cysteine	Scavenger (-SH)	30–300 μM	1 h	++++ (0.3 mM)
2-Mercaptoethanol	Scavenger (-SH)	14 mM	2.5 h	++++
Glutathione	Scavenger (-SH)	10 mM	2.5 h	++
Pyrrolidine dithiocarbamate	Scavenger (>NCS_2)	10 μM–5 mM	1–20 h	++++ (100 μM)
Diethyldithiocarbamate	Scavenger (>NCS_2)	100 μM	1 h	++++
Disulfiram	Scavenger (>NCS_2)	100 μM	1 h	++++
Butylated hydroxyanisol	Scavenger (non S)	10–400 μM	6 h	+++ (400 μM)
Orthophenanthroline	Metal chelator (Cu)	100 μM	1.5 h	+++
Desferrioxamine	Metal chelator (Fe)	100 μM	17 h	+++
Ebselen (PZ 51)	Se peptide with GSH peroxidase activity	50 μM	2.5 h	++
Diclofenac	cyclooxygenase inhibitor	1–50 μM	1.5 h	–
Diphenylene iodonium	NADPH oxidase inhibitor	1–20 μM	1.5 h	–
Mannitol	OH· scavenger	50 mM	1 h	–
Dimethylsulfoxide	OH· scavenger	280 mM	1 h	–
Tetramethylurea	OH· scavenger	10 mM	1 h	–
Nω-Nitro-L-arginine methylester	NO· synthesis inhibitor	5–10 mM	16 h	–
Quinacrine	PLA_2 inhibitor	5 μM	2 h	–
Aminobenzamide		10 mM	2 h	++
Sodium orthovanadate		1–1000 μM	1.25 h	+++

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Israel, Pasteur Institute, personal communication) and ebselen (Table II), a selenium containing peptide with glutathione peroxidase activity.[32] Inhibitory effects on the transactivation by NF-κB were observed with the antioxidants nordihydroquairetic acid and vitamin E in the T cell line MOLT4 stimulated with phorbolester or TNF (N. Israel, Pasteur Institute, personal communication). Agents that showed no inhibiting activity were the hydroxyl radical scavengers mannitol, DMSO and tetramethylurea, the NADPH oxidase inhibitor diphenylene iodonium, and the phospholipase A_2 inhibitor quinacrine.

A novel inducer of NF-κB that is susceptible to antioxidants is shown in Figure 4. Ten micromolar of the anti cancer drug taxol[33] potently induced NF-κB binding activity in the pre-B cell line 70Z/3 (Figure 4A, lane 3). The activation was independent on new protein synthesis because it could occur in the presence of the protein synthesis inhibitor cycloheximide (lane 4). If cells were preincubated for one hour with 100 μM of the antioxidant PDTC, activation of NF-κB by taxol was completely suppressed (Figure 4B, compare lanes 2 and 3). The cytostatic effect of taxol is thought to come from its property to stabilize microtubuli. In that context, it was interesting to observe that 200 μM of the microtubuli destabilizing drug nocodazol could partially suppress the taxol-induced activation of NF-κB (data not shown). This suggested a link between the state of tubulin polymerisation, oxidative stress and NF-κB activity. Intriguingly, this novel activity of taxol was restricted to the pre-B cell line.

