REVIEW

Signaling to NF- κ B

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The transcription factor NF- κ B has been the focus of intense investigation for nearly two decades. Over this period, considerable progress has been made in determining the function and regulation of NF- κ B, although there are nuances in this important signaling pathway that still remain to be understood. The challenge now is to reconcile the regulatory complexity in this pathway with the complexity of responses in which NF- κ B family members play important roles. In this review, we provide an overview of established NF- κ B signaling pathways with focus on the current state of research into the mechanisms that regulate IKK activation and NF- κ B transcriptional activity.

Inducible transcription factors regulate immediate and long-lived cellular responses necessary for organismal adaptation to environmental plasticity. Such responses are mediated to a large degree through changes in gene expression. One transcription factor that serves as a key responder to changes in the environment is NF- κ B, an evolutionarily conserved signaling module that plays a critical role in many biological processes. Understanding how the transcriptional potential, activity, and selectivity of NF- κ B are regulated is therefore a topic of intense investigation in numerous laboratories.

The biological system in which NF- κ B plays the most important role is the immune system (for reviews, see Ghosh et al. 1998; Li and Verma 2002; Bonizzi and Karin 2004). Careful regulation of the transcriptional responses to many different stimuli is crucial to the proper functioning of the mammalian immune system. NF- κ B regulates the expression of cytokines, growth factors, and effector enzymes in response to ligation of many receptors involved in immunity including T-cell receptors (TCRs) and B-cell receptors (BCRs), TNFR, CD40, BAFFR, LT β R, and the Toll/IL-1R family (for reviews, see Ghosh et al. 1998; Silverman and Maniatis 2001; Bonizzi and Karin 2004). NF- κ B also regulates the expression of genes outside of the immune system and, hence, can influence multiple aspects of normal and disease physiology. Recent work has highlighted the role of NF- κ B in embryonic development and in the development and physiology of tissues including mammary gland,

bone, skin, and central nervous system. However, such varied biological roles for NF- κ B raise the intriguing question of whether one common mechanism regulates signaling to NF- κ B in all systems or whether discrete inputs create a diversity of transcriptional responses that are tailored to particular tissues and organs. Understanding how NF- κ B integrates multiple stimuli in multiple systems to generate a unified outcome suitable for specific situations is a challenge that faces researchers in this area. In keeping with the enormous progress that has been made in the study of NF- κ B, there has been a veritable explosion of review articles that have elegantly summarized progress in different aspects of NF- κ B regulation and biology (Ghosh and Karin 2002; Kucharczak et al. 2003; Ruland and Mak 2003; Ben-Neriah and Schmitz 2004; Bonizzi and Karin 2004; Chen and Greene 2004; Karin et al. 2004). Therefore, to avoid duplication, we have decided to focus in this review on a few areas of current activity. The main question that will be discussed in this review is how the different inducers activate NF- κ B and the mechanisms that underlie the regulation of NF- κ B transcriptional activity. The choice of these areas for discussion is, of course, idiosyncratic and we apologize for the narrow focus of this review. However, to help an uninitiated reader delve right into these areas of current research, we have provided a brief overview of the current state of knowledge about this transcription factor. We hope that interested readers will find a sufficiently comprehensive listing of the relevant literature in this article such that they will be able to go on and explore the biology of this fascinating transcription factor in depth.

Overview of the NF- κ B pathway

The five members of the mammalian NF- κ B family, p65 (RelA), RelB, c-Rel, p50/p105 (NF- κ B1), and p52/p100 (NF- κ B2), exist in unstimulated cells as homo- or heterodimers bound to I κ B family proteins. NF- κ B proteins are characterized by the presence of a conserved 300-amino acid Rel homology domain (RHD) that is located toward the N terminus of the protein and is responsible for dimerization, interaction with I κ Bs, and binding to DNA (Fig. 1). Binding to I κ B prevents the NF- κ B:I κ B complex from translocating to the nucleus, thereby maintaining NF- κ B in an inactive state. NF- κ B signaling is generally considered to occur through either the classical or alternative pathway (for review, see Bonizzi and Karin 2004). In the classical pathway of NF- κ B activation, for example, upon stimulation by the proinflammatory

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A NF- κ B/Rel Family **B I κ B Family** **C IKK's**

Figure 1. Schematic representation of NF- κ B, I κ B, and IKK proteins family of proteins. Members of the NF- κ B, I κ B, and IKK proteins families are shown. The number of amino acids in each protein is indicated on the right. Presumed sites of cleavage for p100 (amino acid 447) and p105 (amino acid 433) are shown. Phosphorylation and ubiquitination sites on p100, p105, and I κ B proteins are indicated. (RHD) Rel homology domain; (TAD) transactivation domain; (LZ) leucine zipper domain on IKK α / β and Rel-B; (GRR) glycine-rich region; (HLH) helix-loop-helix domain; (Z) zinc finger domain; (CC1/2) coiled-coil domains; (NBD) NEMO-binding domain; (α) α -helical domain.

tory cytokine tumor necrosis factor α (TNF α), signaling pathways lead to activation of the β subunit of the I κ B kinase (IKK) complex, which then phosphorylates I κ B proteins on two N-terminal serine residues (Fig. 2A). In the alternative pathway, IKK α is activated and phosphorylates p100 (Fig. 2B). Phosphorylated I κ Bs are recognized by the ubiquitin ligase machinery, leading to their polyubiquitination and subsequent degradation, or processing in the case of p100, by the proteasome (for review, see Karin and Ben-Neriah 2000). The freed NF- κ B dimers translocate to the nucleus, where they bind to specific sequences in the promoter or enhancer regions of target genes. Activated NF- κ B can then be down-regulated through multiple mechanisms including the well-characterized feedback pathway whereby newly synthesized I κ B α protein binds to nuclear NF- κ B and exports it out to the cytosol.

There are seven I κ B family members—I κ B α , I κ B β , BCL-3, I κ B ϵ , I κ B γ , and the precursor proteins p100 and p105—which are characterized by the presence of five to seven ankyrin repeats that assemble into elongated cylinders that bind the dimerization domain of NF- κ B dimers (Fig. 1; Hatada et al. 1992). The crystallographic structures of I κ B α and I κ B β bound to p65/p50 or p65/c-Rel dimers revealed that the I κ B proteins mask only the nuclear localization sequence (NLS) of p65, whereas the NLS of p50 remains accessible (Huxford et al. 1998; Jacobs and Harrison 1998; Malek et al. 2001, 2003). The

presence of this accessible NLS on p50 coupled with nuclear export sequences (NES) that are present on I κ B α and p65 results in constant shuttling of I κ B α /NF- κ B complexes between the nucleus and the cytoplasm, although the steady-state localization is in the cytosol (Johnson et al. 1999; Huang et al. 2000). The dynamic balance between cytosolic and nuclear localization is altered upon I κ B α degradation, because it removes the contribution of the I κ B NES and exposes the masked NLS of p65, resulting in predominantly nuclear localization of NF- κ B.

Degradation of I κ B is a tightly regulated event that is initiated upon specific phosphorylation by activated IKK. The IKK activity in cells can be purified as a 700–900-kDa complex, and has been shown to contain two kinase subunits, IKK α (IKK1) and IKK β (IKK2), and a regulatory subunit, NEMO (NF- κ B essential modifier) or IKK γ (for reviews, see Rothwarf and Karin 1999; Ghosh and Karin 2002). In the classical NF- κ B signaling pathway, IKK β is both necessary and sufficient for phosphorylation of I κ B α on Ser 32 and Ser 36, and I κ B β on Ser 19 and Ser 23. The role of IKK α in the classical pathway is unclear, although recent studies suggest it may regulate gene expression in the nucleus by modifying the phosphorylation status of histones. The alternative pathway, however, depends only on the IKK α subunit, which functions by phosphorylating p100 and causing its inducible processing to p52 (Fig. 2B). The alternative path-

Figure 2. Classical and alternative pathways of NF- κ B activation. A model depicting the two signaling pathways to NF- κ B. (A) One pathway is the classical pathway mediated by IKK β and leading to phosphorylation of I κ B. Inputs for the classical pathway include TNFR1/2, TCR and BCR, TLR/IL-1R, and many others. (B) The alternative pathway involves NIK activation of IKK α and leads to the phosphorylation and processing of p100, generating p52:RelB heterodimers. Input signals for the alternative pathway follow ligation of LT β R, BAFFR, and CD40R. These alternative pathway stimuli also activate the classical pathway.

way is activated in response to a subset of NF- κ B inducers including LT β and BAFF.

Upon phosphorylation by IKKs, I κ B proteins are recognized and ubiquitinated by members of the Skp1-Cul1-Roc1/Rbx1/Hrt-1-F-box (SCF or SCRF) family of ubiquitin ligases (for review, see Ben-Neriah 2002). β TrCP (E3RS or Fbw1a), the receptor subunit of the SCF family ubiquitin ligase machinery, binds directly to the phosphorylated E3 recognition sequence (DS*GXXS*) on I κ B (Yaron et al. 1997, 1998; Fuchs et al. 1999; Hatakeyama et al. 1999; Kroll et al. 1999; Spencer et al. 1999; Suzuki et al. 1999; Winston et al. 1999; Wu and Ghosh 1999). Recognition of I κ B α leads to polyubiquitination at conserved residues, Lys 21 and Lys 22 on I κ B α , by the E3 SCF $^{\beta$ -TrCP and the E2 UbcH5 (Alkalay et al. 1995; Scherer et al. 1995; DiDonato et al. 1996). Although it is commonly believed that degradation of I κ B is a cytoplasmic event, β TrCP1 is almost exclusively nuclear with its receptor site occupied by hnRNP-U (Davis et al. 2002). This finding has led to the suggestion that phosphorylated I κ B must out-compete hnRNP-U for binding to β TrCP1. However, β TrCP2, a highly homologous isoform of β TrCP1, is localized in the cytoplasm and can bind I κ B α , although it has lower ligase efficiency than

β TrCP1 (Suzuki et al. 2000; Davis et al. 2002). Cells from β TrCP1-deficient mice display partially decreased rates of I κ B α and I κ B β degradation, indicating that this β TrCP1 function can be partially compensated for by β TrCP2 (Nakayama et al. 2003). Determination of the actual role of β -TrCP isoforms in I κ B degradation will have to await the generation of mice lacking both isoforms.

The precursor protein p105 undergoes constitutive processing, as opposed to degradation, via the proteasome, through a cotranslational mechanism (Fan and Maniatis 1991; Palombella et al. 1994; L. Lin et al. 1998). Limited proteolysis of the precursor protein, which generates p50, is dependent on the presence of a glycine-rich region (GRR) between amino acids 376 and 404 that serves as a stop signal for proteolysis (Lin and Ghosh 1996; Orian et al. 1999). Whether p105 can also undergo inducible processing remains contentious. Multiple reports have demonstrated IKK β -dependent, and IKK α -independent, phosphorylation of p105 C-terminal serines including Ser 923 and Ser 927, followed by inducible degradation, although there is no definitive evidence that this degradation of p105 has a functional role (Fujimoto et al. 1995; MacKichan et al. 1996; Heissmeyer et al.

