



RECENT ADVANCES TOWARDS UNDERSTANDING REDOX MECHANISMS IN THE ACTIVATION OF NUCLEAR FACTOR κ B

YVONNE M. W. JANSSEN-HEININGER,* MATTHEW E. POYNTER,* and PATRICK A. BAEUERLE†

*Department of Pathology, University of Vermont, Burlington, VT, USA; and †Micromet GmbH, Martinsried, Germany

(Received 6 January 2000; Accepted 9 February 2000)

Abstract—The transcription factor, nuclear factor- κ B (NF- κ B) has been studied extensively due to its prominent role in the regulation of immune and inflammatory genes, apoptosis, and cell proliferation. It has been known for more than a decade that NF- κ B is a redox-sensitive transcription factor. The contribution of redox regulation and the location of potential redox-sensitive sites within the NF- κ B activation pathway are subject to intense debate due to many conflicting reports. Redox regulation of NF- κ B has been extensively addressed in this journal and the reader is referred to two comprehensive reviews on the subject [1,2]. With the identification of signaling intermediates proximal to the degradation of the inhibitor, I κ B, the number of potential redox-sensitive sites is rapidly increasing. The purpose of this review is to address recent insights into the NF- κ B signaling cascades that are triggered by proinflammatory cytokines such as TNF- α and IL-1 β . In addition, the role of nitrogen monoxide (NO) in the regulation of NF- κ B will be reviewed. Opportunities for redox regulation that occur upstream of I κ B- α degradation, as well as the potential for redox control of phosphorylation of NF- κ B subunits, will be discussed. Redox-sensitive steps are likely to depend on the nature of the NF- κ B activator, the type of reactive oxygen or nitrogen species involved, the selectivity of signaling pathways activated, as well as the cell type under investigation. Lastly, it is discussed how redox regulation of NF- κ B activation is likely to involve multiple subcellular compartments. © 2000 Elsevier Science Inc.

Keywords—NF- κ B, Redox, Nitrogen monoxide, Signaling, Mitogen-activated protein kinases, Protein kinase A, Phosphoinositide-3-kinase, Free radicals

INTRODUCTION

Investigations that have led to the elucidation of NF- κ B activation pathways have frequently utilized cytokines

Yvonne Janssen-Heininger is an Assistant Professor in the Department of Pathology, University of Vermont, and an Adjunct Assistant Professor and the Department of Pulmonology at the University of Maastricht, The Netherlands. She received her Ph.D. in Health Sciences and Toxicology in Maastricht in 1993. Work in her laboratory focuses on molecular mechanisms of environmental pulmonary diseases with a special focus on reactive oxygen and nitrogen species.

Matthew Poynter is a postdoctoral fellow of the Environmental Pathology Training Grant, in the Department of Pathology, University of Vermont. He received his Ph.D. degree in 1998 from the Department of Pathology, University of Utah. His work emphasized the role of NF- κ B in immune cell activation in aging.

Dr. Patrick Baeuerle is a leading expert in the field of redox regulation of NF- κ B and his laboratory first described in 1991 the importance of oxidants in the activation of NF- κ B. He discovered the association of PI-3-kinase with tyrosine phosphorylated I κ B which is described in this report. He presently is Chief Scientific Officer of Micromet, Inc. where he is developing novel immunotherapeutics for the treatment of cancer.

Address correspondence to: Dr. Yvonne Janssen-Heininger, University of Vermont College of Medicine, Department of Pathology, Med-

including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) as stimulants. These studies have led to the identification of the much sought after kinases that control degradation of the inhibitor molecule I κ B- α . The use of model cytokines has also led to observations that direct phosphorylation of the DNA-binding form of NF- κ B contributes to the transactivation potential of the transcription factor. An enhanced understanding of how cytokines activate NF- κ B DNA-binding and transactivating potential will likely provide valuable insight on how redox events may potentially control the activity of NF- κ B both under normal physiological conditions and in disease states. In this review, the structural and functional characteristics of NF- κ B will be overviewed, recently discovered kinase pathways that control I κ B degradation and NF- κ B transactivation will be described, and the current understanding of redox regulation of

ical Alumni Building, Burlington, VT 05405, USA; Tel: (802) 656-0995; Fax: (802) 656-8892; E-Mail: yjanssen@zoo.uvm.edu.

NF- κ B activation will be addressed, focusing on redox regulation of NF- κ B by nitrogen monoxide (NO). Lastly, experimental approaches that are needed to further address the contribution of redox regulation in activation of NF- κ B will be proposed.

Structural and functional characteristics of NF- κ B

NF- κ B is a transcriptional regulator that consists of homo- and heterodimers of proteins of the Rel family (for recent reviews, see [3–7]). Five members of this family have been identified to date and include p65 (RelA), p50/p105, c-Rel, p52/p100, and RelB. These proteins share structural features and contain a conserved N-terminal sequence called the Rel-homology domain (RHD) consisting of 300 amino acids. The RHD is necessary for homo- and heterodimerization, DNA binding, nuclear localization, and interaction with the inhibitory subunits called I κ B proteins.

NF- κ B is maintained as a latent form in the cytoplasm of cells where it is complexed to I κ B inhibitor proteins. Seven members of the I κ B family of proteins have been identified and include I κ B- α , I κ B- β , I κ B- ϵ , Bcl-3, p100/I κ B- δ , p105/I κ B- γ , I κ B-R. I κ B proteins contain multiple copies of 30–33 amino acid sequences termed ankyrin repeats that mediate the interaction with Rel proteins. Molecular structures of I κ B/NF- κ B complexes were recently revealed [8–11] and have been reviewed [12,13]. The interaction of NF- κ B with I κ B masks the nuclear localization signal and retains NF- κ B in the cytoplasm in a latent form [3–7]. Upon activation of NF- κ B, I κ B is phosphorylated by I κ B kinases (IKK) at two conserved serine residues in the N-terminus, which targets the protein for ubiquitination and degradation by the 26S proteasome [4]. Degradation of I κ B unmasks the nuclear localization signal of NF- κ B, which allows for a rapid translocation of NF- κ B into the nucleus where it binds avidly to DNA (Fig. 1A). NF- κ B proteins bind a DNA motif with the consensus sequence GGGRN-NYYCC, where R = purine, N = any base, and Y = pyrimidine. Based upon this consensus sequence, 128 NF- κ B binding motifs are possible, which may be preferentially recognized by the various dimeric NF- κ B complexes. Curiously, NF- κ B can also bind specifically to DNA sequences that share little similarity with the consensus motif, further expanding the repertoire of genes under transcriptional control by NF- κ B [14].

The number of genes transcriptionally regulated by NF- κ B continues to expand, and includes genes that regulate inflammation and immune responses, viral replication, nitric oxide production (see below), cell-cell interactions, apoptosis, proliferation, etc. (for reviews, see [1–7,15]).

Of particular interest are reports demonstrating that activation of NF- κ B confers a survival signal to many cells. For instance, cells resistant to killing by TNF- α , radiation, or chemotherapeutics can be sensitized by blocking NF- κ B activation using a construct referred to as the I κ B- α super-repressor. This construct contains mutations of serines 32 and 36 or is lacking the entire N-terminus, thereby rendering this molecule refractory to phosphorylation by IKKs and subsequent proteasome-dependent degradation. Expression of such I κ B mutants stably sequesters NF- κ B in the cytoplasm, thereby preventing NF- κ B dependent gene activation. The NF- κ B-dependent survival signal requires transcriptional activation of genes that include Bcl-2, Bcl-X [16], inhibitor of apoptosis protein genes (c-IAP-1, c-IAP-2, etc.) [17], the function of which are still under investigation, and potentially manganese-containing superoxide dismutase [18].

Findings that NF- κ B activation or overexpression was associated with tumorigenesis and metastasis, suggested a role for this transcription factor in the regulation of cell cycle progression. Recent work from the laboratory of A. Baldwin has shed important light on this role when it was demonstrated that NF- κ B activation is important in normal cell cycle progression, at least in part via the transcriptional regulation of cyclin D1 [14].