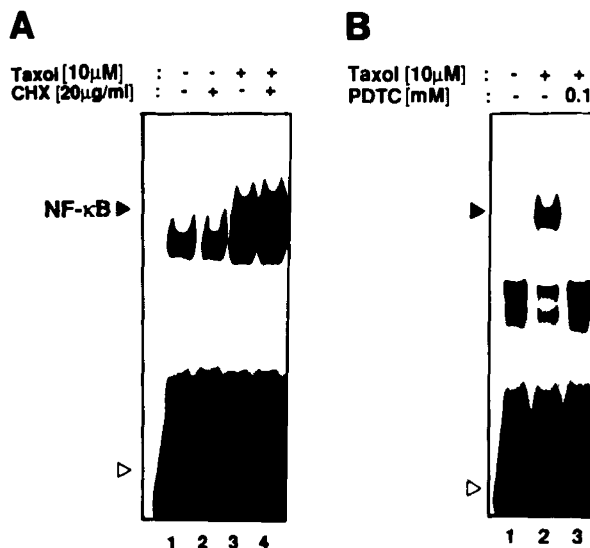

FIGURE 4 Activation of NF-κB by taxol and the effect of pyrrolidine dithiocarbamate (PDTC). (A) Activation of NF-κB by taxol in the presence of a protein synthesis inhibitor. Mouse 70Z/3 pre-B cells were left untreated (lane 1) or treated with 20 μg/ml cycloheximide (lane 2), 10 μM taxol (lane 3), or a combination of both (lane 4). Nuclear extracts were prepared and analyzed for NF-κB DNA binding activity by electrophoretic mobility shift assays. (B) The effect of PDTC. Cells were preincubated for 1 h with 100 μM PDTC prior to addition of 10 μM taxol. For details of illustration, see legend to Figure 2.

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In conclusion, there are now three lines of evidence supporting that NF-κB is an oxidative-stress responsive factor: firstly, its activation in response to the addition of low concentrations of H_2O_2; secondly, the suppressing effect of a variety of antioxidants on its activation in response to all inducers tested; thirdly, observations showing that several NF-κB-inducing agents can stimulate the production of ROI.

Do all inducers of NF-κB cause oxidative stress?

The finding that antioxidants suppress the activation of NF-κB by all inducers tested must raise the question whether all inducers of NF-κB cause oxidative stress. From the literature, there is good evidence that various cell types when treated with PMA, IL-1, TNF, anti-IGM, antigen, UV light and ionizing radiation increase the production of ROI (see Table III). In collaboration with Dr. B. Meier, we recently observed that treatment of a pre-B and an Epstein-Barr virus-transformed B cell line with the endotoxin LPS caused a 6–10 fold increase in the release of superoxide and H_2O_2.[20] In the pre-B cell line, NF-κB could be induced by LPS and antioxidants had a suppressing effect on this activation.

With the viral transactivator proteins Tax, HBx and MHBs^t it is more difficult to demonstrate direct induction of oxidative stress. Because such experiments require *de novo* synthesis of the proteins, it cannot be excluded that the transactivators lead to production of other NF-κB-activating proteins, such as TNF. Recent data obtained with bacterially produced Tax protein are in support for a direct mechanism of the

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TABLE III

NF-κB inducing agents and the effect of antioxidants. References for most inducers of NF-κB can be found in Ref. 7. Not listed there are hydrogen peroxide,[19] butyl peroxide,[20] MHBs^t,[30] anti-IgM,[60,61] okadaic acid[62] and ionizing radiation.[63] The inhibitory effects of antioxidants are documented in Refs. 19–21 and 29–31. Induction of oxidative stress by TNF, PMA and IL-1 is reviewed in Ref. 41. The effects of LPS,[21] T cell mitogens,[64] UV light,[65] peroxides and radiation[66] and anti-IgM[67] are documented elsewhere. n.d., not determined

NF-κB inducing agent	Class	Inhibition by antioxidants	Induction of oxidative stress
Tumor necrosis factor α	Cytokine	yes	yes
Interleukin-1	Cytokine	yes	yes
Lipopolysaccharide	Bacterial lipid	yes	yes
Double-stranded RNA	Viral RNA intermediate	yes	n.d.
Phorbol esters	Protein kinase activator	yes	yes
A23187	Calcium ionophore	yes	n.d.
Lectins (plus phorbol ester)	T cell mitogen	yes	yes
Cycloheximide	Protein synthesis inhibitor	yes	n.d.
Ultraviolet light	Physical stress	n.d.	yes
Ionizing radiation	Physical stress	n.d.	yes
Hydrogen peroxide	Oxidative stress	yes	yes
Butylperoxide	Oxidative stress	n.d.	yes
Tax (HTLV-1)	Viral protein	yes	n.d.
HBx (HBV)	Viral protein	yes	n.d.
MHBs^t (HBV)	Viral protein	yes	n.d.
Anti-IgM	Anti surface antibody	n.d.	yes
Okadaic acid	Phosphatase inhibitor	n.d.	n.d.
Taxol	Microtubuli stabilizer	yes	n.d.

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viral transactivator.[34] Addition of Tax to the culture medium resulted in uptake of some protein into the cytoplasm. Only 15 min after Tax addition, NF-κB was activated. This system would allow to directly measure H_2O_2 and O_2^- release from cells following addition of Tax. In another laboratory, cell lines were established that stably expressed the Tax protein.[35] These cells were desensitized for NF-κB activation by Tax but also by PMA and TNF suggesting that the three inducers relied on a common pathway.