1999, 2001; Orian et al. 2000; Salmeron et al. 2001; Lang et al. 2003; Cohen et al. 2004). It has been suggested that similar to I κ B α , phosphorylation of p105 leads to SCF^{TrCP} binding, resulting in polyubiquitination and degradation of the protein. Polyubiquitination of p105 leading to degradation involves multiple lysine residues, and is dependent on an acidic region between residues 445 and 453 (Harhaj et al. 1996; Orian et al. 1999; Cohen et al. 2004). In contrast, partial processing leading to p50 generation may be carried out by a different ubiquitin ligase machinery (Amir et al. 2002; Lang et al. 2003; Cohen et al. 2004). It was recently demonstrated that SCF^{TrCP} is not responsible for signal-induced processing, and it has been suggested that this event is independent of ubiquitination (Cohen et al. 2004). The p105 precursor protein forms multiple heterodimeric and homodimeric complexes (Ghosh 2004), and this association with NF- κ B proteins inhibits constitutive processing (Harhaj et al. 1996; Cohen et al. 2001). Most likely, processing of p105 is partially determined by the milieu of NF- κ B dimers present in any given tissue.

Processing of p100 bears some similarity to both I κ B α degradation and p105 processing. The phosphorylation of p100 has, so far, only been shown to be catalyzed by IKK α acting downstream from NF- κ B inducing kinase (NIK; Fig. 2B; Senftleben et al. 2001a). Unlike p105, p100 processing is a tightly regulated event, with only minimal constitutive processing in unstimulated cells (Heusch et al. 1999). Interestingly, it appears that NIK can act both as an IKK α -activating kinase as well as a docking protein linking IKK α to p100 (Xiao et al. 2004). The C-terminal death domain (DD) of p100 has been shown to function as a processing inhibitory domain (PID), and expression of a p100 lacking this domain results in increased constitutive processing in a manner dependent on nuclear shuttling (Xiao et al. 2001; Liao and Sun 2003). Similar to I κ B α , phosphorylation of p100 leads to the recruitment of SCF^{TrCP}, polyubiquitination of Lys 855 in a region with sequence homology to Lys 22 of I κ B α , and subsequent processing to p52 (Fong and Sun 2002; Amir et al. 2004; Cohen et al. 2004). Like p105, the p100 GRR is also required for partial processing yielding p52 and release of active p52:NF- κ B complexes (Heusch et al. 1999).

Following degradation of I κ B, the released NF- κ B is able to bind promoter and enhancer regions containing κ B sites with the consensus sequence GGGRNNYYCC (N = any base, R = purine, and Y = pyrimidine). The crystal structures of NF- κ B dimers bound to the κ B enhancer reveal how both immunoglobulin-like domains that make up the RHD contact the DNA of the κ B site. The N-terminal Ig-like domain is primarily responsible for sequence specificity of NF- κ B, whereas hydrophobic residues within the C-terminal domain form the dimerization interface (Ghosh et al. 1995; Muller et al. 1995; F.E. Chen et al. 1998; Y.Q. Chen et al. 1998; Huxford et al. 1998; Huang et al. 2001). RelB, c-Rel, and p65 contain a transactivation domain (TAD) located toward the C terminus that is necessary for transactivation by these proteins. Homodimers of p52 and p50 lack TADs and hence

have no intrinsic ability to drive transcription. In fact, binding of p52 or p50 homodimers to κ B sites of resting cells leads to repression of gene expression (Zhong et al. 2002). The repressive function of p50 or p52 homodimers may provide a threshold for NF- κ B transactivation that can be regulated through the expression and processing of the p100 and p105 precursors. The TADs on p65, c-Rel, and RelB promote transcription by facilitating the recruitment of coactivators and the displacement of repressors. The function of TADs is enhanced through direct modifications of NF- κ B including phosphorylation, and represents another layer of regulation of the NF- κ B-mediated transcriptional response (for review, see Chen and Greene 2004).

Biological roles of NF- κ B and I κ B proteins

All of the mammalian Rel and I κ B family members have been knocked out in mice, and many conditional knockouts and multigene knockouts have also been generated. The phenotype of these various mice with regard to their immune system function and roles in the regulation of apoptosis has recently been reviewed in detail (Kucharczak et al. 2003; Bonizzi and Karin 2004). Therefore, we provide here a succinct overview of the function of each pathway component based on the relevant genetic experiments in support of such a role.

Genetic studies first revealed the crucial role of p65 in mediating protection from apoptosis during TNF α signaling. *p65*^{-/-} mice exhibit lethality caused by liver degeneration at gestational day 15–16 (Beg et al. 1995b). The insult leading to the observed liver apoptosis was shown to be TNF α signaling in the developing liver, as crossing *p65*^{-/-} mice with either *TNF α* ^{-/-} (Doi et al. 1999) or *TNFR*^{-/-} (Alcamo et al. 2001) mice rescues the liver phenotype and allows the study of the effects of p65 knockout on other tissues. p65/TNFR1 double knockouts have increased susceptibility to bacterial infection, highlighting the role of p65 in innate immune responses and the initiation of innate immune responses by non-hematopoietic cells (Alcamo et al. 2001). The knockouts of p65 were also used to show its importance in class switching in B cells and lymphocyte proliferation following various stimuli in chimeric mouse models (Doi et al. 1997).

In contrast to p65-deficient mice, p50 (NF- κ B1)-deficient mice do not show any developmental defects (Sha et al. 1995). Instead, *p50*^{-/-} mice exhibit decreased immunoglobulin production and defective humoral immune responses. B cells from these mice do not respond efficiently to LPS, underscoring the role of the classical p65/p50 heterodimer in Toll/IL-1 signaling pathways, whereas p50 is redundant for antiapoptotic pathways.

Mice lacking p52 (NF- κ B2) fail to develop normal B-cell follicles and germinal centers and have additional defects in their splenic architecture and Peyer's patch development (Caamano et al. 1998; Franzoso et al. 1998; Paxian et al. 2002). These mice display normal B-cell maturation and undergo class-switch recombination, but generate inadequate humoral responses to various

T-cell-dependent antigens. In addition, p52 knockouts fail to generate appropriate class-switched antibodies following influenza infection. Rescue of the T-cell-dependent response by adoptively transferring p52-deficient cells into *rag-1*^{-/-} mice indicates that this deficit is not intrinsic to the B cells (Franzoso et al. 1998). Overall, the p52 knockout mice have a phenotype that is consistent with a role for p52 in LT β R signaling, particularly with regard to development of secondary lymphoid organs (for reviews, see Mebius 2003; Bonizzi and Karin 2004).

RelB is unique in that it does not homodimerize, and further, is unable to heterodimerize with c-Rel or p65. RelB forms heterodimers with p100, p52, and p50 (Ryseck et al. 1992; Dobrzanski et al. 1994) and does not interact with any I κ B molecules other than p100 (Solan et al. 2002). Therefore, RelB/p52 heterodimers exhibit constitutive nuclear localization (Ryseck et al. 1992; Dobrzanski et al. 1993, 1994; Lernbecher et al. 1994). RelB has been implicated in constitutive NF- κ B activity in multiple tissues, and RelB-deficient mice have decreased baseline NF- κ B activity in the thymus and spleen. Interestingly, loss of RelB results in increased inflammatory infiltration in multiple organs as well as severe deficits in adaptive immunity (Weih et al. 1995). Loss of cellular immune responses in these mice is consistent with the observed deficit in thymic medullary epithelial cells and the loss of functional dendritic cells (Burkly et al. 1995). This phenotype is exaggerated when p50 is also knocked out, indicating that p50 and RelB cooperate in the regulation of genes that limit inflammation (Weih et al. 1997). *relB*^{-/-} B cells, although crippled in their proliferative response, undergo normal IgM secretion and class switching in response to various stimuli (Snapper et al. 1996). The importance of RelB in lymphoid organogenesis is evident in RelB knockouts, particularly in the development of Peyer's patches (Paxian et al. 2002; Yilmaz et al. 2003). These findings highlight the role of RelB/p52 dimers (probably in response to LT β R signaling) during lymphoid organogenesis.

c-Rel forms homodimers and heterodimers with p50 and p65. c-Rel-deficient mice fail to generate a productive humoral immune response, and peripheral T and B cells fail to respond to typical mitogenic stimuli (Kontgen et al. 1995). Mice lacking the c-Rel TAD, but with an intact RHD display a more severe phenotype, with marked hyperplasia in secondary lymphoid organs and hypoplastic bone marrow, and support a role for c-Rel in B-cell class switching (Zelazowski et al. 1997; Carrasco et al. 1998).

Transcriptional specificity is partially regulated by the ability of specific NF- κ B dimers to preferentially associate with certain members of the I κ B family. For example, the classical NF- κ B heterodimer (p65/p50) is predominantly, although not exclusively, regulated by I κ B α . Therefore, I κ B α -deficient mice display increased but not constitutive p65/p50 DNA-binding activity, demonstrating that additional mechanisms regulate the activity of this dimer (Beg et al. 1995a; Klement et al. 1996). I κ B α is known to participate in a feedback loop where newly

synthesized I κ B α inhibits the activity of NF- κ B. Consistent with such a role, in the absence of I κ B α the termination of NF- κ B activation in response to TNF- α is significantly delayed. In fact, if one places I κ B β , which otherwise does not participate in the early down-regulation of NF- κ B activity, under control of the I κ B α promoter, the normal kinetics of NF- κ B activation and down-regulation are restored (Cheng et al. 1998). Analysis of cell lines in which multiple isoforms of I κ Bs have been knocked out demonstrates that the functional characteristics of I κ B α , I κ B β , and I κ B ϵ are primarily the result of temporal differences in their degradation and re-synthesis that is attributable to differential transcriptional regulation of their promoters (Hoffmann et al. 2002). Therefore, although I κ B α is the primary regulator of p65:p50 complexes, it remains to be seen whether there are intrinsic properties of the I κ B α protein, in addition to its transcriptional regulation, that contribute to this central function in classical NF- κ B signaling pathways.

