American Journal of Pathology, Vol. 159, No. 2, August 2001

Review

New Insights into the Role of Nuclear Factor-κB in Cell Growth Regulation

Fei Chen, Vince Castranova, and Xianglin Shi

From the Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia

The nuclear factor (NF)-κB family of eukaryotic transcription factors plays an important role in the regulation of immune response, embryo and cell lineage development, cell apoptosis, cell-cycle progression, inflammation, and oncogenesis. A wide range of stimuli, including cytokines, mitogens, environmental particles, toxic metals, and viral or bacterial products, activate NF-κB, mostly through IκB kinase (IKK)-dependent phosphorylation and subsequent degradation of its inhibitor, the IκB family of proteins. Activated NF-κB translocates into the nucleus where it modulates the expression of a variety of genes, including those encoding cytokines, growth factors, acute phase response proteins, cell adhesion molecules, other transcription factors, and several cell apoptosis regulators. During the past few years, tremendous progress has been achieved in our understanding on how intracellular signaling pathways are transmitted in either a linear or a network manner leading to the activation of NF-κB and subsequent cell growth control. However, a detailed molecular mechanism of NF-κB regulating cell growth has yet to be determined. Elucidation of the relationships between NF-κB activation and cell growth will be important in developing new strategies for the treatment of various human diseases, such as chronic autoimmune disorder and cancer. ***(Am J Pathol 2001, 159:387–397)***

After more than a decade of intensive study, a complex body of knowledge has been accumulated, revealing the molecular mechanisms of signal-induced activation of nuclear factor (NF)-κB, a pivotal transcription factor governing the expression of early response genes involved in cell-to-cell interaction, intercellular communication, cell recruitment or transmigration, amplification or spreading of primary pathogenic signals, and initiation or acceleration of tumorigenesis.[1–3] Presently, five mammalian NF-κB family members have been identified and cloned.[4–6] These include NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA(p65), RelB, and c-Rel. All of these NF-κB family members share a highly conserved Rel homology domain responsible for DNA binding, dimerization, and interaction with IκB, the intracellular inhibitor for NF-κB.[7] The C-terminal regions of RelA, RelB and c-Rel contain a transactivating domain that is important for NF-κB-mediated gene transactivation. The C-termini of the precursor molecules for p50 and p52, p105 and p100, contain multiple copies of the so-called ankyrin repeat, which is found in IκB family members, including IκB-α, IκB-β, IκB-ε, Bcl3, and *Drosophila* cactus.

A wide range of signals, which typically include cytokines, mitogens, environmental and occupational particles, toxic metals, intracellular stresses, viral or bacterial products, and UV light, induce expression of early response genes through the NF-κB family of transcription factors.[2,4,8–10] In resting cells, NF-κB is sequestered in the cytoplasm in an inactive form through its association with one of several inhibitory molecules, including IκB-α, IκB-β, IκB-ε, p105, and p100. Activation of the NF-κB-signaling cascade results in a complete degradation of IκB or partial degradation of the carboxyl termini of p105 and p100 precursors, allowing the translocation of NF-κB to the nucleus, where it induces transcription (Figure 1). Activated NF-κB binds to specific DNA sequences in target genes, designated as κB-elements, and regulates transcription of genes mediating inflammation, carcinogenesis, and pro- or anti-apoptotic reactions.

Three IκB proteins, IκB-α, IκB-β, and IκB-ε, have been identified, among which IκB-α is the most abundant inhibitory protein for NF-κB.[4] All IκB proteins contain two conserved serine (S) residues within their N-terminal domain. Phosphorylation of these conserved S residues in response to inducers, leads to the immediate polyubiquitination of IκB proteins by the SCF-β-TrCP complex, a step that has been shown recently to be inhibited by the nonpathogenic *Salmonella* bacteria in gut epithelial

Supported by a Career Development Award in Genetics under a cooperative agreement from the Centers for Disease Control and Prevention through the Association of Teachers of Preventive Medicine (to F. C.).

Accepted for publication April 19, 2001.

Address reprint requests to Dr. Fei Chen, PPRB of NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505. E-mail: lfd3@cdc.gov.

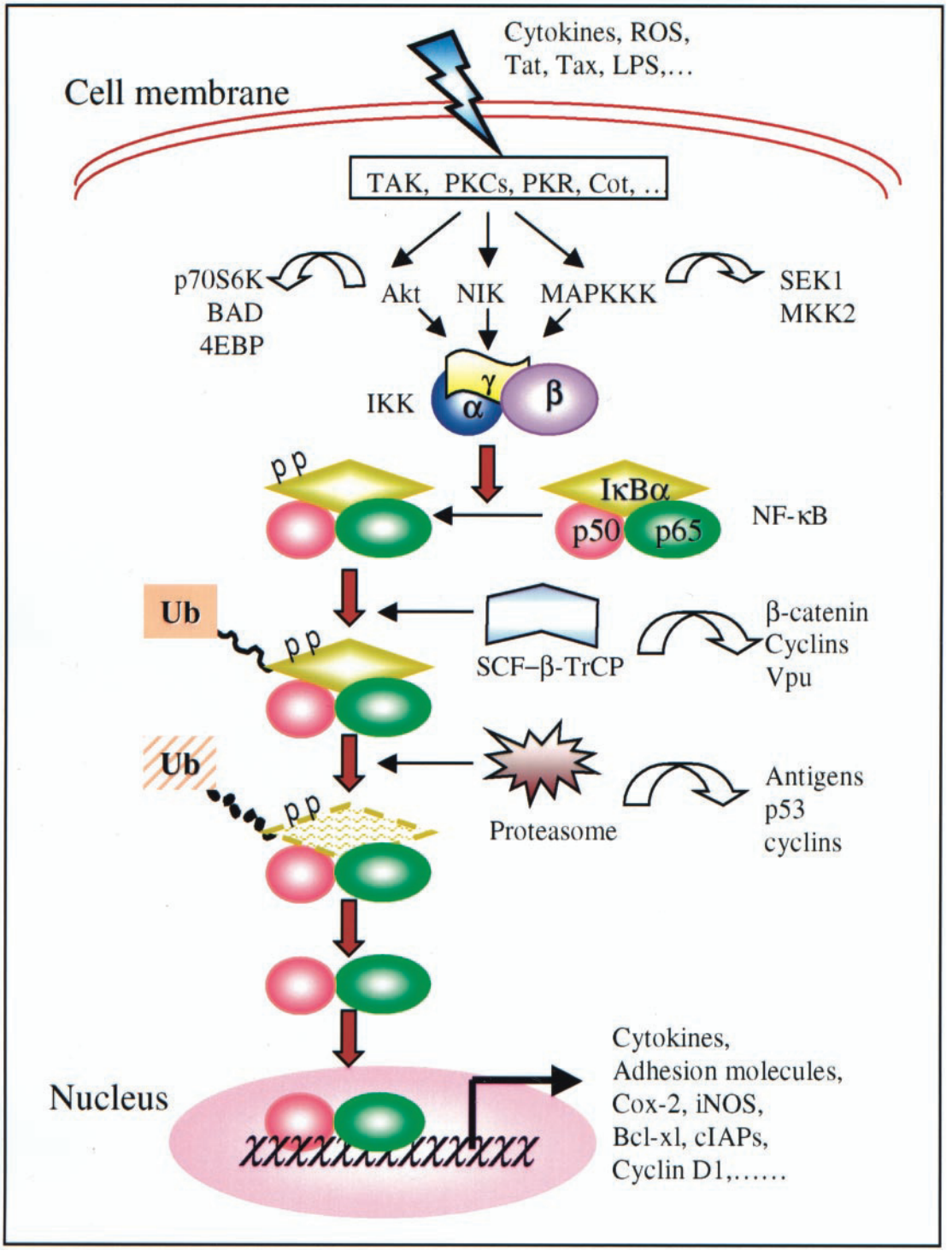

Figure 1. Signaling pathways of NF-κB activation. Extracellular inducers, including cytokines, reactive oxygen species (ROS), and viral and bacterial products, activate IKK through upstream kinases directly or indirectly. Activated IKK phosphorylates N-terminal S32 and S36 residues of IκB-α that is associated with NF-κB p50 and p65 heterodimer. The SCF-β-TrCP complex recognizes phosphorylated IκB-α and modifies IκB-α with polyubiquitin chains. This is followed by proteasome-mediated degradation of IκB-α. After degradation of IκB-α, the activated NF-κB translocates into the nucleus where it binds to the κB-sites of gene promoters or enhancers to up-regulate target gene expression. **Line arrows** and **filled arrows** denote the NF-κB signaling pathways; **open arrows** denote the connections with the by-standing signaling pathways.