Oxidative stress as intracellular signal

ROI are generally considered as cytotoxic agents.[36] High doses of ROI are a complication in chronic and acute inflammatory diseases, upon reperfusion, certain intoxications and environmental stress.[37] In the organism, mainly granulocytes have specialized in the inducible production of large amounts of ROI which are used as agents to kill parasites. Low doses of ROI are produced by every cell type as side products of electron transfer reactions, mainly within mitochondria and peroxisomes but also in the cytosol. There is also now evidence that the radical-producing NADPH oxidase system is not restricted to certain blood cells but that a less active form of the inducible enzyme is more widely expressed.[38] The intracellular concentration of ROI is usually kept at low levels by ubiquitous enzymes that can interconvert or eliminate ROI, and with the help of antioxidants, such as glutathione, ascorbic acid and tocopherol, which can scavenge oxygen radicals. Regulation of levels of these enzymatic activities and compounds provides an alternative means to control the intracellular concentration as well as composition of ROI.

While a cytotoxic role of high ROI doses is well established, the role of moderately increased levels of ROI, also referred to as "oxidative stress", is less clear. There is now a large body of evidence that different cell types are able to enhance the production of ROI following extracellular stimulation with the PKC activator phorbol 12-myristate 13-acetate (PMA) and physiological concentrations of the cytokines TNF and IL-1.[39,40] Our finding that the oxidative stress induced by these agents is involved in mobilization of the inducible transcription factor NF-κB suggests that ROI can be used for the purpose of intracellular signalling.[41] Since the induction of oxidative stress is highly controlled and a rapid elimination of ROI is assured by the action of scavenging enzymes, ROI seem to be particularly suitable for a role as second messenger molecules. Future studies on such a role of ROI might be greatly facilitated by using the dissociation of the NF-κB-IκB complex as a monitoring reaction in intact cells. A messenger role of radicals is not without precedent. It is now well-established that nitric oxide radicals have multiple regulatory functions intra- as well as extracellularly.[42]

A paradigm for the controlled synthesis of ROI is NADPH oxidase from phagocytic blood cells.[43] This multiprotein complex in the plasma membrane requires an iron-containing cytochrome b_{558} subunit and a flavoprotein in order to transfer electrons from NADPH onto dioxygen. The enzyme is strongly induced by activators of PKC most likely by a direct phosphorylation of subunits. Also low molecular weight G proteins seem to play a role in the activation. While in phagocytic cells, the enzyme is responsible for rapid production of toxic ROI concentrations (oxidative burst), a much less active species of the enzyme is found in other cell types and seems to only elicit oxidative stress. Future studies have to explore whether low activity forms of NADPH oxidases are involved in the activation of NF-κB.

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In the case of TNF, a study by Schulze-Osthoff *et al.* indicated that TNF stimulates oxygen radical production in mitochondria.[44] The electron transfer inhibitors rotenon and amytal blocked the cytotoxic effects of TNF while the more downstream inhibitor antimycin A potentiated the effect. This suggested that, upon TNF stimulation of cells, electrons are diverted from the electron transfer chain at the level of complex III and transferred onto oxygen presumably from ubiquinone. Since rotenon also blocks the activation of NF-κB in response to TNF, mitochondria might be involved in cytotoxic as well as signalling activities of TNF (K. Schulze-Osthoff and W. Fiers, University of Gent, personal communication).

It is very likely that the various inducers of NF-κB elicit oxidative stress by more than one means. This might even explain why so many agents can activate the transcription factor. In future studies, the use of pharmacological tools and cell and molecular biology approaches will allow to find out whether a particular inducer of NF-κB increases ROI production via mitochondria, peroxisomes, membrane-bound NADPH oxidases, xanthine oxidase, prostaglandine synthesizing enzymes, downregulation of scavenging enzymes and metabolites, or yet unknown pathways.

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How is IκB released in response to oxidative stress?

