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Oxidative Stress Interference with the Nuclear Factor-κB Activation Pathways

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ABSTRACT. While intracellular redox balance is tightly controlled in many cell types, its modification leads to important cellular changes derived, in part, from a modification of the pattern of gene expression. This modification relies on many transcription factors whose activities are either increased or reduced by a disbalance of the redox environment. Among these transcription factors, nuclear factor-κB (NF-κB) plays a pivotal role in inducing genes involved in the control of the immune system as well as in the response to injury and infection. Because NF-κB can be induced in many cells by a diverse set of stimulating agents, it has been proposed that agents activating it do so by increasing oxidative stress within the cell. However, this model was not found to be universal, since the dependence between NF-κB activation and intracellular reactive oxygen species (ROS) generation was only detected in certain cell lines. The origin of this dependency is still unknown, but could very well be situated in a particular kinase or in adaptator molecules of the signaling cascade, leading to inhibitor κBα (IκBα) phosphorylation. On the other hand, NF-κB can be activated by oxidants in many cell types, but this activation is well characterized only in lymphocytes. This activation is distinct from that of classical activators such as proinflammatory cytokines and phorbol esters, because the activation mechanisms appear to converge on a particular tyrosine residue of IκB-α instead of the two classical N-terminal serines. The nature of the protein kinases or protein phosphatases involved in this process is still undetermined. It will be a challenge in the future to identify the kinases/phosphatases activated by oxidants and to discover why ROS are required in some cells to turn on the transduction pathway leading to NF-κB activation by physiological stimuli. BIOCHEM PHARMACOL **60**;8:1075–1083, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. oxidative stress; NF-κB; signal transduction; tyrosine kinase; cytokines

It has been known for many years that promoter-specific transcription factors exert a primary role in regulating the activity of the basal transcriptional machinery [1, 2]. In order to adjust the expression of target genes according to external requirements, the activity of these transcription factors can be controlled by signaling pathways through a cascade of events involving kinases and phosphatases. The activity of a transcription factor can be regulated at different levels: (i) at the transcriptional level through changes in the activity of the transcriptional unit controlling the expression of the transcription factor itself [3, 4]; (ii) at the pretranslational level through alternative splicing [5, 6; and (iii) at the posttranscriptional level through modifications of the glycosylation [7], acetylation [8], phosphorylation [9], and redox state of the transcription factor [10] or of various cell compartments [11].

THE BASIS FOR A REDOX REGULATION

Redox regulation can be defined as the modulation of protein activity by oxidation and reduction and has been recognized as one of the most important physiological mechanisms for controlling cellular activities [12]. One of the crucial steps in redox regulation is thus the perception of redox-signaling molecules such as superoxide anion, hydrogen peroxide, singlet oxygen, and nitric oxide. The understanding of redox regulation hinges, then, on the identification and characterization of proteins that sense reactive oxygen (ROS†) and nitrogen (RNS) species. In prokaryotic cells, redox-sensitive transcription factors were very early recognized as sensors of elevated levels of superoxide anion and hydrogen peroxide that activate the expression of genes encoding antioxidants [13]. In eukary-

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† *Abbreviations:* ROS, reactive oxygen species; TRX, thioredoxin; IκB, inhibitor kappa B; IKK, IκB kinase; LZ, leucine zipper motif; HLH, helix–loop–helix motif; NF-κB, nuclear factor-kappa B; NIK, NF-κB-inducing kinase; MEKK1, mitogen-activated protein/extracellular signal-regulated kinase kinase 1; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; PKR, double-stranded RNA (dsRNA)-activated serine–threonine protein kinase; MAP3K, mitogen-activated protein kinase kinase kinase; TRAF2, TNF receptor-associated factor 2; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; $pp90^{rsk}$, p90 ribosomal S6 kinase; and PI3-K, phosphoinositide 3-kinase.