cells.[11] This modification subsequently targets IκB proteins for rapid degradation by the 26S proteasome.[12] A high-molecular weight complex that phosphorylates IκB-α or IκB-β has been characterized recently and named IκB kinase (IKK) complex. This complex contains two catalytic subunits, IKK-α and IKK-β, and a structural component named NEMO/IKKγ/IKKAP.[13,14] An earlier report by Cohen and colleagues[15] suggested the presence

of a scaffold protein named IKK complex-associated protein (IKAP) in the IKK complex, which could not be confirmed in other studies. A later study by the group, who originally identified IKAP, indicated that the observed association of IKAP with IKK was because of a nonstrict elusion condition of chromatographic extracts during the purification of IKK.[16] Recently, two groups independently reported the identification of a novel protein, CIKS/Act1, associated with the IKK complex and suggested that CIKS/Act1 functions as an anchoring proteins in the assembly of the IKK complex and in providing a possible connection between IKK and c-Jun-N-terminal kinase signaling.[17,18] IKK-α and IKK-β share 50% sequence homology. Both proteins contain an amino terminal kinase domain, a carboxyl terminal region with a leucine zipper, and a helix-loop-helix domain. *In vitro* or *in vivo* studies indicate that although both IKK-α and IKK-β are capable of phosphorylating IκB-α on ser32 and ser36, IKK-β is more potent in IκB-α phosphorylation induced by proinflammatory stimuli. However, a distinct IKK complex, named IKKi/ϵ that does not contain IKK-α, -β, or -γ, was recently identified in T cells.[3,19] IKKi/ϵ shares 27% homology with IKK-α and IKK-β and possibly mediates NF-κB-activating kinase signaling and PMA/PKCϵ-induced S36 phosphorylation of IκB-α and NF-κB activation.[3,19]

Although the signaling pathways leading to the activation of NF-κB have been well defined, a number of questions remain to be answered. For example, it is unclear exactly how many protein subunits comprise a naïve and activated IKK complex, respectively; how the various signaling pathways converge on this kinase complex; what upstream kinases contribute to the phosphorylation and activation of IKK. It is also unclear whether other substrates, in addition to IκB or NF-κB family members, can be phosphorylated by IKK. Regarding the functional aspects of NF-κB transcription factor, we know that although NF-κB is important and involved in the regulation of cell apoptosis, cell-cycle transition, and carcinogenic transformation,[20] the detailed interconnections among NF-κB activation, cell cycle, and apoptosis are still undefined. Considering the fact that aberrant activation of NF-κB is associated with a wide range of human diseases, elucidation of molecular mechanisms determining NF-κB activation and expression of its various functions may lead to the development of novel preventive and therapeutic strategies for many diseases including chronic inflammation and cancer.

Roles of NF-κB Activation in Cell Apoptosis

Programmed cell death, or apoptosis, is an essential mechanism for any multicellular organism to eliminate cells that are in excess or potentially dangerous.[21] Most apoptotic cells are characterized by unique morphological features, such as membrane blebbing, cell shrinking, cytosolic and nuclear condensation, and breakdown of chromosomal DNA. Depending on the use of different initiating caspases, signal-induced apoptosis can be roughly divided into receptor-mediated extrinsic apoptosis and mitochondrial-mediated intrinsic apoptosis (Figure 2).[22,23] The extrinsic apoptotic pathway is triggered as a consequence of ligand binding to death receptors, including tumor necrosis factor (TNF)-R, Fas, OX40, CD40, and 4–1BB, which contain conserved protein-protein-binding domains termed death domains. These receptors recruit procaspases, mainly caspase-8, via adapter molecules. The intrinsic apoptotic pathway is mediated by mitochondria through release of apoptosis-promoting factors, including cytochrome *c*, apoptosis-inducing factor, and Diablo/Smac.[24–27] Cytochrome *c* forms a complex with a cytosolic protein named Apaf-1, a flavoprotein with homology to plant ascorbate reductases and bacterial NADH oxidases, to activate caspase-9.[27] Whereas apoptosis-inducing factor released from the intermembranous space of damaged mitochondria induces apoptosis in a caspase-independent manner, Smac/Diablo released from mitochondria promotes apoptosis by binding to and antagonizing XIAP, cIAP1, and cIAP2, allowing the activation of caspases.[25,26] Both activated caspase-8 from the extrinsic apoptotic pathway and activated caspase-9 from the intrinsic apoptotic pathway cleave and activate effector caspases, mainly caspase-3 to execute an apoptotic process. These apoptotic pathways, however, were compromised in many cases because of the activation of caspase-independent signaling cascades that function to block the apoptotic responses. A good example is TNF-R-mediated activation of NF-κB that induces expression of anti-apoptotic proteins, including caspase inhibitors, such as cIAP1, cIAP2, and XIAP, and mitochondria membrane stabilizers, such as Bcl-xl and Bfl-1.[28–30]

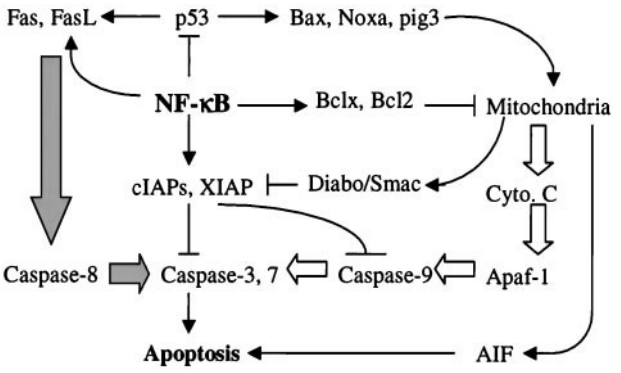

Figure 2. Possible targeting point of anti-apoptotic signals from NF-κB. Intrinsic (**open arrows**) and extrinsic (**filled arrows**) apoptosis pathways are depicted. The effector caspases, such as caspase-3 and caspase-7, are activated by upstream initiator caspases, caspase-8 and caspase-9. The initiator caspases themselves are activated by either ligands binding to the death receptor complex or cytochrome *c* released from damaged mitochondria. An anti-apoptotic effect of NF-κB is achieved through its up-regulation of IAPs that inhibits caspases and Bcl-xl that protects mitochondria from further damaging.→, activation; ⊣, inhibition.

Tumor suppressor protein, p53, has been considered to be one of the major contributors of cell apoptosis in response to a variety of stress inducers. As a transcription factor, p53 is able to up-regulate the expression of genes involved in either reactive oxygen species production or reactive oxygen species metabolism, including quinone oxidoreductase (Pig 3), proline oxidase (Pig 6) homologues, glutathione transferase (Pig 12), and gluta-

thione peroxidase (GPx).[31] Moreover, p53 also activates the expression of several genes that directly control or regulate the process of apoptosis. These genes include Bax, Fas, Fas ligand (FasL), IGF-BP3, PAG608,[32] ei24 (Pig 8),[33] and Noxa.[34]

With the identification of role of NF-κB in transcriptional regulation of several pro-apoptotic genes, such as fas and fasl, controversy raged as reports demonstrating that NF-κB protected cells from apoptosis in some types of cells were matched by a similar number of reports demonstrating that it did not in other types of cells.[35,36] Based primarily on earlier studies, NF-κB was initially considered a pro-apoptotic factor because of its rapid activation in cells in response to apoptotic signals and its involvement in the expression of some apoptotic genes, including TNF-α, c-*myc*, and fasl.[37,38] More recent work, however, has altered this view and revealed an anti-apoptotic effect of NF-κB in response to a variety of apoptotic stimuli.