Oxygen radicals can covalently modify many different amino acid residues in proteins, including cysteine, methionine and histidine residues.[45] Because it is well established that covalent modifications can alter the activity of proteins, one possibility is that direct oxidative damage causes the dissociation of the NF-κB-IκB complex. The target could be either the IκB-binding p65 subunit or IκB itself. Since purified IκB can still react with the NF-κB found in nuclei of stimulated cells, it seems that IκB rather than p65 is the target for a dissociating reaction. It is however possible that p65 is only transiently modified, or that both subunits undergo modification.

A direct oxidative mechanism must very selectively disturb the p65-IκB interaction because p65 has to remain functional in DNA binding and transcription activation. We tried to test the idea of direct oxidation by *in vitro* experiments. Cytosolic fractions or a partially purified complex of NF-κB and IκB were incubated with various concentrations of H_2O_2 in the presence of Fe^{2+}. Such treatments did not result in the activation of NF-κB *in vitro* suggesting that H_2O_2 (and hydroxyl radicals) does not exert a direct role in activation of NF-κB.[19] It is possible that physiologically relevant ROI species could not be produced under the *in vitro* conditions.

Hydroxyl radicals produced from H_2O_2 cannot function as diffusible intracellular messengers as they non-specifically react with the nearest molecule. A more suitable ROI messenger would be the less reactive H_2O_2. Decomposition of H_2O_2 seems nevertheless required because metal chelators and radical scavengers can suppress the activation of NF-κB by peroxide. H_2O_2 as messenger might therefore require a receptor/target with a metal center.

Recently, a tyrosine-specific protein kinase, called ltk, was found to be associated with the endoplasmic reticulum and shown to be activated upon treatment of cells with diamide.[46] Ongoing studies will show whether ltk is a target for ROI and whether protein phosphorylation by ltk or another kinase is required for release of IκB. Alternative effector proteins activated upon oxidative stress could be proteases that selectively degrade IκB. Finally, certain compounds, such as malondialdehyde or 4-hydroxynonenol that are produced from lipids upon oxidative stress, could covalently modify and thereby dissociate the NF-κB-IκB complex. However, we could

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not observe that a treatment of cells with 4-hydroxynonenol over a wide concentration range activated NF-κB (R. Schreck and P. Baeuerle, unpublished observation). Fifty micromolar of the compound rather interfered with the DNA binding of NF-κB.

NF-κB and the regulation of ROI homeostasis

In bacteria, the oxidative stress response serves to newly induce or increase the synthesis or proteins protecting the cell from oxidative damage. For instance, the oxyR factor induces genes coding for catalase and NADPH-dependent alkyl hydroperoxidase.[5] The bacterial transcription factors participate in the oxidative stress response as crucial regulatory elements with sensor as well as effector functions allowing a readjustment to low intracellular ROI levels. Such regulatory circuits also occur in higher organisms explaining, for instance, why reperfusion of hypoxic tissue induces oxidative stress.[47]

If, in higher eukaryotic cells, NF-κB is part of an oxidative stress response system, the factor should be able to activate genes encoding antioxidative functions. There are two such genes that could turn out to be induced by NF-κB. One is coding for the inducible, Mn-dependent, mitochondrial form of SOD. The transcription of the MnSOD gene is activated when cells are stimulated with TNF, LPS and PMA.[48,49] The other gene codes for the human form of thioredoxin, an enzyme involved in the reduction of cysteines in oxygen-damaged proteins.[50] The transcription of the thioredoxin gene is also induced by a spectrum of agents known to induce NF-κB, including the viral transactivator Tax.[51] Future studies have to explore whether NF-κB is indeed a regulator of the two and other oxidant defence genes.

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Are there other oxidative stress-responsive transcription factors?

Another inducible transcription factor which has a relatively wide spectrum of inducers is AP-1.[52] Like NF-κB, the DNA binding form of AP-1 is a heterodimer. It is composed of the proto-oncogene products c-Jun and c-Fos which belong to a multiprotein family binding DNA by a basic leucine zipper motif. Recent reports showed that the mRNA for the c-Jun subunit is induced by H_2O_2.[53,54] This might explain why also the DNA binding activity of AP-1 appears after a 4 h-treatment with 250 μM H_2O_2 in nuclei of HeLa cells.[54] It is thus likely that also AP-1 participates in oxidative stress response. However, in contrast to NF-κB, the recruitment of c-Jun/AP-1 is not direct, but must involve another transcription factor turning on the c-Jun gene. This situation is comparable to the soxRS system of bacteria (see Introduction). Devary *et al.*[54] proposed that the induction of the Jun gene is autoregulative and involves the AP-1 binding site in the Jun promoter. Our data do not support this idea. The antioxidant PDTC was found to induce AP-1 DNA binding activity and transactivation, while, at the same time, it suppressed activation of NF-κB. It is possible that NF-jun, a novel inducible factor with characteristics reminiscent of NF-κB,[55] is responsible for the induction of the c-Jun gene by H_2O_2.