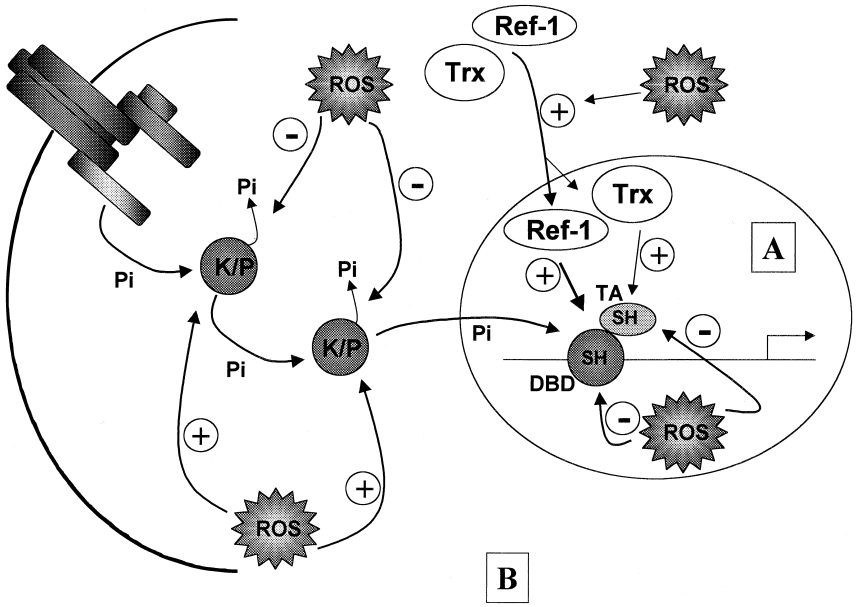

FIG. 1. (A)Effects of ROS on transcription factors within the nucleus. When critical cysteine residues undergo oxidation, the function of the transcription factors can be dramatically altered by preventing their DNA binding or transactivating activity by modifying the contact with the basal transcription machinery. (B)Effects of ROS on transduction pathways where they could possibly activate kinase or inhibit phosphatase (K/P) activities. TA, transactivation domain; SH, SH domain; DBD, DNA-binding domain.

otic cells, redox regulation of transcription factor activity chiefly occurs through reduction/oxidation of specific cysteine residues situated in the DNA-binding domain of transcription factors (Fig. 1). Oxidation of these crucial cysteine residues most often leads to inhibition of transcription factor activity by oxidative stress. Examples of transcription factors that negatively regulate oxidation of reactive cysteines in eukaryotes are NF-I (nuclear factor-I), which possesses redox-sensitive cysteines both in DNA-binding and -transactivating domains an [14, 15], Sp1 [16], several transcription factors containing an HLH motif such as USF (upstream stimulatory factor) [17], MyoD [18], HIF-1α (hypoxia-inducible factor) [19], and the AhR (aryl hydrocarbon receptor), which binds to so-called xenobiotic-responsive elements and is sensitive to strong oxidation conditions [20]. The well-known tumor suppressor protein p53 also contains 12 cyteine residues, nine of which are located in the DNA-binding domain; some of these nine are involved in zinc coordination [21]. *In vitro* oxidation leads to several non-functional p53 conformations and DNA-binding impairment [22]. In addition, *in vivo* DNA binding of p53 is impaired by hydrogen peroxide, with the level of p53 protein being unchanged [23]. *In vitro* DNA-binding activity of NF-κB has also been shown to be inhibited by diamide, a glutathione-oxidizing agent [24]. NF-κB, like other transcription factors, is sensitive to oxidative modification of a particular cysteine at position 62 in p50, which is crucial for DNA-binding activity [25, 26].

Since the activity of a redox-sensitive transcription factor is affected by oxidation, eukaryotic cells have developed rescue mechanisms to keep these critical residues in their reduced state, at least in the nuclear compartment (Fig. 1). Several proteins that are able to reduce cysteine residues have been identified. For instance, TRX, Ref-1, and glutaredoxin (a thioltransferase) have been shown to increase the DNA-binding activity of several transcription factors by targeting crucial cysteines [27, 28]. The case of Ref-1 is very interesting, because it is a multifunctional enzyme identified as a nuclear protein that facilitates AP-1 (activator protein-1) DNA-binding but that also has DNA repair activities [29]. Similarly to TRX, the DNA-binding activity of several transcription factors (Fos, Jun, CCAAT enhancer binding protein C/EBP, Myb, and NF-κB) can be stimulated by Ref-1, and the DNA-binding activity of several other transcription factors (Pax, NF-Y, HIF-1α etc.), which is altered under oxidative conditions, can be protected by Ref-1 [30].