The direct evidence for the anti-apoptotic effects of NF-κB is provided by gene knockout studies in which the genes encoding either members of NF-κB family proteins or upstream kinases were disrupted. RelA (p65)-deficient mice die during embryonic development through apoptosis of hepatocytes.[39] IKK-β gene knockout mice and IKK-β/IKK-α double-knockout mice die as embryos and show massive liver cell apoptosis,[40–42] a phenotype similar to the response of NF-κB p65 gene knockout mice. In addition, knockout of the IKK-α gene results in perinatal lethality of mice with an increased thickness of the skin because of the deficiency of keratinocyte differentiation.[43] Male mice with an inactivated X-linked gene encoding IKK-γ/NEMO, an essential modulator of the IKK complex for NF-κB activation, die at mid-gestation because of a massive apoptosis of cortical and medulla lymphocytes in the thymus, in addition to degeneration of the liver.[44,45] Female mice deficient in the IKK-γ/NEMO gene manifest a unique dermatopathy because of the apoptosis of keratinocytes and consequent abnormal pigmentation, a characteristic strikingly similar to that of the human X-linked dominant, male-lethal genetic disease—incontinential pigmenti or Bloch-Sulzberger Syndrome. Cross-breeding of relA or IKK-β gene knockout mice with TNF-R1 or TNF-α gene knockout mice revealed partial rescue of embryonic lethality, suggesting that NF-κB deficiency sensitized cells in response to TNF-α-mediated cytotoxicity.[46,47] Similarly, mice deficient in both TNF-R1 and IKK-β showed an attenuated embryonic liver apoptosis.[48]

Other compelling evidence linking NF-κB with an anti-apoptotic effect is based on the studies indicating that NF-κB is a priming factor for liver regeneration after partial hepatectomy.[49] This priming effect of NF-κB might be through its transcriptional regulation for survival genes or anti-apoptotic genes whose products can block stress signal-induced cell death, a process critically involved in cell proliferation and transformation. Candidate anti-apoptotic genes targeted by NF-κB include those encoding the cell-cycle regulatory protein cyclin D1,[50–52] the mitochondrial membrane-stabilizing proteins Blf-1 and Bcl-xl,[53,54] the caspase inhibitors cIAP1/cIAP2 and XIAP, and the TNF receptor-associated factors TRAF1 and TRAF2.[55] It should be noted that several reports suggest that NF-κB is also a pro-apoptotic factor in FasL-induced cell death.[56,57] This argument is primarily based on earlier observations that NF-κB can regulate the artificial promoter activity of the fasl gene, a gene encoding an important activator of apoptosis through a CD95/Fas- and Fas-associated death domain (FADD)-mediated caspase-8 activation pathway.[22] However, both promoter truncation studies of the fasl gene and somatic cell mutagenesis studies of IKK-γ indicate that NF-κB is not required for the fasl gene expression.[58,59] Nevertheless, pro-apoptotic or anti-apoptotic effects of NF-κB might depend on the cellular context in combination with a bewildering variety of activators.

Further support for the anti-apoptotic effect of NF-κB comes from observations in which NF-κB can protect cortical neurons from β-amyloid peptide-induced apoptosis in Alzheimer's disease.[60,61] Exposure of cortical neurons to β-amyloid peptide increased levels of IκB-α mRNA and protein and a consequent decrease in NF-κB activity.[60] Elevation of NF-κB activity by pretreatment of these cells with an antisense oligonucleotide to IκB-α protected them from β-amyloid peptide-induced apoptosis. Conversely, blockade of NF-κB activity by κB decoy DNA was associated with enhanced β-amyloid peptide-induced mitochondrial dysfunction and cell apoptosis.[61] Moreover, data from the studies of rodent models of stroke or cardiac arrest suggested that NF-κB might possibly prevent ischemic neuronal degeneration.[62] The protective role of NF-κB on neurons was attributed to NF-κB-mediated transcription of genes encoding Bcl-2, Mn-SOD, and proteins regulating cellular calcium homeostasis.[63]

The vast majority of studies focused on the regulatory roles of NF-κB on apoptosis suggest that NF-κB is acting on the upstream pathways of apoptosis, either negatively or positively. Conversely, a few recent studies have demonstrated the possible regulation of apoptotic molecules on NF-κB. Of potential interest regarding regulation of NF-κB by apoptotic molecules are the observations of cross-competition between NF-κB and p53, a major pro-apoptotic protein.[64–69] The molecular events identified thus far as mediators of cross-competition between NF-κB and p53 can be roughly classified into two categories: the upstream kinases or other regulatory molecules that relay input signals into NF-κB and p53, and co-factors that affect transcriptional activities of NF-κB and p53. An earlier study conducted by Jung and colleagues[70] indicated that ATM, a major kinase responsible for DNA damage-induced N-terminal phosphorylation of p53, was involved in IκB-α phosphorylation in SV40 large T-transformed fibroblasts in response to ionizing radiation. In nontransformed fibroblasts, however, Ashburner and colleagues[71] demonstrated a lack of involvement of ATM in IκB-α phosphorylation. In an *in vitro* study, Liu and colleagues[72] reported that DNA-dependent protein kinase (DNA-PK), a kinase phosphorylating p53 in response to DNA damage, was able to phosphorylate the carboxyl terminus of IκB-α protein. On the functional level, the first evidence of mutual functional regulation

between NF-κB and p53 was from the observation that p53 could antagonize NF-κB activity by cross-competition for a limiting pool of the co-activator, p300.[65] In contrast, two recent studies indicated that p53 might activate NF-κB through an unknown mechanism[73] or stimulate the activity of NF-κB through induction of its target gene, $p21^{Waf1}$, which inhibits cyclin E/Cdk2 activity and blocks its ability to compete with NF-κB for co-factors, such as p300 and CBP.[74]

Further evidence indicating that apoptotic molecules regulate NF-κB comes from study of caspase cleavage of NF-κB p65 subunit or IκB-α protein.[75–77] The cleavage of p65 by caspase-3 leads to a loss of the carboxyl-terminal transactivation domain.[75] The carboxyl terminal truncated p65 is transcriptionally inactive. The cleavage of IκB-α by caspase-3 has been observed in γ-radiation-induced apoptosis and NF-κB inhibition-induced apoptosis.[77,78] A caspase-3 cleavage site has been identified in the region of amino acids 26 to 32 of human IκB-α and 32 to 37 of chicken IκB-α protein.[76] The cleavage site of caspase-3 on IκB-α with the amino acid sequence D-R-H-D-S resembles the consensus caspase-3 cleavage site, D-X-X-D-G/S/A, where X represents any amino acid residue. Cleavage of IκB-α by caspase-3 creates a N-terminal truncated IκB-α protein that is resistant to degradation by proteasome in response to inducers of NF-κB, but is able to bind to and suppress NF-κB. Therefore, the role of caspase-3 cleavage on IκB-α and p65 is to ensure that the anti-apoptotic gene is suppressed and apoptotic process is not interrupted once the cells are committed to apoptotic elimination.

In contrast, several studies indicate that caspases might also participate in the activation of NF-κB under certain circumstances. One example supporting this notion is the involvement of *Dredd*, a caspase encoded by *Drosophila* dredd gene, in the endoprotease cleavage of the *Relish* protein.[79] Structurally similar to human p100 and p105, two precursor proteins of NF-κB family, *Relish* contains a N-terminal Rel homology domain and a C-terminal IκB-like region. On lipopolysaccharide stimulation, *Relish* undergoes a rapid cleavage between the Rel homology domain and IκB-like region. Proteasome inhibitors failed to prevent the cleavage of *Relish*. In contrast, introducing a dominant-negative mutant of *Dredd* to inhibit the caspase activity of *Dredd* significantly blocked the cleavage of *Relish*, indicating caspase, rather than proteasome, is required for the activation of NF-κB-like protein in *Drosophila*. In addition, in mammalian cells, caspase-8, caspase-10, and MRIT, three death effector domain-containing proteins, have been shown to be able to activate upstream signals, such as NIK and IKK, leading to the activation of NF-κB.[80] This activity seems to be mainly dependent on the interaction between the prodomain of caspases and IKK. Furthermore, several recent studies suggested that other apoptosis-inducing proteins, such as Nod2, an Apaf-1 family member, and *Bcl*l0 and vCLAP, two caspase-recruitment domain-containing proteins, could also activate NF-κB through the interaction with IKK-γ subunit of IKK complex.[81–83] However, it is hard to reconcile these observations with the notions that NF-κB is repressed in the cells undergoing apoptosis.[33,77,78]