Another oxidative stress responsive element was identified in the 5′-flanking regions of genes from rat encoding the glutathion S-transferase Ya subunit and NAD(P)H:quinone reductase.[56] The element with the consensus sequence 5′-PuGTGACNNNGC-3′ is referred to as "antioxidant responsive element" (ARE) and confers transcriptional activation upon treatment of cells with antioxidative phenolic compounds. Intriguingly, the same element could activate reporter

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constructs following stimulation of cells with hydrogen peroxide in a μM-range. The transcription factor recognizing this sequence has not yet been identified, and it is not known whether it is activated by ROI in a direct fashion or at a transcriptional level.

CONCLUSIONS

We summarized and discussed in this review experimental evidence suggesting that the inducible cytoplasmic form of the eukaryotic transcription factor NF-κB is responsive to oxidative stress. The factor is selectively activated and transported into the nucleus upon treatment of cells with peroxides, while no evidence could be obtained so far that superoxide or nitric oxide have a similar effect. The mobilization of NF-κB in response to oxidative stress provides an explanation why the factor can be activated by a wide variety of different stimuli and why the activating potential of these stimuli is potently suppressed by antioxidants. Future studies should address the following questions: (1) By what mechanism can the cell inducibly produce ROI for signalling purposes? (2) Is oxidative stress a general response of cells to pathogenic stimulation? (3) How does the cytoplasmic complex of NF-κB senses oxidative stress and by what mechanism is the inhibitory subunit IκB ultimately released? (4) What proteins are involved as upstream messengers in the oxidative stress response? (5) What other transcription factors can directly respond to ROI? NF-κB may provide an important model system to explore a potential role of oxidative stress in intracellular signalling processes.

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References

1. R.I. Morimoto, A. Tissieres and C. Georgopoulos (eds.) (1990) *Stress Proteins in Biology and Medicine*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
2. R.G. Roeder (1991) The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. *Trends in Genetics*, **16**, 402–408.
3. B. Demple and C.F. Amabile-Cuevas (1991) Redox redux: The control of oxidative stress response. *Cell*, **67**, 837–839.
4. S.B. Farr and T. Kogoma (1991) Oxidative stress responses in Escherichia coli and Salmonella typhimurium. *Microbiological Reviews*, **55**, 561–585.
5. G. Storz, L.A. Tartaglia and B.N. Ames (1990) Transcriptional regulation of oxidative stress-inducible genes: Direct activation by oxidation. *Science*, **248**, 189–194.
6. C.F. Amabile-Cuevas and B. Demple (1991) Molecular characterization of the soxRS genes of Escherichia coli: Two genes control a superoxide stress regulon. *Nucleic Acids Research*, **19**, 4479–4484.
7. P.A. Baeuerle (1991) The inducible transcription factor NF-κB: Regulation by distinct protein subunits. *Biochimica et Biophysica Acta*, **1072**, 63–80.
8. M.B. Urban, R. Schreck and P.A. Baeuerle (1991) NF-κB contacts DNA by a heterodimer of the p50 and p65 subunit. *EMBO Journal*, **10**, 1817–1825.
9. M.L. Schmitz and P.A. Baeuerle (1991) The p65 subunit is responsible for the strong activating potential of NF-κB. *EMBO Journal*, **10**, 3805–3817.