Several of these transcription factors that require reducing conditions for DNA-binding *in vitro* become activated *in vivo* by oxidative stress-promoting agents such as hydrogen peroxide [31], anticancer agents [32], cytokines [33], and mitogenic molecules [33]. This apparent contradiction has been explained by the presence of reducing enzymes such as TRX and Ref-1, whose expression is induced by oxidative stress [34, 35], but also by the stimulation of the signaling machinery under oxidative conditions [36] (Fig. 1). It is now widely accepted that certain growth factors and cytokines can trigger a rapid, transient increase in ROS levels; ROS, in turn, participates in the activation of downstream signaling [36]. Although many transduction machineries have been shown to be activated by oxidative stress, the mechanisms by which the activation take place are still largely debated (Fig. 1). Recently, tyrosine phosphatases have been proposed as candidates for the redox regulation of signaling cascades [37]. The attractiveness of tyrosine phosphatases as redox sensors lies in their crucial role in signal transduction and their susceptibility to oxidants. Oxidation of tyrosine phosphatases could well be the primary event in the ligand-independent activation of cell receptors by tyrosine kinase activities such as epithelial growth factor (EGF) and platelet-derived growth factor

(PDGF) [37, 38]. Oxidative stress could either affect the structure of the receptors such that they could no longer be phosphorylated with the same rapid kinetics or inactivate the dephosphorylation enzymes. Both possibilities would result in a shift of the balance from a low level of autophosphorylation to a predominance of spontaneous kinase activity. In the case of signal transduction initiated at receptors without tyrosine kinase activities (e.g. cytokine receptors), the mechanism is still largely unknown.

Among the various transcription factors whose activity can be influenced by oxidative stress, NF-κB is one of the most important. The observation that oxidative stress, such as addition of extracellular hydrogen peroxide, can trigger its nuclear translocation in several cell lines was already made ten years ago by Schreck *et al.* [39], making it one of the first characterized redox-controlled transcription factors.

NF-κB FAMILY MEMBERS

NF-κB and the other members of the Rel family of transcriptional activator proteins are a focal point for understanding how extracellular signals induced the expression of specific sets of genes in higher eukaryotes [40–42]. Unlike most transcription factors, proteins of this family reside in the cytoplasm and must therefore translocate into the nucleus in order to function. The nuclear translocation of Rel proteins is induced by an extraordinarily large number of agents such as bacterial and viral pathogens, immune and inflammatory cytokines, or a variety of agents that damage cells such as oxidizing agents and radiation. Remarkably, an even larger number of genes appear to be targets for activation by Rel proteins.

The Rel protein family has been divided into two groups based upon differences in their structures, functions, and modes of synthesis. The first group consists of p50 (NF-κB1) and p52 (NF-κB2), which are synthesized from precursor proteins of 105 and 100 kDa, respectively. The mature proteins, which are generated by proteolytic processing, have a so-called Rel homology domain that includes motives for DNA binding and dimerization with a nuclear localization signal. The mature proteins form functional Rel dimers with themselves or other members of the family, and dimers containing the unprocessed proteins remain sequestered in the cytoplasm. The second group of Rel proteins, including Rel A, c-Rel, Rel B, v-Rel, and the *Drosophila* Rel proteins Dorsal and Dif are not synthesized as precursors. In addition to the Rel homology domain, they possess one or more transcriptional activation domains. Members of both groups of Rel proteins can form homo- or heterodimers; for example, NF-κB is the classical p50/Rel A heterodimer that binds to the 5′-GGGANNYYCCC-3′ consensus sequence.