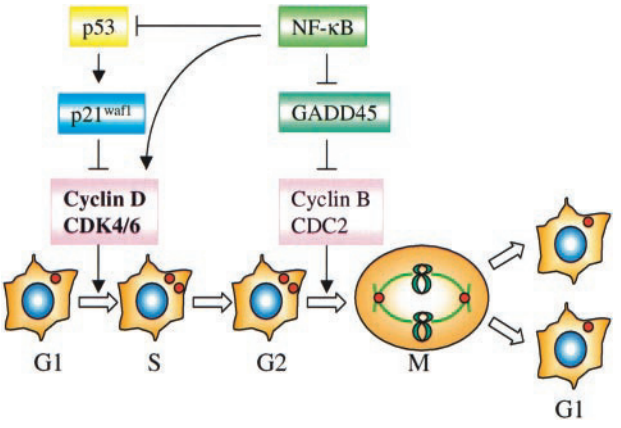

Figure 3. Involvement of NF-κB in cell-cycle regulation. NF-κB may facilitate cell-cycle transition from G_1 to S phase by antagonizing the activation or function of p53 and up-regulating cyclin D1 gene expression. NF-κB may also promote G_2- to M-phase transition by down-regulating the expression of GADD45, a G_2/M phase blocker that inhibits CDC2/cyclin B complex.→, activation; ⊣, inhibition.

NF-κB and Cell-Cycle Regulation

It has been known for decades that multiple signals are required to maintain proper cell growth and tissue homeostasis.[84] Most cells within a normal tissue may be forced out of the active cell cycling into a quiescent (G_0) state from which they may re-enter cell cycling under some future circumstances. In a mature tissue, cells may be induced to terminal differentiation by relinquishing their proliferative or cell-cycling potential. Control of the orderly progression of dividing cells through the G_1, S, G_2, and M phases of the cell cycle in eukaryotic cells relies on a series of cell-cycle regulatory proteins, mainly cyclins that exert their function by binding to and activating a number of specific cyclin-dependent kinases (CDKs). The CDK activity is further modulated by kinases and phosphatases that phosphorylate and dephosphorylate CDK, respectively. Moreover, CDKs are subject to regulation by association with one of a number of specific CDK inhibitors or cell-cycle checkpoint proteins, such as $p21^{Waf1}$, $p16^{INK4a}$, $p27^{Kip1}$,[85] and growth arrest and DNA-damage protein 45 (GADD45) (Figure 3).[86]

Overwhelming evidence during recent years demonstrates that a variety of stress inducers, including DNA-damaging agents, activate checkpoint function of cells, leading to a cell-cycle arrest. Several checkpoints exist in the G_1/S phase, G_2 phase, and M phase of cell cycle. In mammalian cells, the control of the S-phase checkpoint requires the p53 tumor suppressor protein that governs the expression of CDK inhibitor, $p21^{Waf1}$.[87,88] The activation of the G_2/M phase checkpoint is dependent on the phosphorylation-dependent inactivation of CDC25C phosphatase by checkpoint kinases 1 or 2 (Chk1 or Chk2) and the induction of GADD45, an inhibitor for the G_2/M phase cyclin B/CDC2 complex.[89,90] An additional checkpoint, the spindle checkpoint, has been identified

in a later stage of M phase.[84,85] This checkpoint arrests mitotic progression if the spindle is not properly assembled, or if the chromosomes are not correctly oriented and attached to the spindle. All of the checkpoints are essential for maintaining genomic stability by allowing cells to have enough time to repair damage, thus, protecting the organism from the deleterious consequences of mutation.

The relationship between NF-κB and apoptosis has been intensively explored during the last few years, whereas only limited information is available regarding the possible involvement of NF-κB in cell-cycle regulation in cellular response to a variety of stress signals. A critical role for NF-κB in cell-cycle progression was suggested by earlier observations that NF-κB activity was elevated during the G_0 to G_1 cell-cycle transition in mouse fibroblasts.[91] A series of recent studies has begun to elucidate that in addition to fibroblasts, NF-κB activation was required for cell cycling in other types of cells, such as regenerating liver cells and estrogen receptor-negative breast cancer cells.[91–95] It was also found that the levels of NF-κB activation were linked to signaling that controls cell-cycle progression in HeLa cells and Jurkat T cells.[74,96] Inhibition of NF-κB caused impairment of cell-cycle progression in human glioma cells[97] and a retarded G_1/S transition in HeLa cells.[98] The identification of NF-κB binding sites in the promoter region of cyclin D1 gene provided direct evidence for the contributions of NF-κB to the cell cycle.[50–52,95] Cyclin D1, in association with cyclin-dependent kinases, CDK4 and CDK6, promotes G_1/S phase transition through CDK-dependent phosphorylation of pRb, thereby releasing the transcription factor E2F, which is required for the activation of S phase-specific genes.[99–101] Two NF-κB binding sites in the human cyclin D1 promoter have been identified. Inhibition of NF-κB by a degradation-resistant IκB-α caused a pronounced reduction of serum-induced cyclin D1 expression accompanied by a decrease of cyclin D1-associated kinase activity and delayed phosphorylation of pRb.

In contrast, several recent reports also indicated that NF-κB activation is necessary to cause cell-cycle arrest and/or induce cells to commit to terminal differentiation. Overexpression of NF-κB p65 or c-Rel arrests G_1/S cell-cycle transition in pro-B cells and HeLa cells, respectively.[96,102] In HeLa cells, overexpression of c-Rel arrests cells at the G_1/S phase because of the stabilization of p53 protein, which can subsequently activate the expression of $p21^{Waf1}$, a potent inhibitor of CDK2.[96] The elevated levels of $p21^{Waf1}$ correlated with the accumulation of the hypophosphorylated form of pRb and a decrease in E2F DNA binding. It is unclear how overexpression of c-Rel resulted in a prolonged half-life of p53 protein. In pro-B cells, although overexpression of c-Rel exhibited no effect on cell-cycle regulation, overexpression of p65 caused G_1 arrest and subsequent apoptosis. This G_1-arresting effect of p65, however, seems to be dependent on cell developmental stage, because overexpression of p65 did not cause G_1 arrest in mature B cells. It remains unsettled whether manipulation of NF-κB signaling using protein overexpression can lead to consequences that are physiologically relevant. If it is, one may speculate that inhibition of NF-κB should cause over-cycling or hyperproliferation. Indeed, data obtained by gene inactivation of IKK-α in the mouse indicated an unexpected excessive proliferation of the skin basal layer because of the absence of epidermal differentiation.[103,104] NF-κB activity could not be found in keratinocytes from IKK-α-null mouse skin. These results point to a unique role for NF-κB in the epidermis, that is, NF-κB forces keratinocytes out of cell cycle and subsequent terminal differentiation. In this regard, the response of the epidermal keratinocytes to NF-κB seem to be opposite from that of other cell types, such as lymphocytes and macrophages, where NF-κB seems to promote cell-cycle transition.[50–52,95]

Although most of the studies so far addressed the effects of NF-κB on G_1/S phase regulation, the question of whether NF-κB also contributes to G_2/M phase transition has not been explored. In a recent study in human bronchial epithelial cell line, BEAS-2B, we found that NF-κB inhibition by stable expression of a kinase mutated form of IKK-β potentiated toxic metal-induced G_2/M cell-cycle arrest.[105] First, flow cytometric analysis demonstrated that at 48 hours after arsenite treatment, BEAS-2B cells expressing a kinase-mutated form of IKK-β showed a marked dose-dependent increase of cells arrested in the G_2/M phase and a corresponding decrease in the number of cells in G_1 phase. Second, a dose-dependent induction of GADD45 protein was observed in cells treated with arsenite. This induction of GADD45 by arsenite seems to be dependent on the activation of c-Jun-N-terminal kinase, because blockage of c-Jun-N-terminal kinase activation by expression of a dominant-negative SEK1 vector decreased the induction of GADD45. On the other hand, inhibition of NF-κB by expressing a kinase-mutated form of IKK-β increased GADD45 induction by arsenite. Third, analysis for the expression of CDC25 family members revealed that arsenite induced *de novo* CDC25A expression, but markedly reduced the levels of CDC25B and CDC25C proteins, two phosphatases dephosphorylating and activating CDC2/cyclin B complex required for the transition of the cell cycle from G_2 to M phase. The effects of Cr(VI) on the regulation of the cell cycle were also determined and revealed to be more complicated. Although Cr(VI) was able to induce GADD45 and suppress both CDC25B and CDC25C, it had no effect on CDC25A. Cell-cycle-profiling studies showed that whereas a lower concentration of Cr(VI) (0.25 μg/ml) promoted cell-cycle transition, higher concentrations of Cr(VI) (1 to 4 μg/ml) arrested cells at S phase. In the case of vanadate-induced cell-cycle regulation, the cell-cycle-arresting effect of vanadate seems to be dependent on the status of NF-κB activation. In normal epithelial cells, vanadate exhibited less effect on cell-cycle transition. However, in the cells where NF-κB activation was specifically inhibited, vanadate showed a marked G_2/M phase-arresting effect. However, vanadate was unable to induce the expression of GADD45, an inhibitor of cyclin B/CDC2 complex required for G_2/M transition (Chen et al, unpublished observations).