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10. P.A. Baeuerle and D. Baltimore (1988) IκB: A specific inhibitor of the NF-κB transcription factor. *Science*, **242**, 540–546.
11. V. Blank, P. Kourilsky and A. Israel (1992) NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. *Trends in Biochemical Sciences*, **17**, 135–140.
12. S. Ghosh and D. Baltimore (1990) Activation in vitro of NF-κB by phosphorylation of its inhibitor IκB. *Nature*, **344**, 678–682.
13. P.A. Baeuerle and D. Baltimore (1991) The physiology of the NF-κB transcription factor. In *Molecular Aspects of Cellular Regulation—Hormonal Control Regulation of Gene Transcription* (P. Cohen and J.G. Foulkes, etc.), Elsevier, North Holland, Biomedical Press, pp. 409–432.
14. A. Meichle, S. Schütze, G. Hensel, D. Brunsing and M. Krönke (1990) Protein kinase C-independent activation of nuclear factor κB by tumor necrosis factor. *Journal of Biological Chemistry*, **265**, 8339–8343.
15. K. Bomsztyk, W. Rooney, T. Iwasaki, N.A. Rachie, S.K. Dower and C. Hopkins Sibley (1991) Evidence that interleukin-1 and phorbol esters activate NF-κB by different pathways: Role of protein kinase C. *Cell Regulation*, **2**, 329–335.
16. G. Camussi, E. Albano, C. Tetta and F. Bussolino (1991) The molecular action of tumor necrosis factor-α. *European Journal of Biochemistry*, **202**, 3–14.
17. N. Matthews, M.L. Neale, S.K. Jackson and J.M. Stark (1987) Tumour cell killing by tumor necrosis factor: Inhibition by anaerobic conditions, free radical scavengers and inhibitors of arachidonate metabolism. *Immunology*, **62**, 153–155.
18. G.H.W. Wong, J.H. Elwell, L.W. Oberley and D.V. Goeddel (1989) Manganeous superoxide dismutase is essential for cellular resistance by cytotoxicity of tumor necrosis factor. *Cell*, **58**, 923–931.
19. R. Schreck, P. Rieber and P.A. Baeuerle (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *Embo Journal*, **10**, 2247–2258.
20. R. Schreck (1992) PhD thesis. Ludwig-Maximilians-University, Munich, Germany.
21. R. Schreck, B. Meier, D.N. Männel, W. Dröge and P.A. Baeuerle (1992) Dithiocarbamates as potent inhibitors of NF-κB activation in intact cells. *Journal of Experimental Medicine*, **175**, 1181–1194.
22. J.M. Aubry (1985) Search for singlet oxygen in the decomposition of hydrogen peroxide by mineral compounds in aqueous solutions. *Journal of the American Chemical Society*, **107**, 5844–5849.
23. L.I. Ignaro (1990) Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annual Review of Pharmacology and Toxicology*, **30**, 535–560.
24. B. Stein, H.J. Rahmsdorf, A. Steffen, M. Litfin and P. Herrlich (1989) UV-induced DNA damage is an intermediate step in UV-induced expression of Human Immunodeficiency Virus type 1, collagenase, c-fos and metallothionein. *Molecular and Cellular Biology*, **9**, 5169–5181.
25. B. Stein, M. Kramer, H.J. Rahmsdorf, H. Ponta and P. Herrlich (1989) UV-induced transcription from the Human Immunodeficiency Virus Type 1 (HIV-1) long terminal repeat and UV-induced secretion of an extracellular factor that induces HIV-1 transcription in nonirradiated cells. *Journal of Virology*, **63**, 4540–4544.
26. M.B. Toledano and W.J. Leonard (1991) Modulation of transcription factor NF-κB binding activity by oxidation-reduction in vitro. *Proceedings of the National Academy of Sciences (USA)*, **88**, 4328–4332.
27. J.A. Molitor, D.W. Ballard and W.C. Greene (1991) κB-specific DNA binding proteins are differentially inhibited by enhancer mutations and biological oxidation. *The New Biologist*, **10**, 987–996.
28. C. Abate, L. Patel, F.J. Rauscher III and T. Curran (1990) Redox regulation of Fos and Jun DNA-binding activity in vitro. *Science*, **249**, 1157–1161.
29. F.J.T. Staal, M. Roederer, L.A. Herzenberg and L.A. Herzenberg (1990) Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. *Proceedings of the National Academy of Sciences USA*, **87**, 9943–9947.
30. M. Meyer, W.H. Caselmann, V. Schlüter, R. Schreck, P.H. Hofschneider and P.A. Baeuerle (1992) Hepatitis B virus transactivator MHBs[t]: Activation of NF-κB, selective inhibition by antioxidants, and integral membrane localization. *EMBO Journal*, **11**, 2991–3001.
31. R. Schreck, R. Grassmann, B. Fleckenstein and P.A. Baeuerle (1992) Antioxidants selectively suppress the activation of NF-κB by the type I Human T cell leukemia virus protein tax *Journal of Virology*, in press.
32. A. Müller, E. Cadenas, P. Graf and H. Sies (1984) A novel biologically active seleno-organic compound: Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ 51 (ebselen). *Biochemical Pharmacology*, **33**, 3235–3239.
33. E.K. Rowinsky, L.A. Cazenave and R.C. Donehover (1990) Taxol: A novel investigational antimicrotubule agent. *Journal of the National Cancer Institute*, **82**, 1247–1259.