Two types of Rel protein complexes are found in the cytoplasm prior to induction. The first consists of Rel homo- or heterodimers (e.g. p50 and Rel A) bound to a member of the IκB family of inhibitory proteins (IκBα, IκBβ, IκBγ, IκBϵ, Bcl-3, and the *Drosophila* protein cactus). Members of this family share a characteristic ankyrin repeat motif that is required for their interactions with Rel proteins and a C-terminal PEST sequence thought to be involved in protein degradation [43]. The second type of complex consists of a heterodimer formed from a mature Rel protein (e.g. Rel A) and an unprocessed Rel protein precursor (e.g. p105). A third type of complex has been identified in human mammary epithelial cells. It involves the heterodimer p50/Rel A sequestered in the cytoplasm through p100 ankyrin repeats [44].

NF-κB ACTIVATION

The mechanism whereby diverse stimulants lead to the nuclear translocation and DNA-binding activity of NF-κB has been a subject of intense and exciting research. Most work has focused on the p50/Rel A dimer, the predominant form of NF-κB activated in many cells, and its association with IκBα. It is now known that upon stimulation with many NF-κB inducers, IκBα is rapidly phosphorylated on two serine residues (S32 and S36), which targets the inhibitor for ubiquitination and subsequent degradation by the 26S proteasome [45]. The two critical serine residues on IκBβ are serines 19 and 23 [46]. The released NF-κB dimer can then translocate to the nucleus and activate target genes by binding with high affinity to κB elements within the promoter. This series of events is supported by several lines of evidence: (i) various proteasome inhibitors prevent IκBα degradation; (ii) stabilized IκBα is hyperphosphorylated at S32,36 and multi-ubiquitinated; (iii) an S32,36A phosphorylation site mutant is resistant to signal-inducible ubiquitination and degradation; (iv) a K21,22R mutant allows signal-inducible S32,36 phosphorylation but retards ubiquitination and degradation; (v) proteasome inhibitors block the appearance of NF-κB in the nucleus; and (vi) an S32,36A mutant or deletion of the amino-terminal phosphorylation sites produces a dominant negative mutant of IκBα that is capable of preventing NF-κB activation by these signals. The phosphorylation and degradation of IκBα are tightly coupled events, so it was obvious that agents that activate NF-κB do so by stimulating a specific IκBα kinase, or alternatively by inactivating a particular phsophatase.

THE IκBα KINASE (IKK) PATHWAY

Since the key step in NF-κB activation was shown to be IκB phosphorylation, a search for a stimulus-responsive protein kinase catalyzing this event has been the subject of intense work. A protein kinase activity that is specific for the N-terminal regulatory serines of IκBs has been identified [47]. This activity, named IKK, is serine-specific and responsive to a number of potent NF-κB activators, most notably TNF-α and IL-1β, which stimulate its activity with kinetics that match those of IκB degradation. Three IKK polypeptides have been identified. Two of these, IKKα