NF-κB and Oncogenesis

The ability of NF-κB to suppress apoptosis and to regulate cell-cycle transition clearly indicates that NF-κB may participate in many aspects of oncogenesis. Indeed, elevation of NF-κB activity is evident in a number of human cancers, including breast cancer,[106] non-small cell lung carcinoma,[107] thyroid cancer,[108] T- or B- lymphocyte leukemia,[109] melanoma,[110] colon cancer,[111] bladder cancer,[112] and several virally induced tumors.[113–115] The earliest evidence for a role for NF-κB in oncogenic transformation has been derived from the fact that v-Rel, a highly oncogenic retroviral homologue of c-Rel, causes carcinogenesis in avian lymphoid cells.[108] Later studies suggested that v-Rel also has the capacity of transforming mammalian cells *in vivo*.[116] Transgenic mice expressing v-Rel under the control of the T-cell-specific *lck* promoter develop T-cell lymphomas. Inhibition of NF-κB by overexpression of a degradation-resistant IκB-α delays the development of T-cell lymphomas and prolongs the survival of v-Rel transgenic mice.[116]

Chromosomal alterations of NF-κB family genes provided additional evidence for the role of NF-κB in oncogenesis. It has been demonstrated that genes encoding c-Rel, NF-κB2 (p100/p52), p65/RelA, and Bcl-3 proteins are all located within breakpoint regions of the genome that are involved in oncogenic rearrangements or amplifications. Rearrangement of nfkb2 gene by t(10,14) chromosomal translocation causes deletions of sequences encoding the ankyrin repeat motif of p100. Consequently, this carboxyl terminal truncated p100 is constitutively located in the nucleus of cells, which has been originally found in a case of B-cell non-Hodgkin's lymphoma and observed in a number of lymphoid neoplasms, particularly cutaneous lymphomas.[117–119] Rearrangement and amplification of c-Rel gene has also been found in numerous non-Hodgkin's lymphomas and cancer cell lines.[108,120] The bcl-3 gene, which encodes an IκB-like protein that regulates transcriptional activity of NF-κB p50 or p52 homodimer, was identified as a [t(14, 19)(q32; q13.1)] chromosomal translocation in many cases of chronic lymphocytic leukemia.[121] Unlike rearrangement of the nfkb2 gene, alterations at the bcl-3 locus do not truncate or change the coding sequence, but rather cause overexpression of bcl-3 mRNA. In malignant Hodgkin and Reed-Sternberg (H/RS) cells from Hodgkin's lymphoma, mutations in the IκB-α gene have been detected and are suggested to cause a sustained activation of NF-κB.[122]

Accumulating evidence reveals that tumorigenesis or oncogenesis is a multistep process and that these steps reflect defections in regulatory circuits that govern normal cell proliferation, differentiation, and death.[123] Although abnormal activation or function of NF-κB has been clearly demonstrated in the initiation or facilitation of oncogenesis, the central question that has to be answered is: how many and what steps are influenced by NF-κB. NF-κB has been shown to antagonize the function of p53 as discussed earlier.[65] Obviously, this antagonism of p53 by NF-κB will result in the evasion of cells from stress-induced cell-cycle arrest and/or programmed cell death and consequently sensitize the cells for genomic instability. Furthermore, NF-κB could promote cell-cycle transition by a direct transcriptional up-regulation of the cyclin D1 gene.[50–52,95] Although it remains to be confirmed, this increased expression of cyclin D1 may possibly provide cells with an uncontrolled or limitless replicative potential. Up-regulation of anti-apoptotic genes, such as cIAP1, cIAP2, XIAP, and bcl-xl, by NF-κB,[4] is an additional mean of cells to escape from or resist signal-induced apoptosis. Other NF-κB-regulated genes include those encoding intercellular adhesion molecule-1,[5] extracellular matrix protein tenascin-C,[124] vascular endothelial growth factor,[124] chemokines, and cyclooxygenase-2.[124] These gene products are directly associated with the tumor cell metastasis and tumor tissue angiogenesis.

The key role that NF-κB plays on multiple steps of oncogenesis makes this factor a central and favorable target for therapeutic intervention of cancer, especially, certain types of leukemia or lymphomas.[125] Indeed, experimental data suggest that inhibition of NF-κB by antisense oligonucleotides to relA, degradation resistant IκB-α, and aspirin or nonsteroidal anti-inflammatory drugs, could enhance the efficacy of cancer chemotherapies and radiation.[126,127] Studies by Wang and coworkers[55,128] showed that inhibition of NF-κB by infecting the cells with an adenovirus carrying a modified form of IκB-α (superrepressor IκB-α) leads to dramatically enhanced apoptosis of HT1080 fibrosarcoma cells in response to ionizing radiation or daunorubicin treatment. Consistent with these reports, working with pancreatic cancer cell lines exposed to VP16 or doxorubicin, Arlt and colleagues[129] demonstrated recently that NF-κB inhibition by pharmacological proteasome inhibitors or transfection of the cells with a N-terminal-truncated IκB-α variant efficiently reduces chemoresistance of these cells. Using similar or different approaches to inhibit NF-κB, this effect has also been noted in a variety of other cell types including non-small cell lung cancers,[130] head and neck squamous carcinomas,[131] human myeloblastic leukemia cells,[132] colorectal cancer,[133] and bladder cancer cells.[112] Despite these encouraging observations, however, care has to be taken when using different approaches to inhibit NF-κB that might be attributable to the process of oncogenesis. Indeed, different approaches for the inhibition of NF-κB does not necessarily lead to the same extents of inactivation of NF-κB because of the existence of functionally and stoichiometrically different NF-κB complexes that respond to different activation signals.[5] Also, the inhibitory effect of NF-κB inhibitors can vary considerably between different cell types because of unique simultaneous or asynchronous events triggered by these inhibitors in any given cell type.[134]

Summary

The detailed molecular mechanisms by which the NF-κB transcription factor contributes to cell growth control, such as cell apoptosis, cell-cycle transition, and oncogenesis remain to be further determined. One of the major challenges in understanding mechanisms of cell

growth regulation by NF-κB in response to environmental stress is to elucidate how signal transduction pathways are activated and how signaling cross-talk and specificity are achieved when several signaling pathways are activated simultaneously by stress inducers that elicit different cellular responses. For instance, why does activation of the NF-κB, an anti-apoptotic transcription factor, coincide with obvious apoptotic features in cells undergoing stress responses? Because many stress inducers and their mediators are highly reactive but nonspecific, an activation of only one specific signaling pathway is hard to achieve in the cells in response to a particular inducer. Even in a single signaling pathway, because of their highly reactive and nonspecific characteristics, certain stress inducers and their mediators can in principle induce conflicting signals by affecting signaling molecules at different levels. A good example is the effects of oxidative stress on NF-κB signaling pathway. It has been frequently observed in certain types of cells that oxidative stress amplified or potentiated NF-κB activation, whereas at the same time oxidation of IKK or NF-κB proteins inhibited NF-κB function. Translating the knowledge gained by studying the connections among NF-κB activation, cell apoptosis, cell-cycle regulation, and oncogenesis may aid in identifying novel preventive and therapeutic measures for diseases, such as chronic inflammation and cancer.