The activity of NF- κ B is controlled by the transcriptional upregulation of its own inhibitor, I κ B- α through numerous NF- κ B binding motifs in the I κ B- α gene promoter [19]. New expression of I κ B- α disengages NF- κ B from DNA, due to a change of conformation of p65 [3], and sequesters it in the cytoplasm, possibly through the contribution of the nuclear export signal (NES) present in I κ B [20], or exposure of the recently identified putative NES of RelA [21]. Sequestration of NF- κ B by newly synthesized I κ B- α limits prolonged activation of NF- κ B and will cause rapid downregulation of NF- κ B-controlled genes. Interestingly, in response to certain stimuli in specific cell types, the I κ B family member I κ B- β plays a prominent role in mediating the prolonged activation of NF- κ B. After degradation of the existing pool of I κ B- β , newly expressed unphosphorylated I κ B- β can bind NF- κ B [22]. This I κ B- β is a less efficient substrate for IKK- α in comparison to I κ B- α [23,24]. Hypophosphorylated I κ B- β is considered a chaperone that protects NF- κ B from binding I κ B- α . NF- κ B complexed with unphosphorylated I κ B- β can still enter the nucleus, bind to DNA and activate transcription. By this mechanism, I κ B- β can override I κ B- α activity and allow for a prolonged DNA binding and transcriptional activity of NF- κ B [22].

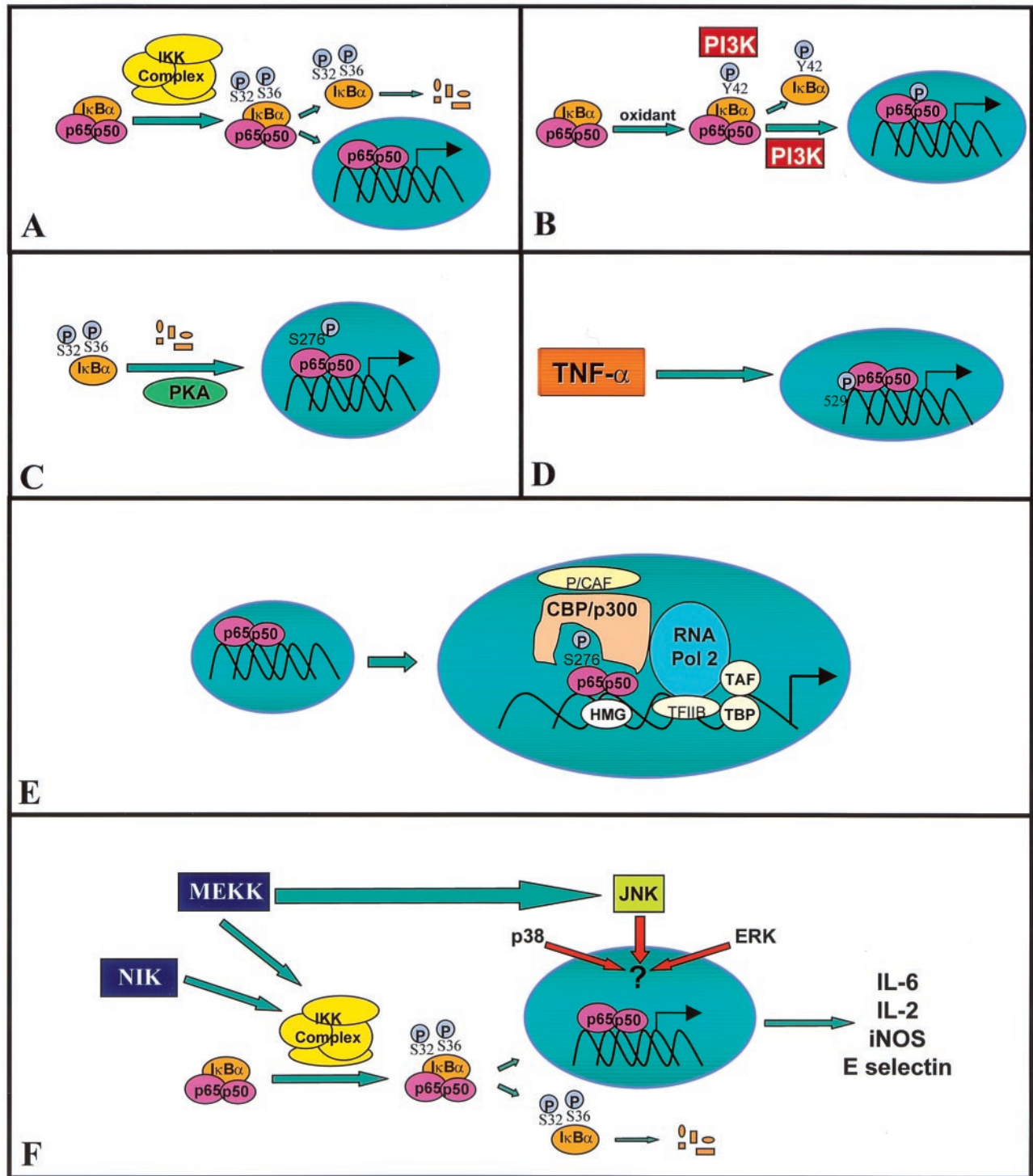


Fig. 1. Pathways that contribute to the activation of NF- κ B. (A) Activation of I κ B kinases cause site specific phosphorylation of I κ B and lead to proteasome-dependent degradation of I κ B. (B) Phosphorylation of I κ B- α at tyrosine 42 causes dissociation of I κ B not associated with degradation via the proteasome pathway. Subsequent activation of NF- κ B ensues via a pathways that involves PI-3 kinase. (C) Activation of NF- κ B by the PKA pathway involves phosphorylation of p65 at serine 276. (D) TNF-induced phosphorylation of p65 at serine 529 augments the transcriptional activity of NF- κ B, which occurs independently of I κ B degradation. (E) Stimulus-induced recruitment of transcriptional coactivators within the nucleus augments the transcriptional activity of NF- κ B. (F) Contribution of MAPKs to the activation of NF- κ B. MAPKs likely regulate I κ B degradation as well as the transcriptional activity of NF- κ B in the nucleus.

Regulation of I κ B via I κ B kinases (IKK)

NF- κ B activation is observed in response to a wide variety of agents that include viruses, cytokines, immune modulators, inorganic compounds, chemotherapeutics, endoplasmic reticulum stressors, oxidants, etc. [3–7,15]. The diversity of stimulants to which it is responsive illustrates that NF- κ B is a versatile transcription factor that regulates many aspects of cellular stress. Because most NF- κ B-activating stimuli cause inducible degradation of I κ B via phosphorylation of two serine residues in the N-terminal portion of the protein, the identification of the kinases that cause inducible phosphorylation of I κ B had been an area of intense investigation. Experimental approaches that employed the cytokine TNF- α as a model NF- κ B activator resulted in the molecular cloning of I κ B kinases in 1997 [23–29].

Binding of TNF- α to its receptor (TNFR-1) leads to the recruitment of the TRADD/TRAF-2 adapter complexes. In some cell types, this causes the localization and activation of NF- κ B-inducing kinase (NIK), which in turn associates with the IKKs. Similarly, binding of IL-1 β to its receptor leads to the association of the TRAF-6, IL-1 receptor accessory protein (IL-1RacP), the adapter protein Myd88, as well as the activation of two serine threonine kinases named IL-1 receptor-associated kinases (IRAK 1 and 2) [4,30]. Assembly of this complex induces its association with IKKs.

IKK was originally purified as an approximately 700 kDa complex containing multiple proteins [31]. Following extensive chemical purification [23,25,26] or as a result of a yeast two-hybrid screening for proteins interacting with NIK [24,27], several laboratories identified the kinases that inducibly phosphorylate I κ B at serines 32 and 36. Several IKK subunits exist: the catalytic subunits IKK- α , IKK- β , and the regulatory subunit IKK (also known as NF- κ B essential modulator [NEMO]) [32]. The core IKK complex consists of a heterodimer of IKK- α and IKK- β and two IKK- γ subunits. IKK- α and - β mediate the phosphorylation of I κ B, whereas IKK- γ links the core to the upstream signaling molecules (see [29] for review). In addition, another protein called IKK-complex-associated protein (IKAP) was found to associate with members of the IKK complex and is thought to act as a scaffold protein [33]. Although homodimeric complexes of IKK- α or IKK- β can be formed *in vitro*, it is thought that in cells IKKs exist primarily as heterodimeric complexes and that phosphorylation of IKK- β is required for activation of the complex. The activity of the IKK complex ultimately mediates the inducible phosphorylation of I κ B- α at serines 32 and 36 and of I κ B- β at serines 19 and 23 [29]. Phosphorylated I κ B- α or I κ B- β are targeted for polyubiquitination of

adjacent lysines followed by degradation through the 26S proteasome (Fig. 1A).