The role of the other I κ B family members is less well understood. The phenotype of I κ B β -deficient mice has not been published, and, consequently, the biological role of this family member remains to be clarified. Early studies suggested that BCL-3 interacts with and removes p50 and p52 homodimers from κ B sites, thereby removing the repressive effects of dimers lacking TADs (Hatada et al. 1992; Kerr et al. 1992; Wulczyn et al. 1992; Franzoso et al. 1993; Naumann et al. 1993). However, subsequent reports suggest BCL-3 forms transcriptionally active complexes with p52 and p50 homodimers (Bours et al. 1993; Fujita et al. 1993). Although these reports have demonstrated that the ability of BCL-3 complexes to promote transcription depends on BCL-3 phosphorylation, the upstream components of this pathway and their regulation have not been well characterized (Nolan et al. 1993). The BCL-3 knockout mouse is viable but is unable to generate an appropriate humoral immune response to multiple pathogens. These mice lack spleen germinal centers, and although they exhibit normal serum antibody levels, they fail to develop antigen-specific responses (Franzoso et al. 1997; Schwarz et al. 1997). This phenotype is partially shared by *p52*^{-/-} mice, supporting the idea that BCL-3 cooperates with p52 to form a transcriptionally active complex (Sha et al. 1995; Caamano et al. 1996; Franzoso et al. 1997; Schwarz et al. 1997; Brasier et al. 2001; Kuwata et al. 2003).

I κ B ϵ has a selective role in the regulation of p65 homodimers and c-Rel:p65 heterodimers (Li and Nabel 1997; Simeonidis et al. 1997; Whiteside et al. 1997). I κ B ϵ degradation occurs in response to many NF- κ B inducers, albeit with considerably slower kinetics than that for I κ B α . Similar to I κ B α , I κ B ϵ has some nuclear-cytoplasmic shuttling ability, although markedly less than I κ B α , and hence, I κ B ϵ -containing complexes are predominantly cytoplasmic (Simeonidis et al. 1997; Tam et al. 2000; Lee and Hannink 2002). I κ B ϵ has a noncanonical NES that is necessary for the cytoplasmic localization of c-Rel and I κ B ϵ itself (Lee and Hannink 2002). As I κ B ϵ is

induced in response to NF- κ B activation (Simeonidis et al. 1997; Whiteside et al. 1997), it appears that it may function in regulating later phases of NF- κ B gene activation by p65:c-Rel complexes. I κ B γ is actually the C-terminal region of mouse p105 (Inoue et al. 1992; Gerondakis et al. 1993; Grumont and Gerondakis 1994). It is still unclear whether I κ B γ , which is synthesized as an alternate mRNA using a separate promoter, has a defined biological role. Finally, I κ B ζ (also called MAIL/INAP), perhaps the eighth member of the mammalian I κ B family, is found in the nucleus and is expressed in response to IL-1 and TLR stimulation of NF- κ B but not TNF (Kitamura et al. 2000; Haruta et al. 2001; Yamazaki et al. 2001; Eto et al. 2003; Muta et al. 2003). Although I κ B ζ has weak homology to BCL-3 and the other I κ Bs, there is not yet any direct evidence that it is functionally an I κ B family member, as it has not yet been shown to interact with any Rel family members.

Signaling pathways to NF- κ B

Numerous pathways lead to the activation of NF- κ B. Almost universally, these pathways proceed via activation

of IKK, degradation of I κ B, and enhancement of the transcriptional activity of NF- κ B. However, there is significant variability in fundamental aspects of these pathways, for example, TNF α signaling via TNFR1 results in the rapid activation of IKK and nearly complete degradation of I κ B α within ~10 min, whereas signal-induced degradation of I κ B α through the TCR takes nearly 45 min. Furthermore, individual NF- κ B responses can be characterized as consisting of waves of activation and inactivation of the various NF- κ B family members (Hoffmann et al. 2002, 2003). This is because of sustained activation of the IKK complex, and its selectivity for different I κ Bs, as well as the differential regulation of I κ B expression by NF- κ B dimers. The baseline complement of NF- κ B complexes present in a cell can therefore influence the nature of the transcriptional response to a given stimulus. The transcriptional activity and specificity of NF- κ B are also differentially regulated. Summation of these effects results in unique transcriptional responses to a given stimulus in a particular cell type. A careful examination of the most-studied pathways—TNFR, TLR/IL-1R, TCR, and BCR—highlights some of these differences, but also reinforces the commonalities that characterize signaling to NF- κ B in general (Fig. 3).

Figure 3. Major signaling pathways that lead to NF- κ B activation. Signal transduction pathways emanating from TNF receptor (A), Toll/IL-1 receptor (B), the BCR (C), and intermediary proteins involved. (Lower left) An outline of pathways leading to NF- κ B as a consequence of cell stress and DNA damage is also indicated. The activation of the TCR is shown using cross-linking anti-CD3/CD28 antibodies.

TNF α : a model NF- κ B stimulus

The TNFR superfamily consists of at least 19 ligands and 29 receptors and exhibits a remarkable diversity in tissue distribution and physiology (for review, see Aggarwal 2003). These receptor/ligand pairs initiate a variety of biological responses, primarily through activation of inducible transcription factors such as NF- κ B and AP-1 (for review, see Wajant et al. 2003). TNF α is probably the most widely studied member of this family of cytokines and performs multiple roles in innate and adaptive immune responses. Most notably, through activation of NF- κ B, TNF signaling directly regulates the expression of antiapoptotic genes such as cIAP1/2 and Bcl-XL (for review, see Kucharczak et al. 2003). If NF- κ B signaling is blocked, then exposure to TNF α induces rapid apoptosis in most cell types. Aberrant TNF signaling has been implicated in many disease states, and anti-TNF antibodies are in clinical use for the treatment of inflammatory conditions such as rheumatoid arthritis (for review, see Aggarwal 2003). Therefore, a detailed understanding of IKK activation through this pathway may provide an opportunity to develop targeted inhibitors with the potential to treat such conditions. However, despite the tremendous clinical relevance of this pathway, and enormous effort in numerous laboratories, there are still significant gaps in our understanding of the mechanisms that underlie TNF α -mediated activation of NF- κ B.

TNF family receptors lack intrinsic enzymatic activity. Instead, signaling is achieved by recruitment of intracellular adapter molecules that associate with the cytoplasmic tail of the TNFR in a signal-dependent manner (for review, see Aggarwal 2003; Wajant et al. 2003). The recruitment of TNFR1 to membrane microdomains, referred to as lipid rafts, with subsequent assembly of the signaling complex, is necessary for signaling to NF- κ B and prevention of apoptosis (Hueber 2003; Legler et al. 2003). Ligation of TNFR1 by trimeric TNF α causes aggregation of the receptor and dissociation of Silencer of death domain (SODD), an endogenous inhibitor of TNF receptor activity, allowing binding of the TNFR-associated death domain protein (TRADD; Fig. 3A; Jiang et al. 1999). SODD may, however, play a redundant role, as studies on *SODD*^{-/-} mice reveal marginal effects on TNF α signaling to NF- κ B (Endres et al. 2003; Takada et al. 2003). TRADD subsequently recruits downstream adapters that result in IKK, p38, JNK, and caspase activation. One critical set of adapter molecules that are recruited to TRADD through direct interaction is the TNF-receptor-associated factor (TRAF) family (for review, see Bradley and Pober 2001; Chung et al. 2002; Dempsey et al. 2003).

Multiple members of the TRAF family including TRAF2, TRAF3, and TRAF5 have been implicated in TNF signaling, however elucidating their functional roles has been complicated because of diverse homotypic and heterotypic interactions that can occur between members of this family. TRAF2 is inducibly recruited to the TNFR and interacts efficiently with TRADD (Hsu et al. 1996). Surprisingly, TRAF2-deficient mice have intact

TNF signaling to NF- κ B; instead, TRAF2 appears to mediate signaling to AP-1 (Yeh et al. 1997). TRAF5 knockouts also exhibit normal NF- κ B activation by TNF; however, TRAF2/5 double knockout cells have substantially reduced TNF-induced IKK activation (Yeh et al. 1997; Nakano et al. 1999; Tada et al. 2001). Therefore, TRAF2 and TRAF5 appear to play a redundant role in TNF signaling to NF- κ B, although the nature of this role remains to be determined. It has been suggested that TRAF2 recruits the IKK complex following TNF α treatment through a direct interaction with the leucine zipper of IKK α or IKK β (Devin et al. 2001). TRAF2 also interacts with the serine/threonine kinase, Receptor interacting protein 1 (RIP1), which can also be independently recruited to TRADD. RIP1 is essential for TNF-induced NF- κ B activation (Hsu et al. 1996), and RIP-deficient cells exhibit increased apoptosis following TNF α stimulation owing to their inability to activate NF- κ B (Kelliher et al. 1998). Intriguingly, RIP may function as a scaffold molecule in TNF signaling because its kinase activity is dispensable for NF- κ B activation (Hsu et al. 1996; Ting et al. 1996; Devin et al. 2000). RIP1 can bind directly to NEMO and thereby recruit IKK to the TNFR1 signaling complex independent of TRAF2 (Zhang et al. 2000). Interestingly, IKK recruitment in the absence of RIP, presumably through TRAF2, is not sufficient for IKK activation (Devin et al. 2001), demonstrating that RIP has a role in addition to or independent of simple recruitment of the IKK complex. RIP may nucleate the assembly of a signaling complex that induces IKK activation through oligomerization of NEMO and subsequent autophosphorylation of IKK (Delhase and Karin 1999). Alternatively, the assembly of a signaling complex may facilitate NF- κ B signaling by bringing the IKK complex into close proximity with an IKK kinase.

An intriguing feature of signaling by TNF is its ability to stimulate both death and survival. As noted above, the balanced induction of both pathways usually leads to activation of cells; however, if activation of NF- κ B, and consequently the survival pathways, are blocked, TNF becomes a potent apoptosis-inducing factor. In general, direct up-regulation by NF- κ B of factors such as IAPs is responsible for the antiapoptotic effects of NF- κ B (for review, see Kucharczak et al. 2003). It appears, however, that the two main target transcription factors of TNF signaling, NF- κ B and AP-1, also participate directly in arbitrating the choice between survival and death. It has been observed that prolonged activation of JNK/AP-1 leads to apoptosis (Guo et al. 1998; De Smaele et al. 2001; Tang et al. 2001). NF- κ B induction can lead to the synthesis of certain effectors that inhibit the JNK pathway, thereby limiting JNK/AP1 activation. One such JNK-inhibiting factor is GADD45 β , which inhibits JNK signaling by blocking the upstream kinase MKK7 (De Smaele et al. 2001; Jin et al. 2002; Papa et al. 2004). Although a more recent study showed that activation of JNK is similar to wild-type cells in *GADD45 β* ^{-/-} MEFs (Jin et al. 2002), it is possible that GADD45 β may cooperate with some other NF- κ B-induced inhibitor of JNK. For example, X-chromosome-linked inhibitor of apoptosis

(XIAP) is induced by NF- κ B, and, in addition to inhibiting multiple proapoptotic caspases, it also blocks JNK activation (Tang et al. 2001). Another NF- κ B-regulated gene, cFLIP (Casper), inhibits JNK at the level of TRAF/RIP (Shu et al. 1997; Kreuz et al. 2001). Furthermore, the roles of JNK and NF- κ B are not entirely antagonistic, as expression of the antiapoptotic protein cIAP1 is coregulated by JNK and NF- κ B, and consequently TNF α -induced apoptosis is increased in JNK double-knockout cells (Lamb et al. 2003; Ventura et al. 2003). Interestingly, TRAF-2-deficient cells are more sensitive to TNF α -induced cell death although NF- κ B signaling remains intact. This may reflect the requirement for TRAF-2 binding to cIAP in order for it to be localized to its site of function or the requirement of JNK-mediated JunD activation for appropriate expression of cIAP (Shu et al. 1996; Wang et al. 1998; Lamb et al. 2003).