Acknowledgments

We thank our colleague Dr Murali Rao for helpful suggestions and critique of the manuscript; and to the many friends whose primary valuable work in the related fields we could not directly acknowledge because of space constrains. Dr. Fei Chen thanks the Health Effects Laboratory Division of National Institute for Occupational Safety and Health for support through a cooperative agreement from the Association of Teachers of Preventive Medicine and the Centers for Disease Control and Prevention of the United States.

References

1. Zandi E, Karin M: Bridging the gap: composition, regulation, and physiological function of the IκB kinase complex. Mol Cell Biol 1999, 19:4547–4551
2. Karin M, Ben-Neriah Y: Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol 2000, 18:621–663
3. Israel A: The IKK complex: an integrator of all signals that activate NF-κB? Trends Cell Biol 2000, 10:129–133
4. Chen F, Castranova V, Shi X, Demers LM: New insights into the role of nuclear factor-κB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem 1999, 45:7–17
5. Baldwin Jr AS: The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol 1996, 14:649–683
6. Ghosh S, May MJ, Kopp EB: NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998, 16:225–260
7. Siebenlist U, Franzoso G, Brown K: Structure, regulation and function of NF-κB. Annu Rev Cell Biol 1994, 10:405–455
8. Pahl HL: Activators and target genes of Rel/NF-κB transcription factors. Oncogene 1999, 18:6853–6866
9. Gilmore TD: The Rel/NF-κB signal transduction pathway: introduction. Oncogene 1999, 18:6842–6844
10. Sun SC, Ballard DW: Persistent activation of NF-κB by the tax transforming protein of HTLV-1: hijacking cellular IκB kinases. Oncogene 1999, 18:6948–6958
11. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL: Prokaryotic regulation of epithelial responses by inhibition of IκBα ubiquitination. Science 2000, 289:1560–1563
12. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. Cell 1994, 78:773–785
13. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV: IκB kinase-β: NF-κB activation and complex formation with IκB kinase-α and NIK. Science 1997, 278:866–869
14. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A: IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. Science 1997, 278:860–866
15. Cohen L, Henzel WJ, Baeuerle PA: IKAP is a scaffold protein of the IκB kinase complex. Nature 1998, 395:292–296
16. Krappmann D, Hatada EN, Tegethoff S, Li J, Klippel A, Giese K, Baeuerle PA, Scheidereit C: The IκB kinase (IKK) complex is tripartite and contains IKKγ but not IKAP as a regular component. J Biol Chem 2000, 275:29779–29787
17. Li X, Commane M, Nie H, Hua X, Chatterjee-Kishore M, Wald D, Haag M, Stark GR: Act1, an NF-κB-activating protein. Proc Natl Acad Sci USA 2000, 97:10489–10493
18. Leonardi A, Chariot A, Claudio E, Cunningham K, Siebenlist U: CIKS, a connection to IκB kinase and stress-activated protein kinase. Proc Natl Acad Sci USA 2000, 97:10494–10499
19. Tojima Y, Fujimoto A, Delhase M, Chen Y, Hatakeyama S, Nakayama K, Kaneko Y, Nimura Y, Motoyama N, Ikeda K, Karin M, Nakanishi M: NAK is an IκB kinase-activating kinase. Nature 2000, 404:778–782
20. Huang S, DeGuzman A, Bucana CD, Fidler IJ: Nuclear factor-κB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res 2000, 6:2573–2581
21. Strasser A, O'Connor L, Dixit VM: Apoptosis signaling. Annu Rev Biochem 2000, 69:217–245
22. Ashkenazi A, Dixit VM: Death receptors: signaling and modulation. Science 1998, 281:1305–1308
23. Green DR, Reed JC: Mitochondria and apoptosis. Science 1998, 281:1309–1312
24. De Laurenzi V, Melino G: Apoptosis. The little devil of death. Nature 2000, 406:135–136
25. Du C, Fang M, Li Y, Li L, Wang X: Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000, 102:33–42
26. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL: Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000, 102:43–53
27. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G: Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999, 397:441–446
28. LaCasse EC, Baird S, Korneluk RG, MacKenzie AE: The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 1998, 17:3247–3259
29. Reed JC: Bcl-2 family proteins. Oncogene 1998, 17:3225–3236
30. Dahl AM, Klein C, Andres PG, London CA, Lodge MP, Mulligan RC, Abbas AK: Expression of bcl-X(L) restores cell survival, but not proliferation off effector differentiation, in CD28-deficient T lymphocytes. J Exp Med 2000, 191:2031–2038
31. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B: A model for p53-induced apoptosis. Nature 1997, 389:300–305
32. Amundson SA, Myers TG, Fornace Jr AJ: Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 1998, 17:3287–3299
33. Gu Z, Flemington C, Chittenden T, Zambetti GP: ei24, a p53 response gene involved in growth suppression and apoptosis. Mol Cell Biol 2000, 20:233–241
34. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T,