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34. P.F. Lindholm, S.J. Marriott, S.D. Gitlin, C.A. Bohan and J.N. Brady (1990) Induction of nuclear factor NF-κB DNA binding activity after exposure of lymphoid cells to soluble Tax_1 protein. *New Biologist*, **2**, 1034–1043.
35. S.M. Ruben and S.A. Rosen (1990) Suppression of signals required for activation of transcription factor NF-κB in cells continuously expressing the HTLV-I Tax protein. *New Biologist*, **2**, 894–902.
36. D. Jamieson (1989) Oxygen toxicity and reactive oxygen metabolites in mammals. *Free Radical Biology and Medicine*, **7**, 87–108.
37. B. Halliwell and J.M.C. Gutteridge (1989) Role of free radicals and catalytic metal ions in human disease: An overview. In *Methods in Enzymology*, **186**, 1–85.
38. A.R. Cross and O.T.G. Jones (1991) Enzymic mechanism of superoxide production. *Biochimica et Biophysica Acta*, **1057**, 281–298.
39. B. Meier, H.H. Radeke, S. Selle, M. Younes, H. Sies, K. Resch and G.G. Habermehl (1989) Human fibroblasts release reactive oxygen species in response to interleukin-1 and tumor necrosis factor-α. *Biochemical Journal*, **263**, 539–545.
40. B.B. Meier, H.H. Radeke, S. Selle, G.G. Habermehl, K. Resch and H. Sies (1990) Human fibroblasts release low amounts of reactive oxygen intermediates in response to the potent phagocyte stimulants serum-treated zymosan, N-formyl-methionyl-leucyl-phenylalanine, leukotriene B4 or 12-o-tetradecanoylphorbol 13-acetate. *Biological Chemistry Hoppe-Seyler*, **371**, 1021–1025.
41. R. Schreck and P.A. Baeuerle (1991) A role for oxygen radicals as second messengers. *Trends in Cell Biology*, **1**, 39–42.
42. T. McCall and P. Vallance (1992) Nitric oxide takes centre-stage with newly defined roles. *Trends in Pharmacological Sciences*, **13**, 1–6.
43. M. Baggiolini and M. Thelan (1991) The phagocytes and the respiratory burst. In *Oxidative Stress: Oxidants and Antioxidants* (H. Sies, ed.), Academic Press, Harcourt Brace Jovanovich, Publishers, London, pp. 399–420.
44. K. Schulze-Osthoff, A.C. Bakker, B. Vanhaesebroeck, R. Beyaert, W.A. Jacob and W. Fiers (1992) Cytotoxic activity of tumor necrosis factor is mediated by early damage to mitochondrial functions—evidence for the involvement of mitochondrial radical generation. *Journal of Biological Chemistry*, **267**, 5317–5322.
45. R.E. Pacifici and K. Davies (1990) Protein degradation as an index of oxidative stress. *Methods in Enzymology*, **186**, 485–502.
46. A.R. Bauskin, I. Alkalay and Y. Ben-Neriah (1991) Redox regulation of a protein tyrosine kinase in the endoplasmic reticulum. *Cell*, **66**, 685–696.
47. B. Omar, J. McCord and J. Downey (1991) Ischaemia-reperfusion. In *Oxidative Stress: Oxidants and Antioxidants* (H. Sies, etc.), Academic Press, Harcourt Brace Jovanovich, Publishers, London, pp. 493–528.
48. G.H.W. Wong and D.V. Goeddel (1988) Induction of manganeous superoxide dismutase by tumor necrosis factor: Possible protective mechanism. *Science*, **241**, 941–944.
49. J. Fujii and N. Taniguchi (1991) Phorbol ester induces manganese-superoxide dismutase in tumor necrosis factor resistant cells. *Journal of Biological Chemistry*, **266**, 23142–23146.
50. A. Holmgren (1989) Thioredoxin and glutaredoxin systems. *Journal of Biological Chemistry*, **264**, 13963–13966.
51. Y. Tagaya, Y. Maeda, A. Mitsui, N. Kondo, H. Matsui, J. Hamuro, N. Brown, K.-I. Arai, T. Yokota, H. Wakasugi and J. Yodoi (1989) ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO Journal*, **8**, 757–764.
52. M. Karin (1991) The AP-1 complex and its role in transcriptional control by protein kinase C. In *Molecular Aspects of Cellular Regulation—Hormonal Regulation of Gene Transcription* (P. Cohen and J.G. Foulkes, eds.), Elsevier, North Holland, Biomedical Press, pp. 235–253.
53. K. Nose, M. Shibanuma, K. Kikuchi, H. Kageyama, S. Sakiyama and T. Kuroki (1991) Transcriptional activation of early-response genes by hydrogen peroxide in a mouse osteoblastic cell line. *European Journal of Biochemistry*, **201**, 99–106.
54. Y. Devary, R.A. Gottlieb, L.F. Lau and M. Karin (1991) Rapid and preferential activation of the c-jun gene during the mammalian UV response. *Molecular and Cellular Biology*, **11**, 2804–2811.
55. M.A. Brach, F. Herrmann, H. Yamada, P.A. Baeuerle and D.W. Kufe (1992) Identification of NF-jun, a novel inducible transcription factor that regulates c-jun gene transcription. *EMBO Journal*, **11**, 1479–1486.
56. T.H. Rushmore, M.R. Morton and C.B. Pickett (1991) The antioxidant responsive element. *Journal of Biological Chemistry*, **266**, 11632–11639.