Lymphotoxin- β R, BAFF-R, and CD40: TNFR family members that signal to NF- κ B through the alternative pathway

The recent discovery of the alternative signaling pathway leading to inducible p100 processing has provided a novel addition to the well-studied pathway of NF- κ B activation through the degradation of I κ B proteins (for review, see Bonizzi and Karin 2004). This represents an important step forward in understanding how NF- κ B family members might be differentially regulated. The alternative pathway is unique in that it is independent of IKK β and NEMO (Claudio et al. 2002; Dejardin et al. 2002). Instead, the functional IKK is thought to be IKK α homodimers, which selectively phosphorylate p100 associated with RelB. Therefore, processing of p100 releases a subset of transcriptionally active NF- κ B dimers, consisting mainly of p52:RelB (Dejardin et al. 2002; Xiao et al. 2004). In contrast to the other pathways discussed here, in the alternative pathway the mechanism of IKK α activation is known. NF- κ B inducing kinase (NIK) is responsible for directly phosphorylating and activating IKK α (Senftleben et al. 2001a; Xiao et al. 2001). However, events that occur upstream of NIK are unclear. The key question concerning signaling by these stimuli is how they are channeled to NIK and IKK α , even though their receptor signaling domains resemble those of other TNF family members. Because LT β , BAFF, and CD40L also activate the classical pathway, it would appear that the intracellular signaling domains of these receptors possess additional sequence motifs that allow their coupling to NIK and the alternative pathway. The creation and systematic analysis of chimeras between these receptors and those that only signal through the classical pathway will probably be necessary to elucidate the mechanism by which the alternative pathway is activated.

Toll/IL-1 receptor signaling to NF- κ B

Signaling to NF- κ B mediates multiple aspects of innate and adaptive immunity (for review, see Ghosh et al. 1998; Silverman and Maniatis 2001; Bonizzi and Karin

2004). NF- κ B plays an essential role in early events of innate immune responses through Toll-like receptor (TLR) signaling pathways. TLRs are evolutionarily conserved Pattern recognition receptors (PRRs) that recognize conserved Pathogen-associated microbial patterns (PAMPs) present on various microbes (for review, see Janeway and Medzhitov 2002; Kopp and Medzhitov 2003; Takeda et al. 2003). The role of TLRs as arbiters of the self-non-self decision means that they play a central role in innate immunity as well as in the initiation of the adaptive immune responses. To date, 11 mammalian TLRs have been described, and each of these signals to NF- κ B. These receptors have varied tissue distribution and recognize many different PAMPs including LPS, dsRNA, nonmethylated CpG DNA, and flagellin. Some members of the TLR family are also capable of heterodimerization, thereby further expanding the repertoire of molecules that are recognized. Significant progress has been made over the past couple of years in deciphering the relevant signaling pathways that operate downstream of TLRs (for review, see Barton and Medzhitov 2003). The intracellular domain of Toll-like receptors bears strong homology with the intracellular domain of the IL-1 receptor, and it is this shared Toll-IL-1R (TIR) domain that mediates interaction with downstream signaling adapters that lead to activation of three key transcription factors, NF- κ B, AP-1, and IRF3.

TLR signaling is initiated by the recruitment of cytosolic adapters that all share the TIR domain (for review, see Dunne and O'Neill 2003). MyD88 was the first TIR domain containing adapter protein characterized and was shown to interact with the TIR domain on TLR/IL-1R cytoplasmic tails by homotypic interaction. MyD88 is crucial for normal NF- κ B induction in response to IL-1, IL-18, and LPS (TLR4; Adachi et al. 1998; Kawai et al. 1999). MyD88 recruitment to TLR4 following receptor aggregation leads to recruitment of another TIR-domain-containing adapter, TIRAP or Mal (Fig. 3B). TIRAP mediates NF- κ B activation downstream of TLR2 and TLR4, but not IL-1R or other TLRs (Hornig et al. 2002; Yamamoto et al. 2002). Currently it is not known how TIRAP selectively acts only in a subset of MyD88-mediated signaling pathways (for review, see McGettrick and O'Neill 2004). MyD88 also contains an N-terminal death domain (DD) that recruits downstream DD-containing signaling molecules such as the serine/threonine kinase IRAK (for review, see Janssens and Beyaert 2003). The exact role played by IRAK family members is somewhat enigmatic because like RIP, IRAK-1 kinase function is also dispensable for its role in TLR/IL-1 signaling (Knop and Martin 1999; X. Li et al. 1999). IRAK-4 is also required for signaling from TLR and IL-1, likely upstream of IRAK-1 (Suzuki et al. 2002). IRAK recruitment and activation are necessary to bring TRAF6 into the signaling complex, although exactly how this is achieved remains mysterious (Qian et al. 2001; Takaesu et al. 2001). TRAF6 is recruited to TLR/IL-1R and is required for MyD88-dependent activation of NF- κ B (Cao et al. 1996; Wesche et al. 1997; Wu and Arron 2003). TRAF6-deficient cells exhibit a complete loss of NF- κ B DNA binding induced

by IL-1R as well as loss of the early NF- κ B response downstream from TLR4 (Lomaga et al. 1999).

The link between TRAF6 and the IKK complex has remained controversial. Two sets of adapters have been proposed to link TRAF6 with IKK. The first involves the Transforming growth factor β activated kinase 1 (TAK1), and two associated adapter proteins TAB1 and TAB2 (Takaesu et al. 2000). The major support for this signaling module came from studies that indicated that they could be copurified with TRAF6 in cells (Wang et al. 2001). The presence of a RING finger domain in TRAF6 led to the suggestion that TRAF6 could function as a ubiquitin ligase (Deng et al. 2000). Upon stimulation, TRAF6 would ubiquitinate itself or components of the TAK1/TAB1/TAB2 complex, thereby priming them for IKK activation (Sun et al. 2004). However, as of yet there is no genetic evidence in mammalian systems supporting such a critical role for TAK1 in TLR/IL-1 signaling. Intriguingly, a recent study indicated that RNAi-mediated knock-down of TAK1 inhibited signaling from both IL-1 and TNF receptors, which was surprising because TRAF6 is not involved in the TNF pathway (Takaesu et al. 2003). One possibility is that TAK1 function is not limited to TRAF6, but rather may extend to other TRAFs that also possess RING finger domains. A more recent report has implicated TAK1 as an essential component in TCR signaling, raising the possibility that contrary to current belief, TRAF proteins are also involved in antigen-receptor signaling (Sun et al. 2004). However, the phenotypes of TAB1 and TAB2 knockouts fail to support any role for these proteins in TLR/IL1 and TNF signaling. Knockout of TAB1 and TAB2 both lead to embryonic lethality at embryonic days 17 (E17) and 12.5 (E12.5), respectively (Komatsu et al. 2002; Sanjo et al. 2003). Fibroblasts isolated from the embryos display completely normal signaling in response to TNF and IL-1 (Sanjo et al. 2003; M.S. Hayden and S. Ghosh, unpubl.). Knocking out TAK1 causes embryonic lethality at E10, thus preventing signaling studies. Therefore, although it remains possible that future development and characterization of conditionally knocked out TAK1 cells will support the proposed role in TLR/IL-1, TNF, and TCR signaling, it is clear that there are significant complexities yet to be resolved.

Besides the TAK1/TAB1/TAB2 proteins, another protein that has been reported to act as a bridge between TRAF6 and the IKK complex is ECSIT (evolutionarily conserved signaling intermediate in toll pathways). ECSIT was identified in a yeast two-hybrid screen as a binding partner of TRAF6 and was shown to be required for TLR and IL-1 signaling, but not TNF-signaling, using RNAi knock-down techniques (Kopp et al. 1999; Xiao et al. 2003). Knockout of ECSIT leads to very early embryonic lethality, thereby preventing isolation of cells suitable for signaling studies. However, the knockouts of ECSIT display phenotypes that are identical to those seen in knockouts of Bmp receptor-1, a member of the TGF β -receptor family that is known to function in early development. In fact, subsequent analysis showed that ECSIT can function as a coactivator for effectors of Bmp/

TGF β signaling, namely, the Smad transcription factors (Xiao et al. 2003). This surprising finding raises the possibility that ECSIT may regulate both TLR and TGF/Bmp pathways, and hence may help provide an explanation for why these pathways cross-repress one another (Moustakas and Heldin 2003). It is also important to point out that the TAK/TAB proteins were also initially identified and characterized as intermediates in TGF β signaling, and hence it is possible that this link between adapters in TLR and TGF signaling pathways may be more extensive than currently imagined.

The initial studies with ECSIT suggested that it might function in TLR signaling by recruiting and activating the kinase MEKK1, which is also involved in TGF β signaling (Kopp et al. 1999; Zhang et al. 2003). However, knockouts of MEKK1 do not display any overt phenotype in TLR or TNF signaling, suggesting that some other kinase might be involved (Xia et al. 2000; Yujiri et al. 2000). More recently, MEKK3 has been strongly implicated in TLR signaling. MEKK3-deficient cells do not transcribe IL-6 following TLR4 or IL-1R stimulation and exhibit delayed and weak NF- κ B DNA binding following LPS stimulation (Huang et al. 2004). Although TRAF6 and MEKK3 inducibly associate in TLR4 signaling, the mechanisms that regulate this process are not known (Huang et al. 2004). Therefore, it is possible, although not proven, that ECSIT exerts its role in TLR signaling by somehow modulating the function of MEKK3.