Tokino T, Taniguchi T, Tanaka N: Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000, 288:1053–1058
35. Barkett M, Gilmore TD: Control of apoptosis by Rel/NF-κB transcription factors. Oncogene 1999, 18:6910–6924
36. Aggarwal BB: Apoptosis and nuclear factor-κB: a tale of association and dissociation. Biochem Pharmacol 2000, 60:1033–1039
37. Hsu SC, Gavrilin MA, Lee HH, Wu CC, Han SH, Lai MZ: NF-κB-dependent Fas ligand expression. Eur J Immunol 1999, 29:2948–2956
38. Matsui K, Fine A, Zhu B, Marshak-Rothstein A, Ju ST: Identification of two NF-κB sites in mouse CD95 ligand (Fas ligand) promoter: functional analysis in T cell hybridoma. J Immunol 1998, 161:3469–3473
39. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. Nature 1995, 376:167–170
40. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M: The IKKβ subunit of IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis. J Exp Med 1999, 189:1839–1845
41. Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, Goeddel DV: Embryonic lethality, liver degeneration, and impaired NF-κB activation in IKK-β-deficient mice. Immunity 1999, 10:421–429
42. Li Q, Estepa G, Memet S, Israel A, Verma IM: Complete lack of NF-κB activity in IKK1 and IKK2 double-deficient mice: additional defect in neurulation. Genes Dev 2000, 14:1729–1733
43. Gerondakis S, Grossmann M, Nakamura Y, Pohl T, Grumont R: Genetic approaches in mice to understand Rel/NF-κB and IκB function: transgenics and knockouts. Oncogene 1999, 18:6888–6895
44. Makris C, Godfrey VL, Krahn-Senftleben G, Takahashi T, Roberts JL, Schwarz T, Feng L, Johnson RS, Karin M: Female mice heterozygous for IKKγ/NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. Mol Cell 2000, 5:969–979
45. Schmidt-Supprian M, Bloch W, Courtois G, Addicks K, Israel A, Rajewsky K, Pasparakis M: NEMO/IKKγ-deficient mice model incontinentia pigmenti. Mol Cell 2000, 5:981–992
46. Rosenfeld ME, Prichard L, Shiojiri N, Fausto N: Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice. Am J Pathol 2000, 156:997–1007
47. Doi TS, Marino MW, Takahashi T, Yoshida T, Sakakura T, Old LJ, Obata Y: Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. Proc Natl Acad Sci USA 1999, 96:2994–2999
48. Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM: Severe liver degeneration in mice lacking the IkB kinase 2 gene. Science 1999, 284:321–325
49. Fausto N, Laird AD, Webber EM: Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. FASEB J 1995, 9:1527–1536
50. Joyce D, Bouzahzah B, Fu M, Albanese C, D'Amico M, Steer J, Klein JU, Lee RJ, Segall JE, Westwick JK, Der CJ, Pestell RG: Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-κB-dependent pathway. J Biol Chem 1999, 274:25245–25249
51. Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M: NF-κB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. Mol Cell Biol 1999, 19:2690–2698
52. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin Jr AS: NF-κB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol 1999, 19:5785–5799
53. Chen F, Demers LM, Vallyathan V, Lu Y, Castranova V, Shi X: Involvement of 5′-flanking κB-like sites within bcl-x gene in silica-induced Bcl-x expression. J Biol Chem 1999, 274:35591–35595
54. Chen C, Edelstein LC, Gelinas C: The Rel/NF-κB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol 2000, 20:2687–2695
55. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin Jr AS: NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 1998, 281:1680–1683
56. Ivanov VN, Lee RK, Podack ER, Malek TR: Regulation of Fas-dependent activation-induced T cell apoptosis by cAMP signaling: a potential role for transcription factor NF-κB. Oncogene 1997, 14:2455–2464
57. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR: DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-κB and AP-1. Mol Cell 1998, 1:543–551
58. Rivera-Walsh I, Cvijic ME, Xiao G, Sun SC: The NF-κB signaling pathway is not required for Fas ligand gene induction but mediates protection from activation-induced cell death. J Biol Chem 2000, 275:25222–25230
59. Latinis KM, Norian LA, Eliason SL, Koretzky GA: Two NFAT transcription factor binding sites participate in the regulation of CD95 (Fas) ligand expression in activated human T cells. J Biol Chem 1997, 272:31427–31434
60. Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E, Paul SM: The NF-κB/Rel family of proteins mediates Ab-induced neurotoxicity and glial activation. Brain Res Mol Brain Res 1998, 57:63–72
61. Guo Q, Robinson N, Mattson MP: Secreted b-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis. J Biol Chem 1998, 273:12341–12351
62. Yu Z, Zhou D, Bruce-Keller AJ, Kindy MS, Mattson MP: Lack of the p50 subunit of nuclear factor-κB increases the vulnerability of hippocampal neurons to excitotoxic injury. J Neurosci 1999, 19:8856–8865
63. Mattson MP, Camandola S: NF-κB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 2001, 107:247–254
64. Yang JP, Hori M, Takahashi N, Kawabe T, Kato H, Okamoto T: NF-κB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. Oncogene 1999, 18:5177–5186
65. Wadgaonkar R, Phelps KM, Haque Z, Williams AJ, Silverman ES, Collins T: CREB-binding protein is a nuclear integrator of nuclear factor-κB and p53 signaling. J Biol Chem 1999, 274:1879–1882
66. Shao J, Fujiwara T, Kadowaki Y, Fukazawa T, Waku T, Itoshima T, Yamatsuji T, Nishizaki M, Roth JA, Tanaka N: Overexpression of the wild-type p53 gene inhibits NF-κB activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. Oncogene 2000, 19:726–736
67. Ariumi Y, Kaida A, Lin JY, Hirota M, Masui O, Yamaoka S, Taya Y, Shimotohno K: HTLV-1 tax oncoprotein represses the p53-mediated trans-activation function through coactivator CBP sequestration. Oncogene 2000, 19:1491–1499
68. Pise-Masison CA, Mahieux R, Jiang H, Ashcroft M, Radonovich M, Duvall J, Guillerm C, Brady JN: Inactivation of p53 by human T-cell lymphotropic virus type 1 Tax requires activation of the NF-κB pathway and is dependent on p53 phosphorylation. Mol Cell Biol 2000, 20:3377–3386
69. Ikeda A, Sun X, Li Y, Zhang Y, Eckner R, Doi TS, Takahashi T, Obata Y, Yoshioka K, Yamamoto K: p300/CBP-dependent and -independent transcriptional interference between NF-κB RelA and p53. Biochem Biophys Res Commun 2000, 272:375–379
70. Jung M, Kondratyev A, Lee SA, Dimtchev A, Dritschilo A: ATM gene product phosphorylates IκB-α. Cancer Res 1997, 57:24–27
71. Ashburner BP, Shackelford RE, Baldwin Jr AS, Paules RS: Lack of involvement of ataxia telangiectasia mutated (ATM) in regulation of nuclear factor-kB (NF-kB) in human diploid fibroblasts. Cancer Res 1999, 59:5456–5460
72. Liu L, Kwak YT, Bex F, Garcia-Martinez LF, Li XH, Meek K, Lane WS, Gaynor RB: DNA-dependent protein kinase phosphorylation of IκBα and IκBβ regulates NF-κB DNA binding properties. Mol Cell Biol 1998, 18:4221–4234
73. Ryan KM, Ernst MK, Rice NR, Vousden KH: Role of NF-κB in p53-mediated programmed cell death. Nature 2000, 404:892–897
74. Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ: Regulation of NF-κB by cyclin-dependent kinases associated with the p300 coactivator. Science 1997, 275:523–527
75. Levkau B, Scatena M, Giachelli CM, Ross R, Raines EW: Apoptosis overrides survival signals through a caspase-mediated dominant-negative NF-κB loop. Nat Cell Biol 1999, 1:227–233
76. Barkett M, Xue D, Horvitz HR, Gilmore TD: Phosphorylation of IκBα