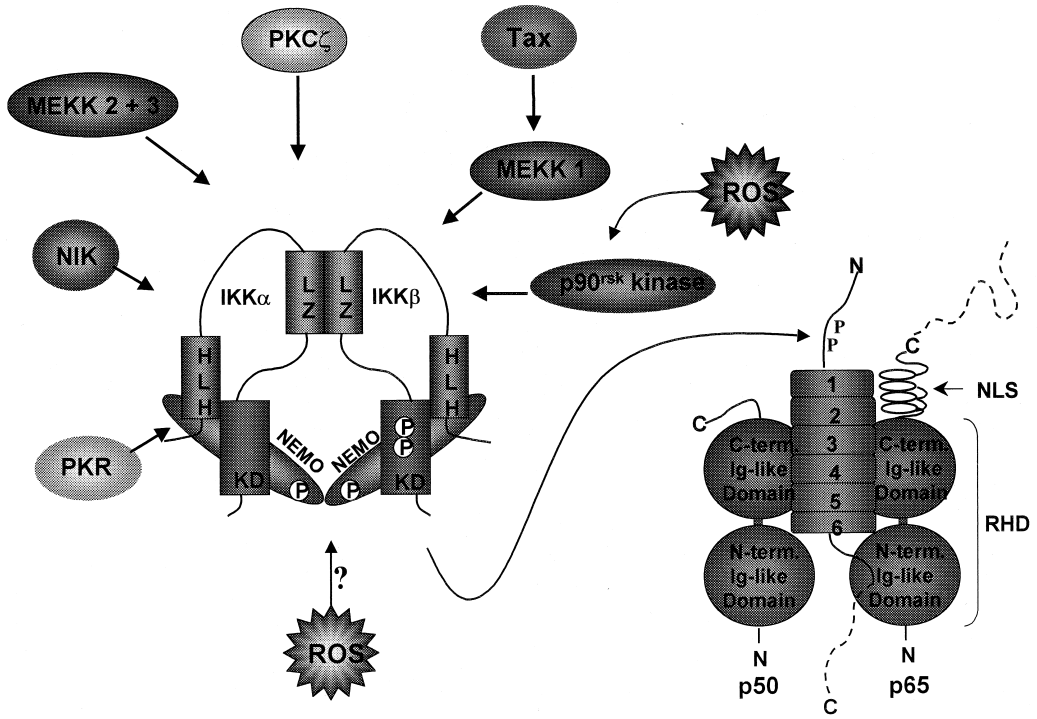

FIG. 2. Schematic representation of the IKKα/IKKβ/IKKγ-NEMO part of the signalsome and of the RelA/p50/IκBα complex. Upstream kinases influencing IKK activity are represented together the Tax protein of human T-lymphotrophic virus type-1 (HTLV-I) and ROS. RHD, Rel homology domain; PKC, protein kinase C; NLS, nuclear localization signal; KD, kinase domain; Ig, immunoglobulin.

(IKK-1) and IKKβ (IKK-2), are catalytic subunits [48], whereas the third polypeptide, IKKγ (also known as NEMO), is regulatory [49, 50] (Fig. 2). IKKα and IKKβ have very similar primary structures with protein kinase domains at their N-terminus, an LZ, and an HLH motif at their C-terminal portion. IKKγ/NEMO does not contain a recognizable catalytic domain, but is composed mostly of three large α-helical regions, including an LZ. Biochemical analysis indicates that the predominant form of IKK is an IKKα/IKKβ heterodimer associated with either a dimer or trimer of IKKγ [50]. Phosphorylation is also involved in negative regulation of IKK activity [51]. A three-step model has been proposed to explain the regulation of IKK activity [51, 52]. Initially, the inactive IKK complex is not phosphorylated on its catalytic subunits. In response to stimuli, upstream kinases are activated and recruited to the complex via IKKγ. This results in phosphorylation of IKKβ and activation of IKKs. Through intramolecular *trans*-autophosphorylation, the activated IKK subunit can phosphorylate the adjacent subunit, which can be either IKKα or IKKβ (in the case of a homodimer), as well as other inactive IKK complexes through an intermolecular reaction. The activated IKK complexes phosphorylate the IκB subunits of NF-κB/IκB complexes, triggering their ubiquitin-dependent degradation and activation of NF-κB. C-terminal autophosphorylation of IKKs induces a low-activity state, facilitating inactivation of IKK by phosphatases once the upstream signal has disappeared.