Multiple TLRs are also capable of signaling in the absence of MyD88. LPS stimulation in *MyD88*^{-/-} cells results in NF- κ B activation with slower kinetics than from normal TLR signaling and leads to expression of only a subset of target genes (Kawai et al. 1999). TRIF (TICAM-1), a TIR-domain-containing adapter, mediates activation of NF- κ B in the absence of MyD88 when cells are stimulated through TLR3 and TLR4 (Oshiumi et al. 2003). TRIF expression is regulated by NF- κ B and is therefore induced by TLR and IL-1R signaling (Hardy et al. 2004). Studies using cells from TRIF-deficient mice demonstrate that TRIF is required for early and late NF- κ B responses and IRF3 responses to LPS, but not for JNK activation (M. Yamamoto et al. 2003a; Akira 2004). Reconstitution of *TRIF*^{-/-} cells with a mutant form lacking the TRAF-binding domain restores induction of IFN β -responsive genes, via activation of IRF3, but not NF- κ B activation, indicating that TRIF is the point of divergence in signaling to NF- κ B and IRF3 by TLR4. Recently it has been reported that TRIF binds RIP1 and that *RIP1*^{-/-} MEFs have decreased NF- κ B signaling from TLR3-poly(I:C) (Meylan et al. 2004). Finally, another TIR-domain-containing adapter, TRAM (TRIF-related adapter molecule), functions upstream of TRIF in MyD88-independent signaling from TLR4. TRAM is required for IRF-3 activation and for the delayed phase of NF- κ B activation following TLR4 engagement. TLR4-induced IRAK activation by MyD88, however, is unaffected by the absence of TRAM (M. Yamamoto et al. 2003b). TRAM does not function in TLR3 or IL-1R signaling pathways (Fitzgerald et al. 2003; M. Yamamoto et al. 2003b).

Two divergent members of the IKK family, IKKi (IKK ϵ) and TBK1 (T2K), have been implicated downstream of TRIF in signaling to IRF-3 and IRF-7, following engagement of TLR3 and TLR4 (Hemmi et al. 2004; Sharma et al. 2003). Although initially both of these kinases were implicated in regulation of NF- κ B activity, recent results have cast doubt on those conclusions. Studies using knockout cells indicate that neither is required for NF- κ B activation by LPS or TNF α (Akira 2004; Hemmi et al. 2004). Also in contrast to the study reporting the knockout of TBK1, a recent study reported that there was no discernible effect on the transcriptional activity of NF- κ B in TBK1 knockout cells following stimulation with multiple PAMPs (McWhirter et al. 2004). The reason for this discrepancy remains to be resolved. IKKi $^{-/-}$ cells have normal induction of IRF3 following stimulation with LPS (hence TLR4), whereas TBK1 $^{-/-}$ cells do not (Hemmi et al. 2004). IKKi expression is regulated by NF- κ B, and it appears that IKKi may be constitutively active once expressed (Mercurio 2004). IKKi may facilitate CCAAT/enhancer-binding protein δ (C/EBP δ) pathways that contribute to the expression of a subset of genes that are induced by IL-1, LPS, TNF, or PMA (Kravchenko et al. 2003). Therefore, TBK1 but not IKKi mediates TLR signaling to IRF-3, likely through direct phosphorylation of IRF-3, and neither kinase appears to be directly involved in TLR signaling to NF- κ B.

The Toll-signaling pathway can also be negatively regulated by proteins that are induced or activated upon TLR signaling and therefore may help to limit signaling from these receptors. For example, Tollip is an adapter protein found in association with IRAK in the IL-1R signaling pathway (Burns et al. 2000; Zhang and Ghosh 2002). Tollip binds to IRAK, and to TLR2, TLR4, or IL-1R upon signaling. Activation of IRAK by MyD88 results in autophosphorylation of IRAK and phosphorylation of Tollip, causing their dissociation (Bulut et al. 2001; Zhang and Ghosh 2002). Tollip can suppress TLR/IL-1R signaling when overexpressed and, therefore, functions as a negative regulator of IRAK activity. It is not known whether Tollip functions in down-regulation of TLR signaling or acts as a constitutive inhibitor of IRAK. It has been suggested that high levels of Tollip in the intestinal epithelium help to prevent inflammation in response to commensals (Melmed et al. 2003). Another well-characterized negative regulator is a member of the IRAK family, IRAK-M. IRAK-M is inducibly expressed in myeloid cells and was initially shown to induce NF- κ B signaling when overexpressed (Wesche et al. 1999). However, in knockout mice, TLR and IL-1R signaling to NF- κ B is enhanced, and the cells do not become tolerant of repeated LPS stimulation (Kobayashi et al. 2002). IRAK-M prevents the release of IRAK-1 and IRAK-4 from the TLR/MyD88 signaling complex, and therefore inhibits the association between IRAKs and TRAF6. Further upstream, alternative splice variants of MyD88 have been reported to inhibit TLR signaling by competitively binding IRAK4 (Janssens et al. 2002, 2003; Burns et al. 2003). Finally, at the level of the membrane, SIGIRR (TIR8), a member of the IL-1R family that contains an extracellu-

lar Ig domain, has been shown to decrease TLR/IL-1R signaling (Thomassen et al. 1999; Wald et al. 2003). In SIGIRR $^{-/-}$ cells, treatment with either LPS or IL-1 leads to enhanced and prolonged activation of both NF- κ B and JNK. SIGIRR binds to other Toll/IL-1 receptors and interacts with IRAK and TRAF6 (Wald et al. 2003). It remains to be seen whether it inhibits signaling by competing for adapters or preventing the dissociation of IRAK from the receptor in a manner analogous to IRAK-M. Interestingly, SIGIRR is most abundantly expressed in epithelial cells, leading one to speculate that it may suppress TLR signaling at sites of exposure to commensal bacteria.

BCR and TCR signaling to NF- κ B

Signaling from BCRs and TCRs is critical for mounting adaptive immune responses and has consequently been of great interest to numerous laboratories. Activation of NF- κ B downstream of BCR and TCR allows antigen-specific proliferation and maturation of lymphocytes into effector cells. Signaling through these two antigen receptors is functionally analogous, although the molecular details differ. From a clinical standpoint, understanding how these signaling pathways operate may provide novel targets for therapies directed at autoimmunity, inflammatory disorders, and transplant rejection.

Signaling through the TCR, or analogously through the BCR, and various costimulatory molecules leads to NF- κ B activation (for review, see Ruland and Mak 2003). TCR ligation induces phosphorylation of key residues on ITAMs present on TCR ζ and CD3 (Fig. 3C). Phosphorylated ITAMs recruit SH2-domain-containing adapters, most notably the Syk family tyrosine kinases Lck and ZAP70. However, the link between the receptor proximal tyrosine kinases and NF- κ B is poorly defined. Recent studies have implicated several potential signaling intermediates that appear to comprise a novel signaling pathway, including PKC θ , CARMA1/CARD11, BCL10, and MALT1 (for reviews, see Lucas et al. 2004; Simeoni et al. 2004; Thome 2004). The IKK complex is rapidly recruited to the immunological synapse through an unknown mechanism and can be colocalized to the TCR by confocal immunofluorescence analysis (Khoshnaw et al. 2000). In ZAP-70-deficient cells, NF- κ B activation induced by TCR stimulation can be rescued with a NEMO-(SH2) $_2$ chimera that targets NEMO to the immunological synapse, indicating that events downstream from ZAP-70 are only required for IKK recruitment (Weil et al. 2003). One intriguing hypothesis that has been proposed is that recruitment of the IKK complex is dependent on changes in cytoskeletal structure during formation of the immunological synapse (Weil et al. 2003). Although the importance of scaffolding molecules in the MAPK pathway has been established, little is known thus far about how these processes affect NF- κ B signaling downstream of antigen receptors.

It is clear from knockout studies that PKC θ is essential for activation of NF- κ B by TCR (Sun et al. 2000), but the specific role played by PKC θ in T cells, and PKC β 1/2 in

B cells (Guo et al. 2004), remains to be elucidated. PKC θ is specifically recruited to the immunological synapse, although how this specificity for PKC θ is achieved remains unclear because T cells express most of the other PKC isoforms. The exact role of PKC θ in the immunological synapse is also unclear, as is the mechanism responsible for recruiting PKC θ . PKC θ is capable of directly interacting with the IKK complex in primary T cells (Khoshnan et al. 2000), and it is possible that PKC θ might act as a scaffold/adaptor to link events in the synapse with the other essential components in this pathway, namely, CARMA1/CARD11, BCL10, and MALT1. Knockouts of each of these genes leads to a specific block in NF- κ B activation in response to antigen-receptor signaling, and therefore current efforts are focused on determining how these proteins are linked to the IKK complex (Ruland et al. 2001; Hara et al. 2003; Ruefli-Brasse et al. 2003).

The MAGUK family protein CARD11/CARMA-1 is required for PKC θ -mediated activation of NF- κ B following TCR ligation (Gaide et al. 2002; Hara et al. 2003). BCL10, which interacts with MALT1 and cIAPs, is also critical for NF- κ B activation via the BCR (Ruland et al. 2001). BCL10 interacts with CARMA-1 and undergoes CARMA-1-dependent phosphorylation, although CARMA-1 lacks kinase activity (Bertin et al. 2001; Gaide et al. 2001). BCL10 knockout mice are unable to activate NF- κ B in response to antigen-receptor stimulation (Ruland et al. 2001). Interestingly, genetic evidence of a role for RIP2 has recently been reported in T-cell signaling (Ruefli-Brasse et al. 2004). RIP2 associates with BCL10 and is necessary for TCR-induced BCL10 phosphorylation and IKK activation. It is not yet clear how upstream mediators of TCR signaling may regulate RIP2 and, in turn, how RIP2 might affect IKK activity. BCL10 oligomerization has been implicated in IKK activation through a process that involves ubiquitination of NEMO (Zhou et al. 2004). This ubiquitination event appears to be mediated by MALT1 and possibly CARMA-1. A surprising recent finding is that TCR-induced ubiquitination of NEMO may depend on TRAF6, which has not previously been thought to play any role in TCR signaling, as T-cell deficits have not been reported in TRAF6-deficient mice (Lomaga et al. 1999; Sun et al. 2004). It is interesting to note that RIP2 associates with TRAF6, and one could speculate that this interaction has a functional role in IKK activation downstream of antigen receptor signaling (McCarthy et al. 1998).

Cell stress

Various intracellular stressors, both physiological and pathological, induce the activation of NF- κ B (Fig. 3). Understanding the pathways responsible will require a significant shift in thinking from traditional signaling paradigms, as there is not an initiating receptor ligation event. Although less conventional, the physiological relevance of these pathways is undeniable. A properly orchestrated response to genotoxic stress is a fundamental defense against transformation and cancer. However, the

biological function of NF- κ B in this response is not immediately clear. Although it is generally hypothesized that activation of NF- κ B provides an antiapoptotic signal, thereby providing a window of opportunity for the cell to repair DNA damage, this has not been formally proven. In fact, under some circumstances, NF- κ B signaling can play a proapoptotic role in response to stimuli such as UV irradiation (Campbell et al. 2004). Nevertheless, cell irradiation and DNA damage have long been known to activate NF- κ B (Brach et al. 1991). However, considerable discrepancies exist in the experimental systems used to investigate cell stress, and this has hindered progress in this area. Many of the agents used to induce genotoxic stress do so through diverse perturbations of cell physiology and could potentially activate NF- κ B through multiple mechanisms. Even when selectively induced, DNA damage elicits a highly complex cellular response that has been the subject of considerable research. The product of the gene mutated in ataxia-telangiectasia (ATM), a phosphoinositide 3-kinase-related kinase (PIKK), has a central role in sensing DNA double-strand breaks (DSBs) and causing the consequent activation of the DNA repair machinery (for review, see Yang et al. 2004). It has been shown using ATM knockout cells, and cells from patients with ataxia-telangiectasia, that ATM is required for NF- κ B activation following ionizing radiation (Li et al. 2001). Topoisomerase inhibitors, such as camptothecin, can also activate NF- κ B, presumably through the generation of DSBs (Piret and Piette 1996). Although NF- κ B activation in response to low-dose radiation could be due to production of reactive oxygen species (ROS; Mohan and Meltz 1994), such a model seems inconsistent with a requirement for ATM in this process.