Gene targeting approaches that knocked out IKK- α or IKK- β in mice have revealed that IKK- β , not IKK- α , is required for the activation of IKK in response to proinflammatory stimuli. In embryos of IKK- β knockout animals, the activation of NF- κ B in response to cytokines was abolished. Furthermore, massive apoptosis of liver cells occurred and mice died at embryonic days 12–13 [34]. This phenotype is identical to that of p65 knockout mice [35]. In contrast, IKK- α -deficient mice die after birth and display a plethora of developmental abnormalities reflective of an inability of keratinocytes to proliferate and differentiate, as well as a defect in interdigital apoptosis. IKK- α $-/-$ mice undergo normal activation of NF- κ B in response to cytokine stimulation [36]. These studies demonstrate the unique functional and regulatory roles of IKK- α and - β . Although tremendous progress has been made regarding the components of the IKK complex, its physiologic regulation by upstream activators, including NIK or MEKK-1 [29,37], or agents that affect the cellular redox state, remains poorly understood and an area of active investigations.

A few reports have emerged that examined the activation of IKKs in certain models of oxidative stress. Ionizing radiation, but not ultraviolet C (UVC) radiation, caused activation of IKKs in HeLa cells [38]. As was expected, ionizing radiation, but not UVC, caused degradation of I κ B- α . Both stresses however enhanced NF- κ B DNA-binding activity, illustrating that nuclear translocation can occur independent of I κ B- α degradation [38]. Additionally, UVC was shown to activate NF- κ B in both an IKK-independent and IKK-dependent fashion, contingent upon the timeframe of exposure [39]. The authors of this study report that acute exposure to UVC causes NF- κ B activation independent of IKK activation or I κ B degradation, similar to observations described above. However, prolonged exposure to UVC causes a second wave of NF- κ B activation that is dependent on IKK activation and I κ B- α degradation. This second wave is associated with release of IL-1 β which, in an autocrine or paracrine fashion, activates NF- κ B. The authors refer to IL-1 β as an alarmone that may be critical in transducing some signals in response a variety of stresses [39]. This is an important concept that is often not considered when studying redox regulation of NF- κ B and may explain some of the discrepancies that currently exist in the literature.

Regulation of I κ B- α via tyrosine phosphorylation

In addition to inducible phosphorylation of serines 32 and 36 of I κ B- α , other sites of the I κ B- α molecules are also subject to both constitutive as well as inducible

phosphorylations. For instance, casein kinase 2 (CK-2) constitutively phosphorylates serine 293 of I κ B- α and appears to be required for the turnover of free I κ B- α [40]. Similarly, I κ B- β is phosphorylated by CK-2 at serines 313 and 315 [41].

A site potentially important for inducible phosphorylation is tyrosine 42 of I κ B- α . In response to the oxidant and tyrosine phosphatase inhibitor, pervanadate, or hypoxia/reoxygenation, tyrosine phosphorylation of I κ B- α occurred instead of serine 32 and 36 phosphorylation, preceding the activation of NF- κ B [42]. Tyrosine 42-phosphorylated I κ B- α also results in dissociation of I κ B- α from NF- κ B, but not its subsequent degradation (Fig. 1B). Tyrosine 42-phosphorylated I κ B- α is not recognized by the proteasome, and interactions with a Src homology 2 (SH2) domain-containing protein may be responsible for mediating the dissociation of NF- κ B and I κ B- α [42]. To address the function of tyrosine 42 of I κ B- α in further detail, a tyrosine 42-phosphorylated I κ B- α peptide was employed in order to determine whether specific proteins associate through SH2 (tyrosine kinase) domain interactions [43]. These studies revealed that the regulatory subunit of phosphoinositide 3 (PI-3)-kinase (p85) specifically associates with tyrosine 42-phosphorylated I κ B- α in vitro and in vivo after treatment of T lymphocytes with pervanadate. Furthermore, the catalytic p110 subunit of PI-3-kinase also contributed to NF- κ B activation by pervanadate, evident from the inhibition of NF- κ B DNA binding and reporter gene induction by the PI-3-kinase inhibitor, wortmannin [43]. The exact mechanisms by which PI-3-kinase regulates NF- κ B activation are unclear but may be exerted via the phosphorylation of nuclear p65, as is discussed below (Fig. 1B).

Importantly, coexposure of Jurkat T cells to pervanadate plus TNF- α protected I κ B- α from degradation that is normally observed in response to TNF- α . Pervanadate and TNF- α cotreatment also resulted in a synergistic activation of an NF- κ B-driven luciferase reporter gene, illustrating enhancement of TNF- α signaling under conditions where tyrosine phosphorylation of I κ B- α occurs [43]. Similar synergisms between oxidant and cytokine or oxidant and phorbol ester-induced reporter gene expression have been observed in numerous cell types, although tyrosine phosphorylation of I κ B- α was not reported in those studies [44–46]. In contrast, pervanadate treatment of U937 cells resulted in I κ B- α tyrosine 42-phosphorylation, which blocked NF- κ B activation in response to TNF- α , possibly by preventing phosphorylation of serines 32 and 36 [47].

Although the studies reviewed above point to an important role of tyrosine 42 in regulating the function of I κ B- α , its exact role in regulating the activation of

NF- κ B in response to different stresses in different cell types still is under investigation.

Regulation of the NF- κ B transactivation potential via DNA-binding subunit phosphorylation

Nearly all inducible transcription factors that translocate to the nucleus are also subject to posttranslational modification in order to regulate their activity. For instance, inducible phosphorylation of residues 63 and 73 of c-Jun by c-Jun-N-terminal kinase (JNK) enhances the transcriptional activation potential of c-Jun [48]. Similarly, evidence has emerged that NF- κ B is also subject to post-translational modification by phosphorylation, which enhances its transactivating potential. Numerous pathways have been identified that can regulate the transcriptional activation potential of NF- κ B via phosphorylation. For instance, the laboratory of S. Ghosh has demonstrated that the catalytic subunit of protein kinase A (PKA) is contained within the inactive NF- κ B-I κ B complex. Degradation of I κ B causes activation of PKA in a cAMP independent manner, which, in turn, phosphorylates serine 276 of p65 and thereby enhances its transcriptional activity [49] (Fig. 1C). Inhibition of PKA activity in cells stimulated with the NF- κ B inducers LPS or the phorbol ester PMA plus the lectin phytohemagglutinin potently inhibits the transcriptional activation by NF- κ B. The inhibition of the transcriptional activation potential of NF- κ B after inhibition of PKA occurs in the absence of changes in DNA binding activity [49] and is one of many examples that DNA binding activity does not necessarily reflect transactivation potential, a concept frequently ignored in reports that assess redox regulation of NF- κ B.

As is described above, activation of PI-3-kinase may play a prominent role in the activation of NF- κ B in certain models, including oxidative stress. NF- κ B is subject to phosphorylation by PI-3-kinase, which may account for an increased transcriptional activity of the protein. Recent data demonstrate that the IL-1 β -mediated activation of PI-3-kinase is responsible for NF- κ B activation and that this requires phosphorylation of p65 by the catalytic subunit (p110) of PI-3-kinase [50]. Moreover, overexpression of the PI-3-kinase-activated kinase AKT also induces p65-mediated transactivation [50]. The downstream effectors of PI-3-kinase and AKT that mediate phosphorylation of p65 and the site(s) of phosphorylation remain to be identified. Intriguingly, the PI-3-kinase-mediated activation of NF- κ B-dependent gene expression occurred independently of I κ B degradation, nuclear translocation of NF- κ B, or NF- κ B DNA-binding activity [50].

Also, in response to TNF- α , p65 is phosphorylated at serine 529 in the C-terminal transactivation domain, an

event that is critical in mediating the transcriptional activation by TNF- α (Fig. 1D) [51]. As described in the PI-3-kinase-dependent activation of NF- κ B, this occurred independently of I κ B degradation, nuclear translocation, and binding of NF- κ B to DNA. To date, the kinase responsible for serine 529 phosphorylation remains to be identified [51].

Phosphorylation of p65 is known to occur at specific sites in a stimulus-specific fashion. Serine 276 phosphorylation of p65 confers an enhanced ability to interact with the transcriptional coactivator CBP/p300 [52]. Interaction with coactivators enhances transactivation potential [53,54], possibly as a result of formation of a molecular bridge that allows binding of other accessory factors or the recruitment of RNA polymerase 2 [53] or high-mobility group proteins I(Y) (HMG I(Y)). If alterations in the nuclear redox status affect the recruitment or conformation of those substrates, it would have profound effects on the transcription of NF- κ B-dependent genes (Fig. 1E).