Two members of the MAP kinase family, NIK and MEKK1, have been shown to directly interact with IKK [53, 54] and activate the kinase subunits [55, 56] (Fig. 2). Signals initiated by IL-1β, T-cell receptor engagement (CD3/CD28 induction), TNF-α, CD95, and Epstein–Barr virus (EBV) latent infection membrane protein 1 (LMP1) converge on NIK [57, 58]. Recently, the interferon-inducible PKR was shown to be physically associated with the IKK complex and the PKR-dependent dsRNA induction of NF-κB to be mediated by NIK and IKK [59]. NF-κB induction by the transforming protein (Tax) of human T-cell leukemia virus type 1 is mediated through MEKK1 [60]. Additionally, TNF-α and IL-1 have been shown to activate and utilize both NIK and MEKK1 coordinately and synergistically [56]. Two other members of the MEKK family (MEKK2 and MEKK3) have been reported to induce IKK activation and site-specific IκBα phosphorylation [61] (Fig. 2). Thus, the consensus with regard to the signal-induced activation of NF-κB appears to be that the core element of the signaling cascade is a MAP3K and an IKK. It should, however, be emphasized that most reports showing the requirements of a signaling cascade involving a MAP3K family member as an upstream kinase for the IKKs have reached their conclusions by using overexpressed wild-type proteins or kinase-dead mutants, a strategy that might not reflect the physiological role of these kinases in activating the IKK complex. In NIK−/− mice for example, TNF-α- and IL-1-β-mediated NF-κB activation does not

seem to be affected, showing either the importance of alternative signal transduction pathways or a propensity of the IKKs to autophosphorylate.

THE IKK PATHWAY: REDOX-SENSITIVE?

This core element (MAP3K/IKK) is likely the focal point for a large diversity of agents that might activate NF-κB. How these NF-κB-activating agents converge on the IKK complex is still largely unknown. An early explanation was proposed by Schreck *et al.* [62], whereby diverse agents would all activate NF-κB by causing an oxidative stress. This proposal has been confirmed by many observations showing that NF-κB activation by diverse stimuli (cytokines, phorbol ester, lipopolysaccharide, CD3 engagement, etc.) generated an increase in the intracellular ROS level. The observation that intracellular ROS generation is required for NF-κB activation is not universal. Indeed, it has recently been shown that proinflammatory cytokines (IL-1 and TNF-α) led to a substantial intracellular ROS generation in lymphoid and monocytic cell lines, whereas in several epithelial cell lines (ovarian, colon, breast, and cervix lines) there was no detectable ROS increase upon activation [63–65]. This lack of ROS generation upon NF-κB activation has also been recorded in endothelial cells (ECV304), where hydrogen peroxide release was not increased upon stimulation with inducers at concentrations and times of exposure that gave strong NF-κB activation [66]. In addition, in epithelial and endothelial cells, NF-κB activation cannot be inhibited by antioxidants and metal chelators as was the case in lymphoid and monocytic cells. This all demonstrates that ROS generation in response to NF-κB inducers is a cell- and stimulus-specific, and not universal, phenomenon. However, in some cell lines, NF-κB activation requires a concomitant ROS generation, since the activation could be abolished in the presence of a large diversity of antioxidant molecules (*N*-acetyl-L-cysteine [NAC], dithiocarbamates, vitamin E derivatives, and glutathione peroxidase activators) that were shown to exert their effects by different mechanisms. How ROS feed into the IKK pathway in lymphoid and monocytic cells is still a mystery because there has been, up to now, no evidence to substantiate the idea that the central IKK, upon which diverse signaling pathways converge, is oxidant-responsive or redox-regulated (Fig. 2). IKKα and IKKβ contain a cysteine residue at position 179 within their activation loop. While the substitution of cysteine 179 into alanine does not modify IKKβ activation by NIK, the activity of the mutated IKKβ cannot be inhibited by anti-inflammatory cyclopentenone prostaglandins [67]. One possibility to explain the role of ROS requirement in NF-κB activation in several cell lines would be that oxidation of cysteine 179 bridges IKKs together or with other subunits of the complex, thereby maintaining them in a more stable conformation to phosphorylate IκBα. Another explanation would be that certain partners of the signalsome are maintained in a non-functional form by a redox-sensitive inhibitor. TRX has been shown to function as an inhibitor of apoptosis signal-regulating kinase 1 (ASK1), which belongs to the MAP3K family [68]. TRX binds to the N-terminal portion of ASK1, with this interaction highly dependent on the redox status of TRX. Upon cell stimulation with TNF-α, TRAF2 fosters ROS production which, in turn, oxidizes TRX, thus freeing ASK1 to permit its homo-oligomerization and subsequent activation of the p38 pathway [69]. From these experiments, TRX turned out to be a physiological inhibitor of a MAP3K, pointing to the possibility that the ROS dependence of NF-κB activation in several cell lines treated with TNF-α or IL-1β relies on the ROS-mediated dissociation of a member of the signalsome that could be inhibited by TRX. Because MEKK1 and NIK are MAP3K family members, it could be postulated that one of these kinases is associated with TRX or other redox-sensitive proteins in lymphoid or monocytic cell lines. The induction of these cells with TNF-α or IL-1 could lead to a ROS-dependent activation of NIK or MEKK1 by oxidation of the potential redox-sensitive inhibitor.