Activation of IKK following treatment with topoisomerase inhibitors is dependent on the zinc finger domain in NEMO (Huang et al. 2002). A recent report showed that modification of NEMO with the small ubiquitin-like modifier (SUMO-1) plays a mechanistic role in the activation of IKK following treatment with topoisomerase inhibitors (Huang et al. 2003). Sumolation of NEMO may be necessary for the recruitment of NEMO to the nucleus resulting in ATM-dependent ubiquitination of NEMO and subsequent activation of IKK. Other studies have suggested that RIP is required for the activation of IKK in response to DNA damage in a manner that is independent of TRAF family members (Hur et al. 2003). It is unclear how these data can be reconciled with the proposed role for SUMO-1 in this process.

NF- κ B has also been shown to be activated following UV-C irradiation; however, this response is not dependent on DNA damage (Devary et al. 1993). NF- κ B activation following UV-C irradiation has been shown to be independent of I κ B α Ser 32/36 phosphorylation and is, therefore, IKK-independent (Li and Karin 1998; Kato et al. 2003; Tergaonkar et al. 2003). Instead, it might operate through activation of p38 pathways leading to the activation of casein kinase 2 (CK2). CK2 then directly phosphorylates I κ B α on C terminus serine residues. Whereas mutation of these serines abolishes the

ability of CK2 to induce I κ B degradation following UV irradiation (Barroga et al. 1995; Lin et al. 1996; McElhinny et al. 1996; Kato et al. 2003), some early transient activation of NF- κ B appears intact in CK2 knockdown cells, p38 knockout cells, or I κ B α knockout cells reconstituted with I κ B α in which the CK2 phosphoacceptor sites were mutated (Kato et al. 2003). A similar pathway of NF- κ B activation has been proposed in cells treated with doxorubicin (Tergaonkar et al. 2003). Doxorubicin's mechanism of action is multipartite, and therefore it is still unclear whether the NF- κ B activation observed is caused by generation of free radicals and ROS via effects similar to UV-C, or caused by topoisomerase inhibition mediated through DNA intercalation. More recently, these findings have been called into question by work showing that doxorubicin and UV-C induce apoptosis through release of NF- κ B dimers that can suppress transcription of antiapoptotic genes by recruiting histone deacetylases (Ashikawa et al. 2004; Campbell et al. 2004). It is not clear whether these findings are generally applicable or are cell-type-specific. In summary, whereas nonlethal UV-C irradiation leads to activation of CK2 downstream from ROS, and consequent degradation of I κ B α , the response to DNA damage is mediated through ATM, and perhaps RIP, to cause IKK activation. In the future, clarification of these issues will require a systematic investigation in multiple cell types using carefully controlled induction of various types of cell stress. In particular, the issue of ROS effects on NF- κ B signaling merits detailed examination, given that this is likely the source of much confusion in the investigation of multiple NF- κ B signaling pathways (Zhang and Chen 2004).

Regulation of IKK

With the possible exception of CK2, the common feature of all pathways leading to the activation of NF- κ B is the activation of one of the I κ B kinases. Consequently, this is the most important regulatory step in determining the NF- κ B response to a given stimulus. Current evidence points toward a mechanism that depends on the assembly of complex signalosomes for the activation of IKK where the key event is induced proximity allowing cross-phosphorylation between the IKK subunits. However, a role for an activating IKK kinase cannot be completely ruled out. In fact, activation of IKK α in the alternative pathway by NIK is well established. Determining whether such an event also occurs in the activation of the classical pathway will require a more comprehensive analysis of the composition of the IKK complexes in various tissues and cells under different physiological conditions.

Structure and function of the IKK complex

The initial efforts to identify the I κ B kinase led to the purification of a high-molecular-weight kinase complex from unstimulated cells that was capable of phosphory-

lating I κ B α on the appropriate serine residues (Chen et al. 1996; Lee et al. 1997). Subsequently, multiple groups identified a stimulus-dependent kinase activity that was termed the I κ B kinase (DiDonato et al. 1997; Mercurio et al. 1997; Woronicz et al. 1997; Zandi et al. 1997). The first component of this 700–900-kDa complex was shown to be CHUK, a serine/threonine kinase that, remarkably, had been proposed to be a regulator of gene transcription based solely on sequence homology (Connelly and Marcu 1995). CHUK, a 745-amino acid protein, has since been renamed IKK α . IKK β was discovered shortly thereafter based on sequence homology and biochemical purification. IKK α and IKK β are serine/threonine kinases that are characterized by the presence of an N-terminal kinase domain, a C-terminal helix–loop–helix (HLH) domain, and a leucine zipper domain (Fig. 1). NEMO (also known as IKK γ , IKKAP1, and Fip-3), the regulatory subunit of the IKK complex, was initially isolated through genetic complementation cloning in an NF- κ B-unresponsive cell line, and subsequently by affinity purification and as a factor interacting with an adenoviral inhibitor of NF- κ B (Rothwarf et al. 1998; Yamaoka et al. 1998; Y. Li et al. 1999; Mercurio et al. 1999). NEMO is a 48-kDa protein that is not related to IKK α and IKK β and contains a C-terminal zinc finger-like domain, a leucine zipper, and N-terminal and C-terminal coiled-coil domains (Fig. 1).

IKK α and IKK β share 52% overall sequence identity and 65% identity within the catalytic domain. Both IKK α and IKK β can be inactivated through the mutation of a single lysine, Lys 44, within the predicted ATP-binding site (Fig. 1; Mercurio et al. 1997; Woronicz et al. 1997; Zandi et al. 1997). IKK α and IKK β are capable of phosphorylating multiple members of the I κ B family at multiple sites. These kinases also have substrates outside of the I κ B family, most notably p65. In vitro studies indicate that the IKK α /IKK β heterodimers have higher catalytic efficiency than either homodimer (Huynh et al. 2000). Although IKK α and IKK β display different specificities for different I κ B family members, it is not known whether they are independently regulated. Although both IKK α and IKK β are capable of phosphorylating I κ B α at Ser 32 and Ser 36, and I κ B β at Ser 19 and Ser 23, IKK α is less efficient and cannot replace IKK β in knockout studies (DiDonato et al. 1997; Mercurio et al. 1997; Regnier et al. 1997). Both IKK α and IKK β are less efficient kinases for I κ B β than for I κ B α , and this difference may explain the delayed degradation kinetics of I κ B β following stimulation with TNF α (Wu and Ghosh 2003). I κ B α bound to NF- κ B is a better substrate for IKK β than free I κ B, which may prevent the degradation of newly synthesized I κ B, thus allowing it to accumulate and down-regulate NF- κ B activity (Zandi et al. 1998). It is unclear, however, how the enzymology of the IKKs is affected by heterodimerization and complex formation in vivo.

IKK α and IKK β dimerization is dependent on the leucine zipper domain, which is therefore required for kinase activity (Mercurio et al. 1997; Woronicz et al. 1997; Zandi et al. 1997). Available data indicate that IKK α and IKK β preferentially form heterodimers in vivo. However,

the sequence features that determine this preference have not been elucidated. Studies using knockout cells show that both IKK α and IKK β are capable of homodimerization, although evidence for homodimers in unmanipulated cells is more controversial. Immunodepletion studies have demonstrated the presence of IKK β /NEMO complexes activated by anti-CD3/CD28 in primary T cells (Khoshnan et al. 1999). An IKK β -only complex was also shown to exist in HeLa cells by affinity purification, anion exchange chromatography, and immunodepletion, although this complex appeared to be only weakly activated by TNF α (Mercurio et al. 1999). Also, because in the alternative pathway, phosphorylation of p100 in response to BAFF or LT β stimulation can occur in the absence of IKK β and NEMO (Senftleben et al. 2001a), it has been suggested that there is a discrete IKK α -only complex. However, there is not yet definitive evidence that IKK α homodimers, which are not bound to NEMO, exist *in vivo* and mediate signaling via the alternative pathway.

The HLH domain is required for full IKK β activity and is also involved in the down-regulation of kinase activity. Deletion mutants lacking the HLH domain have diminished kinase activity following overexpression or stimulation (Delhase et al. 1999). Interestingly, a C-terminal deletion mutant of IKK β (1–559) can be functionally rescued through the coexpression of the HLH domain of IKK β (amino acids 558–756), indicating that a direct interaction between the HLH domain and kinase domain is necessary for activity. However, when the C-terminal domain of IKK β is expressed *in trans*, or when the C-terminal serine residues are mutated, IKK activity is prolonged. More recent data have shown that removing the HLH region also abrogates the binding of IKK to NEMO, and hence the role of the HLH domain may be in facilitating the assembly of functional IKK–NEMO complexes (May et al. 2002).

IKK α and IKK β interact with NEMO through a C-terminal hexapeptide sequence (Leu-Asp-Trp-Ser-Trp-Leu) termed the NEMO binding domain (NBD; Fig. 1; May et al. 2000, 2002). A peptide encompassing this sequence when coupled to the cell-permeable antennapedia domain blocks NF- κ B signaling by disrupting the IKK complex (May et al. 2000, 2002). NEMO binding to IKK requires residues 135–231, located within the first coiled-coil motif, and can interact with the NBD of both IKK α and IKK β . Although *in vitro* assembly of the complex indicates that only IKK β assembles with NEMO, data from IKK β knockout mice clearly indicate otherwise, as IKK α /NEMO complexes are readily formed (Rothwarf et al. 1998; Yamaoka et al. 1998; Mercurio et al. 1999). Competition experiments using the NBD peptide indicate that IKK β binds to NEMO with considerably higher affinity (May et al. 2002). Furthermore, IKK α is less sensitive to mutation of two residues within the NBD (Asp 749 and Trp 742) that abolish IKK β binding to NEMO, indicating that the binding of IKK α is less stringent. Although IKK α or IKK β produced in baculovirus or yeast systems is catalytically active, association with NEMO is required for *in vivo* I κ B phosphorylation. In fact, ex-

change of C-terminal portions of IKK α and IKK β , which contain the NBD and HLH domain, confers some increase in IKK β -like behavior on IKK α (Kwak et al. 2000). Taken together, these results indicate that the C-terminal portion of IKK, either through the interaction with NEMO or through inducing a conformational change, strongly affects kinase activity and specificity.