Regulation of NF- κ B activation by mitogen-activated protein kinases (MAPKs)

In response to a wide variety of cytokines, chemical, physical, or biological stresses, including oxidants, the family of mitogen-activated protein kinases (MAPKs) become rapidly activated, as is the subject of review by Rincon and Davis in this Forum. For the purpose of this review, note that three subclasses of MAPKs have been described: the extracellular signal-regulated kinase (ERK), sometimes also referred to simply as MAPK. This subclass is often implicated in the regulation of cell proliferation. Representatives of the other two subclasses of MAPKs are c-Jun-N-terminal kinase (JNK) and p38. The latter two pathways are often induced as a result of cellular stress or under conditions leading to apoptosis [55]. Stimulation of cell surface receptors causes recruitment of adaptor and small G-proteins, which, in turn, activate a series of phosphorylation reactions to activate the MAPKs in the cytoplasm. Activated MAPKs in turn translocate to the nucleus where they phosphorylate specific targets, many of which are subunits of transcription factors. Various outcomes of activation of these pathways have been described that are likely to depend upon the target of phosphorylation within a cell as well as the extent and duration of activation of these pathways [55].

Many agents that activate MAPKs also activate NF- κ B, suggesting that cross talk occurs between these pathways (Fig. 1F). In support of this notion, it has been demonstrated that in response to TNF- α receptor stimulation, TRAF-2 induces activation of JNK via the downstream activation of receptor interacting protein (RIP) [56], thereby implicating the TRAF-2 molecule as a

point of convergence between the JNK and NF- κ B pathway. Recruitment of NIK proceeds to the activation of IKK and NF- κ B in certain cell types. It is of interest that NIK structurally resembles MAPK kinase kinases [57]. In addition, MEKK-1, an upstream kinase in the JNK pathway, was demonstrated to phosphorylate the 700–900 kDa IKK kinase complex and IKK- α and IKK- β [58], and is required for the ability of TNF- α to activate NF- κ B [31]. Coordinate regulation of IKKs by MEKK and NIK has been demonstrated in HeLa and Cos-1 cells exposed to TNF- α or IL-1 β . Formation of MEKK-1-IKK versus NIK-IKK complexes may provide a molecular basis for the selective regulation of IKK in response to diverse stimuli [59]. Despite these observations, physiological substrates for IKKs remain controversial, as is apparent from the literature [37,58]. More recently, the MAPK family members, MKK 2 and 3, were shown to target IKK- α and IKK- β and thus, mediate activation of NF- κ B [60].

The cooperation between MAPK and NF- κ B in the regulation of gene expression may also occur at the level of transcriptional activation (Fig. 1F). IL-6, inducible nitric oxide synthase (iNOS), E-selectin, and IL-2 are genes known to be transcriptionally regulated by NF- κ B. A number of reports have recently demonstrated that various MAPKs may augment or cooperate with NF- κ B to transcriptionally activate expression of these genes [61–68]. For instance, TNF- α causes activation of the p38 subclass of MAPK and transcriptional upregulation of IL-6. Inhibition of p38 using the pharmacologic inhibitor, SB203580, or a dominant negative mutant of MKK-6, inhibited NF- κ B-dependent reporter gene activation [68,69]. Overexpression of MKK-6 in myocardial cells activates NF- κ B and inhibits apoptosis in a p38-dependent manner, suggesting an important role for p38 and NF- κ B in the survival of some cell types [70].

In contrast to this work are observations that activation of p38 by sodium salicylate inhibit I κ B- α degradation and NF- κ B activation by TNF- α [71]. Furthermore, ERK, JNK, and the JNK target, c-Jun, are important in the NF- κ B-dependent activation of IL-6 in human monocytes [65], suggesting that different MAPKs may regulate the same gene in different cell types. The LPS-mediated induction of iNOS in RAW macrophages is in part dependent upon NF- κ B (see below). Chen and Wang recently demonstrated that p38, but not ERK, is required for iNOS induction in response to stimulation with LPS [63]. Blockage of p38 also blocked iNOS expression in mouse astrocytes [62]. In addition, pharmacological inhibition of ERK or p38 directly repressed the transactivation function of p65 in cells exposed to TNF- α [68]. Inhibition occurred without apparent effect of NF- κ B DNA-binding activity, again illustrating a

marked discrepancy between DNA-binding activity and the transcriptional activity of NF- κ B.

Further evidence for relationships between MAPK and NF- κ B pathways stem from observations that demonstrate a direct interaction between JNK and c-Rel using the yeast two-hybrid system [72]. Of importance is the identification of a novel cytokine entitled thioredoxin peroxidase-related activator of NF- κ B and c-Jun N-terminal kinase (TRANK), a protein highly homologous to thiol specific antioxidant proteins. This protein, as its name implies, was shown to activate both NF- κ B and JNK and caused expression of the NF- κ B regulated genes, iNOS and intracellular adhesion molecule (ICAM) [73]. This illustrates that redox-sensitive pathways may activate MAPKs and NF- κ B in a coordinated fashion.

Work from our laboratory examined the contribution of MAPKs in the activation of an NF- κ B-dependent reporter gene in rat lung epithelial cells. Overexpression of MEKK-1 activated an NF- κ B-dependent luciferase reporter, and synergized with hydrogen peroxide (H_2O_2) or the ONOO⁻ generator, 3-morpholinosydnonimine (SIN-1), to activate NF- κ B-dependent luciferase gene activity. In contrast, a dominant negative form of MEKK-1 decreased or inhibited the ability of TNF- α or oxidants to induce NF- κ B-dependent reporter gene activity [46]. Interestingly, selective inhibition of the ERK pathway with the MEK-1 inhibitor PD98059 caused constitutive activation of NF- κ B-dependent luciferase activity and dramatically enhanced the ability of TNF- α or SIN-1 to activate NF- κ B. This work suggests that the MEKK-1 pathway, and potentially JNK as a downstream effector, positively regulates NF- κ B activity in response to oxidants, whereas the ERK pathway negatively regulates NF- κ B [46]. These results also imply that coordinate activation of multiple MAPK pathways may, at the same time, transduce both positive and negative signals towards NF- κ B activation and the extent and duration of activation of these pathways likely dictates whether or not NF- κ B activation ensues.

At present, the targets of phosphorylation by MAPK that enhance NF- κ B-dependent gene activation are unknown. The contrasting effects of different MAPK in regulating NF- κ B-dependent gene expression in different cell types may be the result of different activation profiles of these pathways, and hence their relative sensitivity to inactivation by pharmacological agents or dominant negative constructs.

Redox regulation of NF- κ B: nitrogen monoxide ($\cdot$ NO)

In addition to peroxides, which have been implicated in the activation of NF- κ B [1,2], it has become apparent that nitrogen monoxide (nitric oxide, $\cdot$ NO) can also reg-

ulate the activity of NF- κ B. Interesting analogies exist between the activities of $\cdot$ NO and NF- κ B, which suggest that they may be regulated coordinately. For instance, like NF- κ B, $\cdot$ NO has been associated with protection against apoptosis (see [74] for review) although contradictory reports also exist [75]. As is discussed below, the effects of $\cdot$ NO are probably cell type and concentration dependent. Consequently, both activation [76–78] and inhibition [76,79–85] of NF- κ B have been observed in response to $\cdot$ NO.