Recently, it was shown that oxidative stress can activate $p90^{rsk}$ via Fyn and Ras [70]. This stimulation occurs in various cell lines (fibroblasts, lymphocytes, and endothelial cells), is extensive, rapid, and transient, and is observed with micromolar concentrations of hydrogen peroxide. Since $p90^{rsk}$ was shown to be capable of phosphorylating the N-terminal domain of IκBα and stimulating its degradation [71], it could be argued that in the case of several inducers (not TNF-α and Tax), $p90^{rsk}$ could collaborate or synergize with IKKs in NF-κB activation, thereby accounting for the redox dependence observed in some cell lines with a particular subset of inducers.

IKK-INDEPENDENT PATHWAYS

There are exceptions to the MAP3K/IKK pathway for NF-κB activation. The first is activation of NF-κB in response to UV-C radiation (254-nm wavelength) which, although dependent on IκB phosphorylation and on ubiquitin-mediated proteasomal degradation, does not involve IκB phosphorylation at the N-terminal serine residues [72, 73]. Critical IκBα aminoacids for UV activation are serine/threonine residues located in the C-terminal domain (a stretch between amino acid positions 277 and 287 of the carboxy-terminal part of IκBα) (Fig. 3) [73]. Since this process is not dependent on serines 32 and 36, it is thus independent of IKK activity. The second exception occurs in reoxygenated hypoxic cells or in cells treated with pervanadate, which stimulate phosphorylation of IκBα at tyrosine 42 (Fig. 3) [74, 75]. This phosphorylation induces the dissociation of the inhibitor from NF-κB rather than its degradation. The regulatory subunit (p85α) of PI3-K stably interacts with tyrosine-phosphorylated IκBα, providing a potential mechanism for sequestering tyrosine-phosphorylated IκBα from NF-κB. However, other IκB proteins lack a site homologous to tyrosine 42 in IκBα, casting doubt on

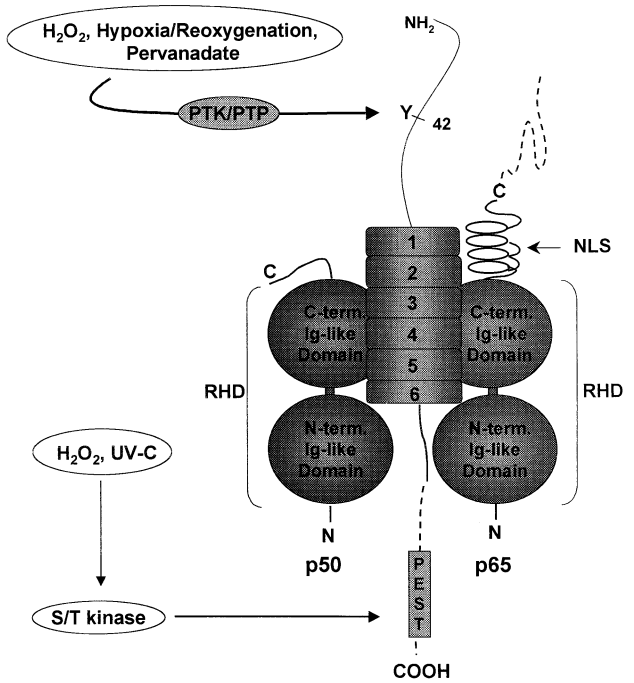

FIG. 3. Hydrogen peroxide, hypoxia/reoxygenation, pervanadate, and UV do not activate NF-κB through the IKK pathway. These stresses converge on tyrosine 42 and on the C-terminal region of the IκBα molecule. NLS, nuclear localization signal; RHD, Rel homology domain; S/T, serine/threonine; Ig, immunoglobulin.