NEMO has been shown to form tetramers *in vitro*, and it appears that NEMO can be detected in several oligomeric states *in vivo* (Tegethoff et al. 2003). In contrast, it has been reported recently that NEMO multimerization is cell-type-specific, with NEMO existing as dimer, trimer, or both, but never as tetramer (Israel 2004). This discrepancy is likely attributable to differences in experimental systems, such as the use of different homobifunctional cross-linking agents. It has also been argued that NEMO exists as a monomer in resting cells and undergoes regulated trimerization—NEMO/IKK α /IKK β $\rightarrow$ NEMO₃/IKK α /IKK β (Israel 2004). Upon trimerization, the second coiled-coil domain (CC2, amino acids 248–291) and leucine zipper domain (LZ, amino acids 294–341) align in an antiparallel fashion with an overall structure similar to the HIV-1 gp41 ectodomain (Agou et al. 2004). When recombinant NEMO peptides encompassing the LZ and CC2 domains are mixed *in vitro*, they self-assemble into a pseudohexameric state. These results are consistent with previous reports that oligomerization of NEMO may occur through association of the C-terminal domain with RIP, and that forced oligomerization of NEMO activates IKK (Poyet et al. 2000). Indeed, peptide mimics of CC2 and the LZ domain have been found to inhibit NEMO oligomerization and NF- κ B activation without interfering with NEMO binding to the IKKs (Israel 2004).

Thus far, IKK α , IKK β , and NEMO are the only components that have been conclusively demonstrated to be a part of the IKK complex. The stoichiometry of these components in the complex continues to be debated, although many researchers have supported the idea of a heterodimer of IKK α and IKK β associating with NEMO (Rothwarf and Karin 1999; Miller and Zandi 2001). This debate about the composition of the complex has been driven by the discrepancy between the apparent molecular weight observed during gel filtration chromatography (700–900 kDa) and the predicted size based on the actual molecular weight of the components (~200–350 kDa). There are, however, certain difficulties in defining new components of the IKK complex with any certainty. One problem has been the assumption that there is only one IKK complex. Most studies have, therefore, not made efforts to distinguish whether all or only a subset of the IKK complexes contain a given constituent. Partial purification efforts have identified proteins that likely associate only with a small subset of IKK, if at all, and may do so in a tissue-specific manner. Therefore, the only definitive components of the complex remain IKK α , IKK β , and NEMO.

Perhaps not surprisingly, various members of the NF- κ B and I κ B family can associate with the IKK complex (Cohen et al. 1998; Heilker et al. 1999; Bouwmeester et

al. 2004). It has been shown more recently that the chaperone HSP-90/Cdc37 is constitutively associated with the IKK complex (G. Chen et al. 2002). A role for HSP-90 in IKK was supported by functional data generated using the HSP-90 inhibitor geldanamycin (GA), which inhibits activation of IKK by TNF- α . HSP-90, however, can associate with multiple other kinases that are involved in the NF- κ B pathway, including RIP, MEKK3, MEKK1, NIK, AKT, and TBK1 (Fisher et al. 2000; Sato et al. 2000; Goes and Martin 2001). It has also been speculated that through binding to NEMO, HSP-90 or HSP-70 may regulate NEMO's oligomeric state (Agou et al. 2002; G. Chen et al. 2002). Therefore, it is difficult to ascribe a specific role for HSP-90/Cdc37 as a bona fide component of the IKK complex. Instead, HSP-90 may function as a chaperone during assembly of the complex or during its regeneration following signaling.

ELKS is a 105-kDa regulatory protein that has recently been proposed to be an IKK-interacting protein (Sigala et al. 2004). ELKS was copurified with IKK β by immunoprecipitation, and has structural motifs reminiscent of NEMO with coiled-coil and leucine zipper domains. In unstimulated cells, ELKS appears to be a stoichiometric component of the IKK complex based on immunodepletion experiments. Knock-down of ELKS using RNAi blocks the early activation of NF- κ B following TNF α or IL-1 stimulation. However, it appears that the late phase of NF- κ B activation may be intact in these cells. Furthermore, it was shown that when ELKS is knocked down, the IKK complex fails to associate with I κ B α , suggesting that ELKS mediates interaction of the IKK complex with I κ B α but not I κ B β (Sigala et al. 2004). Therefore, it is tempting to speculate that ELKS provides the link between MEKK3, as discussed below, and assembly of the IKK/I κ B α complex necessary for activation of the early NF- κ B response. Surprisingly, whereas overexpression of full-length ELKS does not activate IKK, expression of an N-terminal deletion mutant of ELKS does. The biological significance of this regulatory component has not yet been verified genetically.

There are significant emerging data arguing against the existence of additional components for the IKK complex. Recombinant NEMO when mixed with either IKK α or IKK β can self-assemble into a complex of apparent molecular weight that is similar to the purified complex (Krappmann et al. 2000). Coproduction of NEMO with IKK α and IKK β in yeast also yields an appropriately sized complex (Miller and Zandi 2001). This may be because NEMO has a large Stoke's radius, which explains why NEMO trimers migrate with an apparent molecular mass of 550 kDa during gel filtration chromatography (Agou et al. 2004). Alternatively, this may simply reflect an IKK α / β /NEMO complex of higher-order stoichiometry—a dimer of trimers. An additional IKK complex, of an apparent molecular mass of ~300 kDa has been reported by multiple groups (Zandi et al. 1997; Yamaoka et al. 1998). This complex seems to consist of only IKK α and IKK β (Yamaoka et al. 1998; Zandi et al. 1998); however, the physiological role of this IKK complex remains to be determined.

Genetic analysis of the role of IKK subunits

Knockouts of each of the three IKK subunits have been generated. The IKK β knockout phenotype is similar to that observed for the p65 knockout, supporting an argument for a central role for IKK β in mediating NF- κ B signaling via TNF α (Q. Li et al. 1999b; Z.W. Li et al. 1999; Tanaka et al. 1999). Surprisingly, even *ikk β ^{+/-}* heterozygous animals have decreased NF- κ B activity (Z.W. Li et al. 1999). The similarity of their phenotypes and the ability to rescue both by knocking out TNFR1 demonstrate that IKK β is required for TNF α signaling to NF- κ B and the inhibition of TNF-induced apoptosis (Z.W. Li et al. 1999; Sentleben et al. 2001b). Of note, TNFR/IKK β double knockouts show a more pronounced defect in innate immune responses to bacterial infection, only surviving a few days after birth, whereas the p65 knockouts can survive for many months. These data indicate that there is no partial compensation for IKK β by IKK α or any other kinase.

The *ikk α ^{-/-}* mice are born live, but die perinatally from multiple morphological defects, particularly in epidermal and skeletal development (Hu et al. 1999; Q. Li et al. 1999a; Takeda et al. 1999). In contrast to the *ikk β ^{-/-}* mice, *ikk α ^{-/-}* mice do not exhibit liver apoptosis, and cells derived from these mice have only mildly reduced NF- κ B activation in response to TNF α . These initial characterizations indicated that IKK α had little role in NF- κ B signaling, and perhaps had some unrelated role in development, as knockout mice exhibit altered limb bud morphology at E12.5 (Q. Li et al. 1999a; Takeda et al. 1999). The epidermal layer of the skin in IKK α -deficient mice fails to differentiate properly, and they exhibit multiple skeletal abnormalities. Subsequent analysis has shown that these defects are NF- κ B-independent. The *ikk α ^{-/-}* mouse can be rescued by knocking in an IKK α in which both of the activation loop serines are mutated to alanines (IKK α AA; Cao et al. 2001; Hu et al. 2001), indicating that the role of IKK α in epidermal development is independent of its kinase activity. More recently, conditional expression of IKK α or kinase dead IKK α in basal keratinocytes has been shown to be sufficient to rescue the morphogenetic defects observed in the *IKK α ^{-/-}* mice (Sil et al. 2004). The mechanism of NF- κ B-independent control of keratinocyte differentiation by IKK α is unknown; however, it is dependent on the presence of a previously unrecognized NLS in IKK α (amino acids 232–240; Sil et al. 2004). IKK α / β double-knockout mice show further defects in NF- κ B signaling. The mice are characterized by failure of neurulation during development, and MEFs from double-knockout embryos do not respond to TNF α , IL-1, or LPS (Q. Li et al. 2000). Interestingly, the neurulation defect is not found in NEMO-deficient mice.

NEMO, like IKK β , is required for signaling through the classical NF- κ B pathway. Studies using deletion mutants of NEMO demonstrate that the C-terminal portion is required for stimulus-induced activation of IKK through interaction with upstream adapters, whereas binding to IKK α and IKK β occurs using sequences from

the N terminus (Rothwarf et al. 1998; Makris et al. 2002). NEMO-deficient mice die at E12.5–E13 from massive liver apoptosis (Rudolph et al. 2000), and MEFs from *NEMO*^{-/-} mice have a complete block in NF- κ B activation by TNF, LPS, or IL-1. Therefore, based on studies using knockouts of the different IKK subunits, it was concluded that only IKK β and NEMO participate in signaling to NF- κ B in response to proinflammatory cytokines. One notable observation from the characterization of the *NEMO*^{-/-} MEFs is a slight increase in the baseline IKK activity, although this activity is not inducible. Finally, the female *NEMO*^{+/-} mouse replicates the findings from human Incontinentia pigmenti patients, where female heterozygotes display a severe inflammatory skin disease that is probably the result of random X-inactivation, as NEMO is located on the X chromosome (Makris et al. 2000; Schmidt-Supprian et al. 2000).

Mechanism of IKK activation

Activation of the IKK complex is dependent on phosphorylation, and can be decreased by phosphatase treatment of IKK purified from activated cells (DiDonato et al. 1997). Most research has focused on the phosphorylation events on IKK β , as the importance of IKK α has been appreciated more recently. IKK β is phosphorylated on two sites within the activation loop of the kinase domain: Ser 177 and Ser 181 (Delhase et al. 1999). IKK β is also phosphorylated outside of the activation loop at multiple serines that are C-terminal of the HLH motif, most likely through autophosphorylation following activation. Deletion mutants that lack the C-terminal serine-rich segment are activated normally in response to TNF α but remain active for longer periods of time, indicating that autophosphorylation or *trans*-autophosphorylation of IKK β is a negative regulatory mechanism (Delhase et al. 1999).

Multiple upstream kinases have been suggested to act as IKK kinases (IKK-K). However, most such candidates failed to survive the test of gene knockouts. Such accumulation of negative results has led to the increasing belief that the IKK complex is primarily regulated through *trans*-autophosphorylation. Indeed, many of the proposed IKK-Ks have been found to play the role of adaptors instead of kinases. *Trans*-autophosphorylation caused by induced proximity and/or conformational change is an appealing mechanism of IKK activation and could be mediated through recruitment of the IKK complex by multimeric adapter/scaffolding complexes such as RIP or TRAF family members (Delhase et al. 1999; Inohara et al. 2000; Poyet et al. 2000; Tang et al. 2003). To date, however, these studies have relied on overexpression of IKK components that may have altered the stoichiometry of the IKK complex. Therefore, the role of IKK-K or autophosphorylation must be evaluated on a case-by-case basis.