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57. W. Bossart, H. Loeffler and K. Bienz (1975) Enucleation of cells by density gradient centrifugation. *Experimental Cell Research*, **96**, 360–366.
58. M.H. Wiegler and I.B. Weinstein (1975) A preparative method for obtaining enucleated mammalian cells. *Biochemical and Biophysical Research Communication*, **63**, 669–674.
59. S. Mihm, J. Ennen, U. Pessara, R. Kurth and W. Dröge (1991) Inhibition of HIV-1 replication and NF-κB activity by cysteine and cysteine derivatives. *AIDS* (*Phila.*), **5**, 497–505.
60. J. Liu, T.C. Chiles, R. Sen and T.L. Rothstein (1991) Inducible nuclear expression of NF-κB in primary B cells stimulated through the surface Ig receptor. *Journal of Immunology*, **146**, 1685–1691.
61. J.W. Rooney, P.M. Dubois and C. Hopkins Sibley (1991) Crosslinking of surface IgM activates NF-κB in B lymphocyte. *European Journal of Immunology*, **21**, 2993–3998.
62. C. Thevenin, S.-J. Kim, P. Rieckmann, H. Fujiki, M.A. Norcross, M.B. Sporn, A.S. Fauci and J.H. Kehri (1991) Induction of nuclear factor-κB and the human immunodeficiency virus long terminal repeat by okadaic acid, a specific inhibitor of phosphatases 1 and 2A. *New Biologist*, **2**, 793–800.
63. M.A. Brach, R. Hass, M.L. Sherman, H. Gunji, R. Weichselbaum and D.W. Kufe (1991) Ionizing radiation induces expression and binding of the nuclear factor κB. *Journal of Clinical Investigation*, **88**, 691–695.
64. G. Benichou, J.M. Kanellopoulos, F. Mitenne, P. Galanaud and G. Leca (1989) T-cell chemiluminescence. *Scandinavian Journal of Immunology*, **30**, 265–269.
65. R.M. Tyrrell (1991) UVA (320–380 nm) radiation as an oxidative stress. In *Oxidative Stress: Oxidants and Antioxidants* (H. Sies, ed.), Academic Press, Harcourt Brace Jovanovich, Publishers, London, pp. 57–83.
66. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine*, 2nd edition, Clarendon Press, Oxford.
67. F.-E. Maly, A. Urwyler, R. Tengler, M. Steinitz, B. Golding and A.L. de Weck (1990) Oxidative burst in human EBV-transformed B cell lines triggered by immobilized specific antigen. *Scandinavian Journal of Immunology*, **32**, 661–668.

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