Various laboratories have convincingly described that NF- κ B sites in the promoter region of the gene encoding inducible nitric oxide synthase (iNOS) are required for transcriptional activity. In many cases, agents that block NF- κ B also block iNOS gene expression and $\cdot$ NO production [86] (Fig. 2). It is believed that $\cdot$ NO produced by iNOS may serve in a negative feedback loop to block prolonged activation of NF- κ B and transcription of its target genes [79–82], thereby limiting chronic inflammation [80,81]. The inhibitory effect of high concentrations of $\cdot$ NO on NF- κ B activation may occur as a result of nitrosylation of cysteine 62 of the p50 protein, which prevents NF- κ B from binding DNA [87,88], although a subsequent study demonstrated that immunoprecipitated, nitrosylated p50 retained DNA-binding activity [85]. In models of LPS-induced NF- κ B activation, simultaneous presence of an iNOS inhibitor augmented NF- κ B DNA-binding activity and transactivation, findings again illustrating that production of high concentrations of $\cdot$ NO decrease NF- κ B activation [85,89,90]. The decreased activity of NF- κ B was present under conditions where s-nitroso p50 could be immunoprecipitated from cells, suggesting that the regulation of NF- κ B occurs via p50 nitrosylation in vivo [85]. In addition, stabilization of I κ B by high concentrations of $\cdot$ NO may also contribute to NF- κ B inactivation. For example, $\cdot$ NO donors decreased IL-1 β -induced NF- κ B DNA-binding activity and iNOS mRNA levels, which was associated with a prevention of I κ B- α phosphorylation and degradation [84]. It will be of interest to determine whether I κ B or its upstream activators, the IKKs, are subject to nitrosylation to block their function. Sequence analysis has revealed multiple cysteines in the kinase domain of IKKs [23,24], which make IKKs potential targets of attack by $\cdot$ NO.

Interestingly, conditions that lead to iNOS expression also result in a coordinated upregulation of extracellular superoxide dismutase (EcSOD) [91] or MnSOD [92]. Because SOD rapidly scavenges superoxide ($O_2^{\cdot-}$), this action will spare $\cdot$ NO as it prevents the reaction between $O_2^{\cdot-}$ and $\cdot$ NO that generates peroxynitrite (ONOO⁻). Cells that upregulate SOD will therefore maintain the high $\cdot$ NO concentration required for negative feedback signaling. The importance of this mechanism is sug-

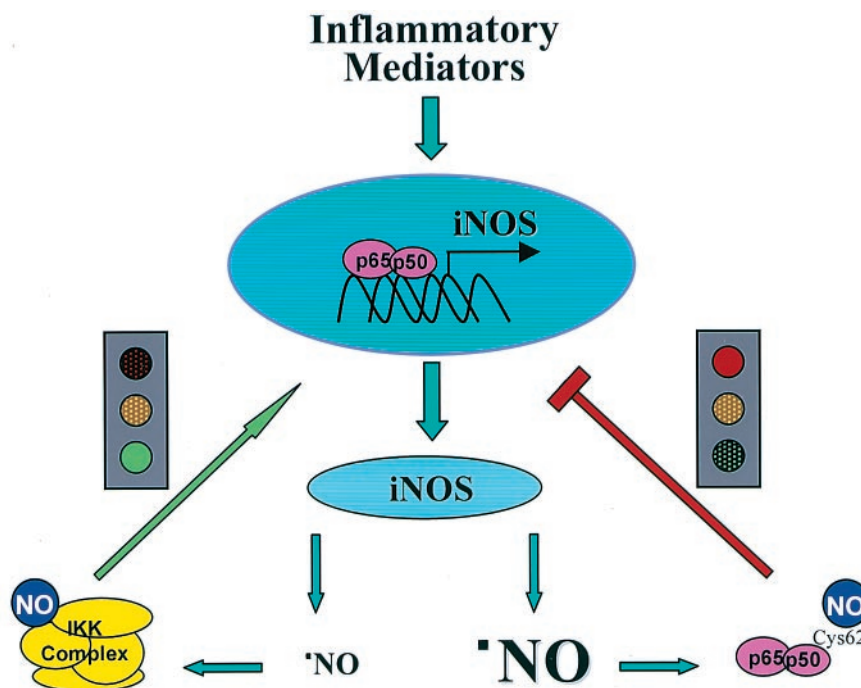


Fig. 2. Control of NF- κ B activation by $\cdot$ NO. Low levels of $\cdot$ NO augment NF- κ B activity potentially via regulation of the activity of IKKs. In contrast, high levels of $\cdot$ NO produced from iNOS or high concentrations of donors inhibit NF- κ B in part via nitrosylation of cysteine 62 of p50 and provides a negative feedback loop to block prolonged activation.

gested by studies demonstrating a potential role of ONOO^- as a proinflammatory stimulus [93] and the presence of nitrotyrosine residues in many models of inflammation [94]. Moreover, work from our laboratory has demonstrated that SIN-1, a generator of ONOO^- , activates NF- κ B [46]. Additionally, the proinflammatory oxidant gas, nitrogen dioxide, a potential reaction product of ONOO^- , also resulted in upregulation of an NF- κ B-dependent reporter gene, and inflammatory genes transcriptionally controlled by NF- κ B (Poynter *et al.*, manuscript in preparation).

The concentration dependence of $\cdot$ NO on the activation of NF- κ B was convincingly demonstrated in studies using $\cdot$ NO donors at different concentrations. Low concentrations of an $\cdot$ NO donor enhanced the TNF- α or PMA induced NF- κ B DNA-binding activity and transactivation, which was associated with an increased activation of IKK- α [76]. It is not clear how this activating effect of $\cdot$ NO is exerted, although s-nitrosylation of Ras and its subsequent activation leading to NF- κ B activation has been observed [77,78]. These data illustrate that low concentrations of $\cdot$ NO provide a self-amplifying signal in the inflammatory response. In contrast, high levels of $\cdot$ NO decreased the ability of cytokines to activate NF- κ B DNA-binding and transactivation of NF- κ B-dependent reporter genes [76,81,83], in the absence of cytotoxic effects [76], confirming aforementioned observations of a negative feedback loop imposed by high

concentrations of $\cdot$ NO. The pathways depicting the concentration-dependent regulation of NF- κ B by $\cdot$ NO are illustrated in Fig. 2.

CONCLUSIONS

In this review, pathways that mediate the activation of NF- κ B have been described. From these events, depicted in Figs. 1A through 1F, it has become clear that NF- κ B activation is exceedingly more complex than release from the inhibitor, I κ B. In addition to I κ B degradation, phosphorylation of NF- κ B subunits is critical for the transcriptional activation of NF- κ B-regulated genes. Subunit phosphorylation is regulated independently of I κ B degradation, and provides an additional level of control of the activity of NF- κ B. Subunit phosphorylation is an important regulatory event in the activation of other transcription factors, such as activator protein 1 (AP-1) [48]. It is unclear whether phosphorylation of NF- κ B occurs in the cytoplasm or the nucleus. The identification of critical kinases that regulate NF- κ B phosphorylation, as well as the sites of phosphorylation, may answer this question. In consideration of the above, redox regulation also can occur at levels that are distinct from I κ B degradation. Documented discrepancies between DNA binding and transactivation clearly illustrate that frequently used EMSA analysis are often times inadequate for evaluating the activity of NF- κ B.

With the discoveries of additional kinases that regulate I κ B degradation and NF- κ B phosphorylation, the sites subject to redox regulation have become more complex and potentially increased in number. When evaluating the link between oxidant stress and NF- κ B, is it of critical importance to consider the site of oxidant generation, the type of oxidant encountered (i.e., *NO vs. ONOO⁻), and the time frame by which NF- κ B activation occurs. For instance, oxidants acting at cell membranes may activate cell surface receptors or Ras directly and trigger downstream MAPK cascades, which thereby contribute to NF- κ B activation. In contrast, oxidants derived from mitochondria or oxidants that readily diffuse across membranes may target proteins that act further downstream in the activation cascade. Alternatively, some effects of oxidants on NF- κ B activation may be caused by autocrine or paracrine release of alarmones such as IL-1 β [39], which, in turn, stimulate a well-characterized activation pathway. The development of assays that allow identification of proteins that have undergone redox-dependent modifications in intact cells will greatly enhance our understanding in this field.

Although numerous laboratories have demonstrated a direct activation of NF- κ B in response to oxidants, other reports have not, thereby causing speculation as to the relevance of oxidants as physiological activators of NF- κ B (see [95] for review). Although the use of many different cell types, agonists, endpoints, timeframes of investigation, and a general lack of consideration of the antioxidant status preclude an answer at this time, it is safe to state that in many cases oxidants at least provide costimulatory signals towards the activation of NF- κ B.

Acknowledgements — The authors thank Laurie Sabens for her help in the preparation of this manuscript. Supported by a grant from the National Institutes of Health (RO1 HL60014).