inhibits its cleavage by caspase CPP32 in vitro. J Biol Chem 1997, 272:29419–29422
77. Reuther JY, Baldwin Jr AS: Apoptosis promotes a caspase-induced amino-terminal truncation of IκBα that functions as a stable inhibitor of NF-κB. J Biol Chem 1999, 274:20664–20670
78. Jung M, Zhang Y, Dimtchev A, Dritschilo A: Impaired regulation of nuclear factor-κB results in apoptosis induced by γ radiation. Radiat Res 1998, 149:596–601
79. Silverman N, Zhou R, Stoven S, Pandey N, Hultmark D, Maniatis T: A drosophila IκB kinase complex required for relish cleavage and antibacterial immunity. Genes Dev 2000, 14:2461–2471
80. Chaudhary PM, Eby MT, Jasmin A, Kumar A, Liu L, Hood L: Activation of the NF-κB pathway by caspase 8 and its homologs. Oncogene 2000, 19:4451–4460
81. Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G: Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. J Biol Chem 2000, 276:4812–4818
82. Ruland J, Duncan GS, Elia A, del Barco Barrantes I, Nguyen L, Plyte S, Millar DG, Bouchard D, Wakeham A, Ohashi PS, Mak TW: Bcl10 is a positive regulator of antigen receptor-induced activation of NF-κB and neural tube closure. Cell 2001, 104:33–42
83. Poyet JL, Srinivasula SM, Alnemri ES: vCLAP, a caspase recruitment domain-containing protein of equine herpesvirus-2, persistently activates the IκB kinases through oligomerization of IKKγ. J Biol Chem 2001, 276:3183–3187
84. Nurse P: A long twentieth century of the cell cycle and beyond. Cell 2000, 100:71–78
85. Shackelford RE, Kaufmann WK, Paules RS: Cell cycle control, checkpoint mechanisms, and genotoxic stress. Environ Health Perspect 1999, 107(Suppl 1):S5–S24
86. Sheikh MS, Hollander MC, Fornance Jr AJ: Role of Gadd45 in apoptosis. Biochem Pharmacol 2000, 59:43–45
87. Sandor V, Senderowicz A, Mertins S, Sackett D, Sausville E, Blagosklonny MV, Bates SE: P21-dependent g(1)arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. Br J Cancer 2000, 83:817–825
88. Jimenez GS, Khan SH, Stommel JM, Wahl GM: p53 regulation by post-translational modification and nuclear retention in response to diverse stresses. Oncogene 1999, 18:7656–7665
89. Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, O'Connor PM, Piwnica-Worms H: The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. J Biol Chem 2000, 275:5600–5605
90. Hutchins JR, Hughes M, Clarke PR: Substrate specificity determinants of the checkpoint protein kinase Chk1. FEBS Lett 2000, 466: 91–95
91. Baldwin Jr AS, Azizkhan JC, Jensen DE, Beg AA, Coodly LR: Induction of NF-κB DNA-binding activity during the G0-to-G1 transition in mouse fibroblasts. Mol Cell Biol 1991, 11:4943–4951
92. Cressman DE, Greenbaum LE, Haber BA, Taub R: Rapid activation of post-hepatectomy factor/nuclear factor κB in hepatocytes, a primary response in the regenerating liver. J Biol Chem 1994, 269: 30429–30435
93. Duckett CS, Perkins ND, Leung K, Agranoff AB, Nabel GJ: Cytokine induction of nuclear factor κB in cycling and growth-arrested cells. Evidence for cell cycle-independent activation. J Biol Chem 1995, 270:18836–18840
94. Yamada Y, Kirillova I, Peschon JJ, Fausto N: Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 1997, 94:1441–1446
95. Biswas DK, Cruz AP, Gansberger E, Pardee AB: Epidermal growth factor-induced nuclear factor κB activation: a major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. Proc Natl Acad Sci USA 2000, 97:8542–8547
96. Bash J, Zong WX, Gelinas C: c-Rel arrests the proliferation of HeLa cells and affects critical regulators of the G1/S-phase transition. Mol Cell Biol 1997, 17:6526–6536
97. Otsuka G, Nagaya T, Saito K, Mizuno M, Yoshida J, Seo H: Inhibition of nuclear factor-κB activation confers sensitivity to tumor necrosis factor-a by impairment of cell cycle progression in human glioma cells. Cancer Res 1999, 59:4446–4452
98. Kaltschmidt B, Kaltschmidt C, Hehner SP, Droge W, Schmitz ML: Repression of NF-κB impairs HeLa cell proliferation by functional interference with cell cycle checkpoint regulators. Oncogene 1999, 18:3213–3225
99. Nevins JR: E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science 1992, 258:424–429
100. Sherr CJ, Roberts JM: Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 1995, 9:1149–1163
101. Weinberg RA: The retinoblastoma protein and cell cycle control. Cell 1995, 81:323–330
102. Sheehy AM, Schlissel MS: Overexpression of RelA causes G1 arrest and apoptosis in a pro-B cell line. J Biol Chem 1999, 274:8708–8716
103. Takeda K, Takeuchi O, Tsujimura T, Itami S, Adachi O, Kawai T, Sanjo H, Yoshikawa K, Terada N, Akira S: Limb and skin abnormalities in mice lacking IKKα. Science 1999, 284:313–316
104. Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M: Abnormal morphogenesis but intact IKK activation in mice lacking the IKKα subunit of IκB kinase. Science 1999, 284: 316–320
105. Chen F, Lu Y, Zhang Z, Vallyathan V, Ding M, Castranova V, Shi X: Opposite effect of NF-κB and c-Jun-N-terminal kinase on p53-independent GADD45 induction by arsenite. J Biol Chem 2001, 276: 11414–11419
106. Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE: Aberrant nuclear factor-κB/Rel expression and the pathogenesis of breast cancer. J Clin Invest 1997, 100:2952–2960
107. Mukhopadhyay T, Roth JA, Maxwell SA: Altered expression of the p50 subunit of the NF-κB transcription factor complex in non-small cell lung carcinoma. Oncogene 1995, 11:999–1003
108. Gilmore TD, Koedood M, Piffat KA, White DW: Rel/NF-κB/IκB proteins and cancer. Oncogene 1996, 13:1367–1378
109. Bargou RC, Leng C, Krappmann D, Emmerich F, Mapara MY, Bommert K, Royer HD, Scheidereit C, Dorken B: High-level nuclear NF-κB and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. Blood 1996, 87:4340–4347
110. Devalaraja MN, Wang DZ, Ballard DW, Richmond A: Elevated constitutive IκB kinase activity and IκBα phosphorylation in Hs294T melanoma cells lead to increased basal MGSA/GRO-α transcription. Cancer Res 1999, 59:1372–1377
111. Dejardin E, Deregowski V, Chapelier M, Jacobs N, Gielen J, Merville MP, Bours V: Regulation of NF-κB activity by IκB-related proteins in adenocarcinoma cells. Oncogene 1999, 18:2567–2577
112. Sumitomo M, Tachibana M, Ozu C, Asakura H, Murai M, Hayakawa M, Nakamura H, Takayanagi A, Shimizu N: Induction of apoptosis of cytokine-producing bladder cancer cells by adenovirus-mediated IκBα overexpression. Hum Gene Ther 1999, 10:37–47
113. Miwa M, Kushida S, Maeda N, Fang J, Kawamura T, Kameyama T, Uchida K: Pathogenesis and prevention of HTLV-1-associated diseases. Leukemia 1997, 11(Suppl 3):S65–S66
114. Berger C, Brousset P, McQuain C, Knecht H: Deletion variants within the NF-κB activation domain of the LMP1 oncogene in acquired immunodeficiency syndrome-related large cell lymphomas, in prelymphomas and atypical lymphoproliferations. Leuk Lymphoma 1997, 26:239–250
115. Nasti G, Vaccher E, Errante D, Tirelli U: Malignant tumors and AIDS. Biomed Pharmacother 1997, 51:243–251
116. Carrasco D, Rizzo CA, Dorfman K, Bravo R: The v-rel oncogene promotes malignant T-cell leukemia/lymphoma in transgenic mice. EMBO J 1996, 15:3640–3650
117. Neri A, Chang CC, Lombardi L, Salina M, Corradini P, Maiolo AT, Chaganti RS, Dalla-Favera R: B cell lymphoma-associated chromosomal translocation involves candidate oncogene lyt-10, homologous to NF-κB p50. Cell 1991, 67:1075–1087
118. Fracchiolla NS, Lombardi L, Salina M, Migliazza A, Baldini L, Berti E, Cro L, Polli E, Maiolo AT, Neri A: Structural alterations of the NF-κB transcription factor lyt-10 in lymphoid malignancies. Oncogene 1993, 8:2839–2845
119. Liptay S, Seriu T, Bartram CR, Schmid RM: Germline configuration of nfkb2, c-rel and bcl3 in childhood acute lymphoblastic leukemia (ALL). Leukemia 1997, 11:1364–1366
120. Houldsworth J, Mathew S, Rao PH, Dyomina K, Louie DC, Parsa N, Offit K, Chaganti RS: REL proto-oncogene is frequently amplified in extranodal diffuse large cell lymphoma. Blood 1996, 87:25–29
121. Michaux L, Dierlamm J, Wlodarska I, Bours V, Van den Berghe H, Hagemeijer A: t(14;19)/BCL3 rearrangements in lymphoproliferative

disorders: a review of 23 cases. Cancer Genet Cytogenet 1997, 94:36–43
122. Cabannes E, Khan G, Aillet F, Jarrett RF, Hay RT: Mutations in the IκBα gene in Hodgkin's disease suggest a tumour suppressor role for IκBα. Oncogene 1999, 18:3063–3070
123. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000, 100:57–70
124. Mettouchi A, Cabon F, Montreau N, Dejong V, Vernier P, Gherzi R, Mercier G, Binetruy B: The c-Jun-induced transformation process involves complex regulation of tenascin-C expression. Mol Cell Biol 1997, 17:3202–3209
125. Schwartz SA, Hernandez A, Mark Evers B: The role of NF-κB/IκB proteins in cancer: implications for novel treatment strategies. Surg Oncol 1999, 8:143–153
126. Mayo MW, Baldwin AS: The transcription factor NF-κB: control of oncogenesis and cancer therapy resistance. Biochim Biophys Acta 2000, 1470:M55–M62
127. Baldwin AS: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. J Clin Invest 2001, 107:241–246
128. Wang CY, Cusack JC, Liu R, Baldwin AS: Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-κB. Nat Med 1999, 5:412–417
129. Arlt A, Vorndamm J, Breitenbroich M, Folsch UR, Kalthoff H, Schmidt WE, Schafer H: Inhibition of the NF-κB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide (VP16) or doxorubicin. Oncogene 2001, 20:859–868
130. Jones DR, Broad RM, Madrid LV, Baldwin AS, Mayo MW: Inhibition of NF-κB sensitizes non-small cell lung cancer cells to chemotherapy-induced apoptosis. Ann Thorac Surg 2000, 70:930–937
131. Kato T, Duffey DC, Ondrey FG, Dong G, Chen Z, Cook JA, Mitchell JB, Van Waes C: Cisplatin and radiation sensitivity in human head and neck squamous carcinomas are independently modulated by glutathione and transcription factor NF-κB. Head Neck 2000, 22: 748–759
132. Romano MF, Lamberti A, Bisogni R, Tassone P, Pagnini D, Storti G, Del Vecchio L, Turco MC, Venuta S: Enhancement of cytosine arabinoside-induced apoptosis in human myeloblastic leukemia cells by NF-κB/Rel-specific decoy oligodeoxynucleotides. Gene Ther 2000, 7:1234–1237
133. Cusack JC, Liu R, Baldwin AS: Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-κB activation. Cancer Res 2000, 60:2323–2330
134. Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P, Delhalle S, Benoit V, Merville MP: Nuclear factor-κB, cancer, and apoptosis. Biochem Pharmacol 2000, 60:1085–1089