the universality of this pathway. In U937 cells, it has been shown that NF-κB activation by pervanadate requires residue Y42 and IκBα degradation through the 26S proteasome [76]. Very recently, the mechanism of NF-κB activation by hydrogen peroxide was delineated in murine T lymphocytes [77]. In these cells, NF-κB activation by micromolar concentrations of hydrogen peroxide follows a rather slow kinetics compared to the rapid and transient induction observed with proinflammatory cytokines or phorbol ester. Again, activation does not rely on the two N-terminal serine residues, but on phosphorylation of tyrosine 42 and serine/threonine residues in the PEST domain within the C-terminal part of IκBα. This dual-site phosphorylation likely involves two different kinases that should be tandemly activated (Fig. 3). Contrarily to what has been observed with hypoxia/reoxygenation or pervanadate, IκBα is degraded after hydrogen peroxide treatment. Because this degradation is sensitive to calpain inhibitors, it was deduced that phosphorylated IκBα protein on S/T residues in the PEST sequence becomes a substrate for digestion by calpains (Fig. 3). This conclusion is reinforced by recent data showing that the PEST sequence of IκBα is critical for its calpain-dependent degradation [78]. Furthermore, the IκBα-PEST domain binds to the calmodulin-like domain of the large subunit of μ-calpain and acts as a modulator in promoting the physical association and subsequent degradation by μ-calpain [79].

Both hypoxia/reoxygenation and hydrogen peroxide seem to activate NF-κB via an IKK-independent pathway, since N-terminal serine residues do not appear to be required. A still-unidentified tyrosine kinase activity would be needed to phosphorylate tyrosine 42, which will either be responsible for IκBα displacement from the p50/Rel A complex or the target of supplementary phosphorylation in the PEST domain and subsequent degradation by calpain proteases. Many tyrosine kinase activities have been shown to be activated by oxidative stress [80]. In T cells, oxidants such as hydrogen peroxide, pervanadate, and ultraviolet light have been found to mimic the intracellular signals initiated by T-cell receptor (TCR) aggregation [81]. The accumulation of tyrosine-phosphorylated proteins after oxidative stress in T lymphocytes is the result of a shift in the net balance of competing PTK and PTP activities and result, in part, from the activation of several PTKs, including members of the Src, Syk/ZAP-70, and Btk/Itk families [82, 83]. Oxidative stress has also been shown to trigger tyrosine phosphorylation in B lymphocytes through a mechanism that is sensitive to the level of intracellular thiols [84]. Taking together these data with those reporting that PTP functions can be impaired by oxidants, it is obvious that the net increase in tyrosine kinase activity after oxidative stress could be associated with NF-κB activation via tyrosine 42 phosphorylation. The exact nature of the tyrosine kinases involved in the reaction is still to be determined.

CONCLUSIONS

Like several other transcription factors, NF-κB exhibits a dual response to oxidative stress. Its DNA-binding domain must be kept in a reduced form by TRX to bind, *in vitro*, to its responsive elements. *In vivo*, oxidative stress can induce its nuclear translocation after degradation or displacement of IκBα. Tyrosine phosphorylation has been shown to be important for this event, but the tyrosine kinase activity responsible for this phosphorylation is still unknown. On the other hand, NF-κB activation through proinflammatory cytokine receptors appears to be associated with an intracellular increase in ROS. This event is often cell- and stimulus-specific because in many cell lines, in particular in epithelial cells, pathways leading to NF-κB activation do not require ROS generation. The challenge now will be to understand why NF-κB activation can be redox-modulated in some cell types but not in others and what differences among the transduction pathways could explain these apparent discrepancies.

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