REFERENCES

- [1] Flohe, L.; Brigelius-Flohe, R.; Saliou, C.; Traber, M. G.; Packer, L. Redox regulation of NF- κ B activation. *Free Radic. Biol. Med.* **22**:1115–1126; 1997.
- [2] Ginn-Pease, M. E.; Whisler, R. L. Redox signals and NF- κ B activation in T cells. *Free Radic. Biol. Med.* **25**:346–361; 1998.
- [3] Baeuerle, P. A. I κ B–NF- κ B structures: at the interface of inflammation control. *Cell* **95**:729–731; 1998.
- [4] May, M. J.; Ghosh, S. Signal transduction through NF- κ B. *Immunol. Today* **19**:80–88; 1998.
- [5] Verma, I. M.; Stevenson, J. K.; Schwarz, E. M.; Van Antwerp, D.; Miyamoto, S. Rel/NF- κ B/I κ B family: intimate tales of association and dissociation. *Genes Dev.* **9**:2723–2735; 1995.
- [6] Baeuerle, P. A.; Baltimore, D. NF- κ B: ten years after. *Cell* **87**:13–20; 1996.
- [7] Ghosh, S.; May, M. J.; Kopp, E. B. NF- κ B and Rel proteins: evolutionarily conserved mediators of immune responses. *Ann. Rev. Immunol.* **16**:225–260; 1998.
- [8] Li, T.; Narhi, L. O.; Wen, J.; Philo, J. S.; Sitney, K.; Inoue, J.; Yamamoto, T.; Arakawa, T. Interactions between NF- κ B and its inhibitor I κ B: biophysical characterization of a NF- κ B/I κ B-alpha complex. *J. Protein Chem.* **17**:757–763; 1998.
- [9] Simeonidis, S.; Stauber, D.; Chen, G.; Hendrickson, W. A.; Thanos, D. Mechanisms by which I κ B proteins control NF- κ B activity. *Proc. Natl. Acad. Sci. USA* **96**:49–54; 1999.
- [10] Huxford, T.; Huang, D. B.; Malek, S.; Ghosh, G. The crystal structure of the I κ B α /NF- κ B complex reveals mechanisms of NF- κ B inactivation. *Cell* **95**:759–770; 1998.
- [11] Jacobs, M. D.; Harrison, S. C. Structure of an I κ B- α /NF- κ B complex. *Cell* **95**:749–758; 1998.
- [12] Baeuerle, P. A. I κ B–NF- κ B structures: at the interface of inflammation control. *Cell* **95**:729–731; 1998.
- [13] Cramer, P.; Muller, C. W. A firm hand on NF- κ B: structures of the I κ B- α –NF- κ B complex. *Structure Fold Des.* **7**:R1–6; 1999.
- [14] Guttridge, D. C.; Albanese, C.; Reuther, J. Y.; Pestell, R. G.; Baldwin, A. S. Jr. NF- κ B controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol. Cell. Biol.* **19**:5785–5799; 1999.
- [15] Pahl, H. L. Signal transduction from the endoplasmic reticulum to the cell nucleus. *Physiol. Rev.* **79**:683–701; 1999.
- [16] Tamatani, M.; Che, Y. H.; Matsuzaki, H.; Ogawa, S.; Okado, H.; Miyake, S.; Mizuno, T.; Tohyama, M. Tumor necrosis factor induces Bcl-2 and Bcl-x expression through NF- κ B activation in primary hippocampal neurons. *J. Biol. Chem.* **274**:8531–8538; 1999.
- [17] Wang, C. Y.; Mayo, M. W.; Korneluk, R. G.; Goeddel, D. V.; Baldwin, A. S. Jr. NF- κ B antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**:1680–1683; 1998.
- [18] Manna, S. K.; Zhang, H. J.; Yan, T.; Oberley, L. W.; Aggarwal, B. B. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor κ B and activated protein 1. *J. Biol. Chem.* **273**:13245–13254; 1998.
- [19] LeBail, O.; Schmidt-Ullrich, R.; Israel, A. Promoter analysis of the gene encoding the I κ B- α /MAD3 inhibitor of NF- κ B: positive regulation by members of the rel/NF- κ B family. *EMBO J.* **12**:5043–5049; 1993.
- [20] Arenzana-Seisdedos, F.; Turpin, P.; Rodriguez, M.; Thomas, D.; Hay, R. T.; Virelizier, J. L.; Dargemont, C. Nuclear localization of I κ B- α promotes active transport of NF- κ B from the nucleus to the cytoplasm. *J. Cell Sci.* **112**:369–378.
- [21] Harhaj, E. W.; Sun, S. C. Regulation of RelA subcellular localization by a putative nuclear export signal and p50. *Mol. Cell. Biol.* **19**:7088–7095; 1999.
- [22] Suyang, H.; Phillips, R.; Douglas, I.; Ghosh, S. Role of unphosphorylated, newly synthesized I κ B- β in persistent activation of NF- κ B. *Mol. Cell. Biol.* **16**:5444–5449; 1996.
- [23] DiDonato, J. A.; Hayakawa, M.; Rothwarf, D. M.; Zandi, E.; Karin, M. A cytokine-responsive I κ B kinase that activates the transcription factor NF- κ B. *Nature* **388**:548–554; 1997.
- [24] Regnier, C. H.; Song, H. Y.; Gao, X.; Goeddel, D. V.; Cao, Z.; Rothe, M. Identification and characterization of an I κ B kinase. *Cell* **90**:373–383; 1997.
- [25] Zandi, E.; Rothwarf, D. M.; Delhase, M.; Hayakawa, M.; Karin, M. The I κ B kinase complex (IKK) contains two kinase subunits, IKK α and IKK β , necessary for I κ B phosphorylation and NF- κ B activation. *Cell* **91**:243–252; 1997.
- [26] Mercurio, F.; Zhu, H.; Murray, B. W.; Shevchenko, A.; Bennett, B. L.; Li, J. W.; Young, D. B.; Barbosa, M.; Mann, M. IKK-1 and IKK-2: cytokine-activated I κ B kinases essential for NF- κ B activation. *Science* **278**:860–866; 1997.
- [27] Woronicz, J. D.; Gao, X.; Cao, Z.; Rothe, M.; Goeddel, D. V. I κ B kinase- β : NF- κ B activation and complex formation with I κ B kinase- α and NIK. *Science* **278**:866–869; 1997.
- [28] Maniatis, T. Catalysis by a multiprotein I κ B kinase complex. *Science* **278**:818–819; 1997.
- [29] Zandi, E.; Karin, M. Bridging the gap: composition, regulation, and physiological function of the I κ B kinase complex. *Mol. Cell Biol.* **19**:4547–4551; 1999.
- [30] Muzio, M.; Ni, J.; Feng, P.; Dixit, V. M. IRAK (Pelle) family member IRAK-2 and Myd88 as proximal mediators of IL-1 signaling. *Science* **278**:1612–1615; 1997.

- [31] Lee, F. S.; Hagler, J.; Chen, Z. J.; Maniatis, T. Activation of the I κ B- α kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* **88**:213–222; 1997.
- [32] Yamaoka, S.; Courtois, G.; Bessia, C.; Whiteside, S. T.; Weil, R.; Agou, F.; Kirk, H. E.; Kay, R. J.; Israel, A. Complementation cloning of NEMO, a component of the I κ B kinase complex essential for NF- κ B activation. *Cell* **93**:1231–1240; 1998.
- [33] Cohen, L.; Henzel, W. J.; Bauerle, P. A. I κ AP is a scaffold protein of the I κ B kinase complex. *Nature* **395**:292–296; 1998.
- [34] Li, Q.; Van Antwerp, D.; Mercurio, F.; Lee, K. F.; Verma, I. M. Severe liver degeneration in mice lacking the I κ B kinase 2 gene. *Science* **284**:321–325; 1999.
- [35] Beg, A. A.; Sha, W. K.; Bronson, R. T.; Ghosh, S.; Baltimore, D. Embryonic lethality in mice lacking the RelA component of NF- κ B. *Nature* **376**:167–170; 1995.
- [36] Hu, Y.; Baud, V.; Delhase, M.; Zhang, P.; Deerincx, T.; Ellisman, M.; Johnson, R.; Karin, M. Abnormal morphogenesis but intact IKK activation in mice lacking the IKK α subunit of I κ B kinase. *Science* **284**:316–320; 1999.
- [37] Karin, M.; Delhase, M. JNK or IKK, AP-1 or NF- κ B, which are the targets for MEK kinase 1 action? *Proc. Natl. Acad. Sci. USA* **95**:9067–9069; 1998.
- [38] Li, N.; Karin, M. Ionizing radiation and short wavelength UV activate NF- κ B through two distinct mechanisms. *Proc. Natl. Acad. Sci. USA* **95**:13012–13017; 1998.
- [39] Bender, K.; Gottlicher, M.; Whiteside, S.; Rahmsdorf, H. J.; Herrlich, P. Sequential DNA damage-independent and -dependent activation of NF- κ B by UV. *EMBO J.* **17**:5170–5181; 1998.
- [40] Barroga, C. F.; Stevenson, J. K.; Schwarz, E. M.; Verma, I. M. Constitutive phosphorylation of I κ B- α by casein kinase II. *Proc. Natl. Acad. Sci. USA* **92**:7637–7641; 1995.
- [41] Czu, Z. L.; McKinsey, T. A.; Liu, L.; Qi, X.; Ballard, D. W. Basal phosphorylation of the PEST domain in the I κ B- β regulates its functional interaction with the c-rel proto-oncogene product. *Mol. Cell. Biol.* **16**:5974–5984; 1996.
- [42] Imbert, V.; Rupec, R. A.; Livolsi, A.; Pahl, H. L.; Traenckner, B. M.; Mueller-Dieckmann, C.; Farahifar, D.; Rossi, B.; Auburger, P.; Baeuerle, P. A.; Peyron, J. F. Tyrosine phosphorylation of I κ B- α activates NF- κ B without proteolytic degradation of I κ B- α . *Cell* **86**:787–798; 1996.
- [43] Beraud, C.; Henzel, W. J.; Baeuerle, P. A. Involvement of regulatory and catalytic subunits of phosphoinositide 3-kinase in NF- κ B activation. *Proc. Natl. Acad. Sci. USA* **96**:429–434; 1999.
- [44] Israel, N.; Gougerot-Pocidalo, M. A.; Aillet, F.; Virelizier, J. L. Redox status of cells influences constitutive or induced NF- κ B translocation and HIV long terminal repeat activity in human T and monocytic cell lines. *J. Immunol.* **149**:3386–3393; 1992.
- [45] Ginn-Pease, M. E.; Whisler, R. L. Optimal NF- κ B-mediated transcriptional responses in Jurkat T cells exposed to oxidative stress are dependent on intracellular glutathione and costimulatory signals. *Biochem. Biophys. Res. Comm.* **226**:695–702; 1996.
- [46] Janssen-Heininger, Y. M. W.; Macara, I.; Mossman, B. T. Cooperativity between oxidants and tumor necrosis factor in the activation of nuclear factor (NF)- κ B: requirement of Ras/mitogen-activated protein kinases in the activation of NF- κ B by oxidants. *Am. J. Respir. Cell Mol. Biol.* **20**:942–952; 1999.
- [47] Singh, S.; Darnay, B. G.; Aggarwal, B. B. Site-specific tyrosine phosphorylation of I κ B- α negatively regulates its inducible phosphorylation and degradation. *J. Biol. Chem.* **271**:31049–31054; 1996.
- [48] Derijard, B.; Hibi, M.; Wu, I. H.; Barrett, T.; Su, B.; Deng, T.; Karin, M.; Davis, R. J. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**:1025–1037; 1994.
- [49] Zhong, H.; SuYang, H.; Erdjument-Bromage, H.; Tempst, P.; Ghosh, S. The transcriptional activity of NF- κ B is regulated by the I κ B-associated PKA α subunit through a cyclic AMP-independent mechanism. *Cell* **89**:413–424; 1997.
- [50] Sizemore, N.; Leung, S.; Stark, G. R. Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF- κ B p65/RelA subunit. *Mol. Cell. Biol.* **19**:4798–4805; 1999.
- [51] Wang, D.; Baldwin, A. S., Jr. Activation of nuclear factor κ B-dependent transcription by tumor necrosis factor α is mediated through phosphorylation of RelA/p65 on serine 529. *J. Biol. Chem.* **273**:29411–29416; 1998.
- [52] Zhong, H.; Voll, R. E.; Ghosh, S. Phosphorylation of NF- κ B by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Molec. Cell* **1**:661–671; 1998.
- [53] Gerritsen, M. E.; Williams, A. J.; Neish, A. S.; Moore, S.; Shi, Y.; Collins, T. CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc. Natl. Acad. Sci. USA* **94**:2927–2932; 1997.
- [54] Sheppard, K. A.; Rose, D. W.; Haque, Z. K.; Kurokawa, R.; McInerney, E.; Westin, S.; Thanos, D.; Rosenfeld, M. G.; Glass, C. K.; Collins, T. Transcriptional activation by NF- κ B requires multiple coactivators. *Mol. Cell. Biol.* **19**:6367–6378; 1999.
- [55] Karin, M. Mitogen-activated protein kinase cascades as regulators of stress responses. *Ann. NY Acad. Sci.* **851**:139–146; 1998.
- [56] Song, H. Y.; Regnier, C. H.; Kirschning, C. J.; Goeddel, D. V.; Rothe, M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor κ B and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc. Natl. Acad. Sci. USA* **94**:9792–9796; 1997.
- [57] Malinin, N. L.; Boldin, M. P.; Kovalenko, A. V.; Wallach, D. MAP3K-related kinase involved in NF- κ B induction by TNF, CD95 and IL-1. *Nature* **385**:540–544; 1997.
- [58] Lee, F. S.; Peters, R. T.; Dang, L. C.; Maniatis, T. MEKK1 activates both I κ B kinase α and I κ B kinase β . *Proc. Natl. Acad. Sci. USA* **95**:9319–9324; 1998.
- [59] Nemoto, S.; DiDonato, J. A.; Lin, A. Coordinate regulation of I κ B kinases by mitogen-activated protein kinase kinase 1 and NF- κ B-inducing kinase. *Mol. Cell. Biol.* **18**:7336–7343; 1998.
- [60] Zhao, Q.; Lee, F. S. Mitogen-activated protein kinase/ERK kinase kinases 2 and 3 activated nuclear factor κ B through I κ B kinase α and I κ B kinase β . *J. Biol. Chem.* **274**:8355–8358; 1999.
- [61] Rand, M. A.; Whitley, M. Z.; Gupta, S.; Pierce, J. W.; Best, J.; Davis, R. J.; Collins, T. Tumor necrosis factor α -induced E-selectin expression is activated by the nuclear factor κ B and c-Jun N-terminal kinase/p38 mitogen-activated protein kinase pathways. *J. Biol. Chem.* **272**:2753–2761; 1997.
- [62] Da Silva, J.; Pierrat, B.; Mary, J. L.; Lesslauer, W. Blockade of p38 mitogen-activated protein kinase pathway inhibits inducible nitric-oxide synthase expression in mouse astrocytes. *J. Biol. Chem.* **272**:28373–28380; 1997.
- [63] Chen, C. C.; Wang, J. K. P38 but not p44/42 mitogen-activated protein kinase is required for nitric oxide synthase induction mediated by lipopolysaccharide in RAW 264.7 macrophages. *Mol. Pharmacol.* **55**:481–488; 1999.
- [64] Nick, J. A.; Avdi, N. J.; Young, S. K.; Lehman, L. A.; McDonald, P. P.; Frasch, S.; Billstrom, M. A.; Henson, P. M.; Johnson, G. L.; Worthen, G. S. Selective activation and functional significance of p38 α mitogen-activated protein kinase in lipopolysaccharide-stimulated neutrophils. *J. Clin. Invest.* **103**:851–858; 1999.
- [65] Tuyt, L. M.; Dokter, W. H.; Birkenkamp, K.; Koopmans, S. B.; Lummen, C.; Kruijer, W.; Vellenga, E. Extracellular-regulated kinase 1/2, Jun N-terminal kinase, and c-Jun are involved in NF- κ B-dependent IL-6 expression in human monocytes. *J. Immunol.* **162**:4893–4902; 1999.
- [66] Kempf, S. J.; Hiura, T. S.; Nel, A. E. The Jun kinase cascade is responsible for activating the CD28 response element of the IL-2 promoter: proof of cross talk with the I κ B kinase cascade. *J. Immunol.* **162**:3176–3187; 1999.
- [67] Beyaert, R.; Cuenda, A.; Vanden Berghe, W.; Plaisance, S.; Lee, J. C.; Haegeman, G.; Cohen, P.; Fiers, W. The p38/RK mitogen-activated protein kinase pathway regulates interleukin 6 synthesis in response to tumour necrosis factor. *EMBO J.* **15**:1914–1923; 1996.
- [68] Vanden Berghe, W.; Plaisance, S.; Boone, E.; De Bosscher, K.; Schmitz, M. L.; Fiers, W.; Haegeman, G. P38 and extracellular signal-regulated kinase mitogen-activated protein kinase path-

- ways are required for nuclear factor- κ B p65 transactivation mediated by tumor necrosis factor. *J. Biol. Chem.* **273**:3285–3290; 1998.
- [69] Wesselborg, S.; Bauer, M. K.; Vogt, M.; Schmitz, M. L.; Schulze-Osthoff, K. Activation of transcription factor NF- κ B and p38 mitogen-activated protein kinase is mediated by distinct and separate stress effector pathways. *J. Biol. Chem.* **272**:12422–12429; 1997.
- [70] Zechner, D.; Craig, R.; Hanford, D. S.; McDonough, P. M.; Sabbadini, R. A.; Glembotski, C. C. MKK6 activates myocardial cell NF- κ B and inhibits apoptosis in a p38 mitogen-activated protein kinase-dependent manner. *J. Biol. Chem.* **273**:8232–8239; 1998.
- [71] Schwenger, P.; Alpert, D.; Skolnik, E. Y.; Vilcek, J. Activation of p38 mitogen-activated protein kinase by sodium salicylate leads to inhibition of tumor necrosis factor-induced I κ B- α phosphorylation and degradation. *Mol. Cell. Biol.* **18**:78–84; 1998.
- [72] Meyer, C. F.; Wang, X.; Chang, C.; Templeton, D.; Tan, T. H. Interaction between c-Rel and the mitogen-activated protein kinase kinase 1 signaling cascade in mediating κ B enhancer activation. *J. Biol. Chem.* **271**:8971–8976; 1996.
- [73] Haridas, V.; Ni, J.; Meager, A.; Su, J.; Yu, G. L.; Zhai, Y.; Kyaw, H.; Akama, K. T.; Hu, J.; Van Eldik, L. J.; Aggarwal, B. B. Cutting edge: TRANK, a novel cytokine that activates NF- κ B and c-Jun N-terminal kinase. *J. Immunol.* **161**:1–6; 1998.
- [74] Kim, Y. M.; Bombeck, C. A.; Billiar, T. R. Nitric oxide as a bifunctional regulator of apoptosis. *Circ. Res.* **84**:253–256; 1999.
- [75] Wong, H. R. Nuclear factor κ B and nitric oxide regulating life and death: nonsense or harsh reality? *Crit. Care Med.* **26**:1470–1471; 1998.
- [76] Umansky, V.; Hehner, S. P.; Dumont, A.; Hofmann, T. G.; Schirmacher, V.; Droge, W.; Schmitz, M. L. Costimulatory effect of nitric oxide on endothelial NF- κ B implies a physiological self-amplifying mechanism. *Eur. J. Immunol.* **28**:2276–2282; 1998.
- [77] Lander, H. M.; Jajjar, D. P.; Hempstead, B. L.; Mirza, U. A.; Chait, T. B.; Campbell, S.; Quilliam, L. A. A molecular redox switch on p21ras: structural basis for the nitric oxide-p21ras interactions. *J. Biol. Chem.* **272**:4323–4326; 1997.
- [78] Yun, H. Y.; Gonzalez-Zulueta, M.; Dawson, V. L.; Dawson, T. M. Nitric oxide mediates N-methyl-D-aspartate receptor-induced activation of p21ras. *Proc. Natl. Acad. Sci. USA* **95**:5773–5778; 1998.
- [79] Park, S. K.; Lin, H. S.; Murphy, S. Nitric oxide regulates nitric oxide synthase-2 gene expression by inhibiting NF- κ B binding to DNA. *Biochem. J.* **322**:609–613; 1997.
- [80] Taylor, B. S.; Kim, Y. M.; Wang, Q.; Shapiro, R. A.; Billiar, T. R.; Geller, D. A. Nitric oxide down-regulates hepatocyte-inducible nitric oxide synthase gene expression. *Arch. Surg.* **132**:1177–1183; 1997.
- [81] Peng, H. B.; Spiecker, M.; Liao, J. K. Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation. *J. Immunol.* **161**:1970–1976; 1998.
- [82] Sekkai, D.; Aillet, F.; Israel, N.; Lepoivre, M. Inhibition of NF- κ B and HIV-1 long terminal repeat transcriptional activation by inducible nitric oxide synthase 2 activity. *J. Biol. Chem.* **273**:3895–3900; 1998.
- [83] Shin, W. S.; Hong, Y. H.; Peng, H. B.; De Caterina, R.; Libby, P.; Liao, J. K. Nitric oxide attenuates vascular smooth muscle cell activation by interferon- γ . The role of constitutive NF- κ B activity. *J. Biol. Chem.* **271**:11317–11324; 1996.
- [84] Katsuyama, K.; Shichiri, M.; Marumo, F.; Hirata, Y. NO inhibits cytokine-induced iNOS expression and NF- κ B activation by interfering with phosphorylation and degradation of I κ B- α . *Arterioscler. Thromb. Vasc. Biol.* **18**:1796–1802; 1998.
- [85] De la Torre, A.; Schroeder, R. A.; Punzalan, C.; Kuo, P. C. Endotoxin-mediated S-nitrosylation of p50 alters NF- κ B-dependent gene transcription in ANA-1 murine macrophages. *J. Immunol.* **162**:4101–4108; 1999.
- [86] Griscavage, J. M.; Wilk, S.; Ignarro, L. J. Inhibitors of the proteasome pathway interfere with induction of nitric oxide synthase in macrophages by blocking activation of transcription factor NF- κ B. *Proc. Natl. Acad. Sci. USA* **93**:3308–3312; 1996.
- [87] De la Torre, A.; Schroeder, R. A.; Kuo, P. C. Alteration of NF- κ B P50 DNA-binding kinetics by S-nitrosylation. *Biochem. Biophys. Res. Commun.* **238**:703–706; 1997.
- [88] Matthews, J. R.; Botting, C. H.; Panico, M.; Morris, H. R.; Hay, R. T. Inhibition of NF- κ B DNA-binding by nitric oxide. *Nucl. Acids Res.* **24**:2236–2242; 1996.
- [89] Walley, K. R.; McDonald, T. E.; Higashimoto, Y.; Hayashi, S. Modulation of proinflammatory cytokines by nitric oxide in immune acute lung injury. *Am. J. Respir. Crit. Care Med.* **160**:698–704 1999.
- [90] Chen, F.; Lu, Y.; Castranova, V.; Rejanasakul, Y.; Miyahara, K.; Shizuta, Y.; Vallyathan, V.; Shi, X.; Demers, L. M. Nitric oxide inhibits HIV tat-induced NF- κ B activation. *Am. J. Pathol.* **155**:275–284; 1999.
- [91] Brady, T. C.; Chang, L. Y.; Day, B. J.; Crapo, J. D. Extracellular superoxide dismutase is upregulated with inducible nitric oxide synthase after NF- κ B activation. *Am. J. Physiol.* **273**:L1002–L1006; 1997.
- [92] Wong, G. H. W.; Goedel, D. V. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* **242**:941–943; 1988.
- [93] Miller, M. J.; Sandoval, M. Nitric oxide. III. A molecular prelude to intestinal inflammation. *Am. J. Physiol.* **276**:G795–799; 1999.
- [94] van der Vliet, A.; Eiserich, J. P.; Shigedage, M. K.; Cross, C. E. Reactive nitrogen species and tyrosine nitration in the respiratory tract: epiphenomena or a pathobiologic mechanism of disease? *Am. J. Respir. Crit. Care Med.* **160**:109; 1999.
- [95] Li, N.; Karin, M. Is NF- κ B the sensor of oxidative stress? *FASEB J.* **13**:1137–1143; 1999.

ABBREVIATIONS

CK-2—casein kinase 2
H₂O₂—hydrogen peroxide
ICAM—intracellular adhesion molecule
IKAP—IKK-complex associated protein
IKK—I κ B-kinases
IL-1 β —interleukin-1 β
iNOS—inducible nitric oxide synthase
JNK—c-Jun-N-terminal kinase
MAPKs—mitogen-activated protein kinases
NES—nuclear export signal
NF- κ B—nuclear factor κ B
NIK—NF- κ B-inducing kinase
 $\cdot$ NO—nitrogen monoxide
PI-3—phosphoinositide 3
PKA—protein kinase A
RHD—Rel-homology domain
RIP—receptor interacting protein
SIN-1—3-morpholininosydnonimine
TNF- α —tumor necrosis factor α