The strongest evidence for an IKK-K is in the alternative pathway of NF- κ B signaling. NF- κ B inducing kinase (NIK) is a MAP3K family member that directly phosphorylates and activates IKK α (Regnier et al. 1997; X. Lin

et al. 1998; Ling et al. 1998; Uhlik et al. 1998) in response to activation of the alternative pathway through select members of the TNFR family including LT β R and BAFFR. Although NIK has also been implicated in the canonical signaling pathway, and does associate in complexes with IKK β and NEMO (Regnier et al. 1997; Woronicz et al. 1997; X. Lin et al. 1998; Bouwmeester et al. 2004), the phenotype of *NIK*^{-/-} and *aly/aly* mice, which are severely deficient in lymph node development because of a dominant negative point mutation in the NIK kinase domain, is consistent with NIK primarily functioning only in the alternative pathway (Shinkura et al. 1999). NIK-deficient mice fail to activate NF- κ B downstream from LT β R and BAFFR, whereas canonical signaling remains intact.

Data in support of the MAP3K member MEKK3 (MAPK/ERK kinase kinase) functioning as an IKK-K are relatively strong, but are incomplete. MEFs from *MEKK3*^{-/-} mice are unable to degrade I κ B α following TNF- α stimulation; however, the block in NF- κ B DNA binding is not complete (Yang et al. 2001). MEKK3 is able to phosphorylate IKK β in *in vitro* kinase assays, and can induce IKK activation in RIP-deficient cells (Yang et al. 2001). It has been suggested that the residual NF- κ B activity in these cells is due to MEKK1 activity, but there is very little evidence to support this hypothesis, and MEKK1-deficient cells have normal NF- κ B signaling (Xia et al. 2000; Yujiri et al. 2000). Furthermore, there is no *in vivo* evidence to support the hypothesis that the requirement for MEKK3 in TNF signaling is through direct phosphorylation of IKK by MEKK3. MEKK3 binds to the IKK complex following stimulation, and is required for the assembly of the active IKK complex with I κ B α /NF- κ B leading to the early phase of NF- κ B activation (Schmidt et al. 2003). However, it is not yet clear whether MEKK3 directly phosphorylates IKK following TNF stimulation or mediates docking of IKK with I κ B α /NF- κ B through phosphorylation of another substrate, such as ELKS, or by acting directly as a scaffold. In this scenario it has been proposed that MEKK3 mediates assembly of IKK/I κ B α complexes, whereas late stage signaling, which depends on I κ B β degradation, is mediated by MEKK2-dependent assembly of IKK/I κ B β complexes (Schmidt et al. 2003). This may represent another regulatory step through which active IKK complexes might be targeted to the appropriate I κ B/NF- κ B complexes.

Another MAP3K with a mysterious role in IKK activation is Tpl2 (also known as Cot, MAP3K8). Tpl2 is a serine/threonine kinase that when overexpressed can activate IKK, ERK, JNK, and p38 pathways. Expressed Tpl2 can also associate with IKK α and NIK (Lin et al. 1999). One report implicated Tpl2 in T-cell signaling to NF- κ B downstream of Akt, a serine/threonine kinase that has also been implicated in NF- κ B activation (Kane et al. 2002). Tpl2 has also been shown to interact with p105, and to induce p105 processing when overexpressed (Belich et al. 1999). More recently it has been shown that there exists a reciprocal relationship between Tpl2 and p105 such that p105 regulates Tpl2 activity and stability (Beinke et al. 2003; Waterfield et al. 2003). Overall, the

relationship between Tpl2 and upstream components of the NF- κ B signaling pathway remain unclear. Tpl2-deficient mice are resistant to the induction of shock by LPS administration that is independent of NF- κ B-induced TNF transcription (Dumitru et al. 2000) probably because of a failure to activate ERK1/2, resulting in a post-transcriptional block in TNF α production (Dumitru et al. 2000). Tpl2-deficient macrophages likewise fail to up-regulate COX-2 following LPS treatment because of a decrease in CREB activity downstream of ERK1/2, Msk1, and p90RSK (Eliopoulos et al. 2002). The induction of NF- κ B by LPS in Tpl2-deficient mice, however, is relatively normal. Therefore, it remains to be seen whether the interaction between Tpl2 and IKK α /NIK is of biological consequence, probably by examining other NF- κ B signaling pathways in Tpl2-deficient cells.

The atypical protein kinase C (aPKC) proteins PKC ζ and PCK λ have also been implicated as potential IKK-Ks (Lallena et al. 1999; Sanz et al. 2000). However, these data are complicated by the observed interactions of aPKCs with RIP and TRAFs, and in the case of PKC ζ , by evidence that this kinase directly phosphorylates p65 on Ser 311 (Duran et al. 2003, 2004). Surprisingly, the PKC ζ ^{-/-} mice demonstrate that although PKC ζ is not required for activation of IKK in MEFs, it is required in the lungs of the adult animal (Leitges et al. 2001). Therefore, it appears that the role of aPKCs in IKK activation is cell-type-specific, and requires further characterization.

Act-1 (CIKS) has been proposed to cause IKK activation (X. Li et al. 2000; Mauro et al. 2003). Act-1 was reported to interact with TRAF6/TRAF3 and NEMO, and consequently it may have a role in signaling from Toll/IL-1R and CD40 to the IKK complex (Kanamori et al. 2002; Qian et al. 2002). However, *Act1*^{-/-} mice have recently been made, and exhibit splenomegaly and lymphadenopathy caused by massive B-cell expansion, possibly from increased Bcl-XL levels (Li 2004). The B cells in these mice display increased NF- κ B and AP-1 responses following CD40 ligation, indicating the function of Act-1 is quite different from previously thought.

Stimulation of cells by dsRNA activates NF- κ B signaling through TLR-dependent and TLR-independent mechanisms. The dsRNA-responsive protein kinase PKR is induced by IFN γ and is activated by viral dsRNA, resulting in a blockade of protein synthesis through phosphorylation of eIF-2 α (for review, see D'Acquisto and Ghosh 2001). Activation of IKK and JNK are also an important component of the antiviral response through PKR (Kumar et al. 1994; Chu et al. 1999). Activation of NF- κ B by PKR is probably mediated through a direct interaction with IKK, most likely IKK β , but is independent of NEMO, and is independent of PKR kinase activity (Bonnet et al. 2000; Ishii et al. 2001). Therefore, like RIP and IRAK, PKR probably serves as a scaffold in this NF- κ B signaling pathway.

As discussed above, autophosphorylation of the C-terminal domain of the IKKs is likely to play an important role as part of a negative feedback loop. Degradation of I κ B may allow IKK to phosphorylate the C-terminal

serines due to decreased substrate competition. Phosphorylation of the C-terminal domain of IKK may recruit a phosphatase capable of dephosphorylating the activation-loop serines, such as protein phosphatase-2C β (Prajapati et al. 2004). Supporting evidence for such a model comes from experiments where treatment of cells with the phosphatase inhibitor okadaic acid leads to increased NF- κ B activity and I κ B degradation (DiDonato et al. 1997; Miskolci et al. 2003). However, it is difficult to determine unequivocally whether the phosphatases act directly on the phosphorylated IKKs or on other pathway components.

A widely studied negative regulator of NF- κ B is A20. A20 is an NF- κ B-regulated gene, containing a zinc finger and TRAF-binding domain, that is expressed rapidly following TNF α induction (Krikos et al. 1992; Laherty et al. 1992). A20 has been shown by multiple groups to inhibit TNF α induction of NF- κ B (Cooper et al. 1996; Jaattela et al. 1996; Song et al. 1996). It appears that A20 inhibits NF- κ B transcriptional activity independent of nuclear localization, and it is unclear if A20 affects the activity of IKK β or blocks I κ B degradation (Zetoune et al. 2001). Interestingly, A20 interacts with IKK α but not with IKK β , and thus may provide a means to negatively regulate NF- κ B activity downstream of I κ B degradation (Zetoune et al. 2001). A20-deficient mice exhibit an inflammatory phenotype and increased sensitivity to TNF and LPS, and cells from these mice show prolonged NF- κ B activation in response to TNF (Lee et al. 2000). A20 might also act indirectly by blocking IKK and thereby allowing newly synthesized I κ B α to accumulate, translocate to the nucleus, and remove NF- κ B bound to DNA (Song et al. 1996; Zhang et al. 2000). A20 has also been recently reported to be a deubiquitinating enzyme (DUB), although the significance of this function is unknown (Evans et al. 2004).

There is increasing interest in the role of nonphosphate modifications in the regulation of signaling pathway components (for review, see Lynch and Gadina 2004). In particular, recent studies have shown that multiple components of NF- κ B signaling pathways act as ubiquitin ligases, substrates, or both (Ben-Neriah 2002). There has been tremendous interest recently in the role of regulatory ubiquitination, particularly Lys 63-linked monoubiquitination, in signaling to NF- κ B. Members of the TRAF family have E3 ligase activity, and have been proposed to catalyze an autoubiquitination necessary for activation of IKK downstream of TNF signaling. Genetic evidence for the role of ubiquitin in TNF signaling comes from investigations of the cylindratomosis gene product, CYLD, which has been proposed to serve as a deubiquitinating enzyme for TRAF2 (Brummelkamp et al. 2003; Kovalenko et al. 2003; Trompouki et al. 2003). Hence, CYLD may negatively regulate NF- κ B activation by TNFR family members, although how ubiquitination of TRAF2 participates in activation/recruitment of the IKK complex is not clear. It is assumed that an ubiquitination event might be required for the aggregation of the IKK complex, but as yet, no clear mechanism to explain such a connection exists. The protein cIAP1 has

been shown to ubiquitinate NEMO, and perhaps the interaction between NEMO and cIAP1 is mediated by ubiquitinated TRAF2 (Tang et al. 2003). Ubiquitination by cIAP1 has been proposed to cause TRAF2 degradation, which is surprising because Lys 63 ubiquitination, which is mediated by Ubc13 and MMS2, is a regulatory event and is the target of CYLD DUB activity. It is therefore not yet clear whether the ubiquitination of NEMO directly leads to activation of the IKK complex. It is tempting to speculate that ubiquitination of NEMO might result in the formation of NEMO oligomers that can mediate IKK activation through conformational change and autophosphorylation.

TRAF6 ubiquitin ligase activity has recently been shown to be able to activate IKK in cell lysates (Sun et al. 2004). In the Toll/IL-1 signaling pathway, biochemical studies implicated the ubiquitin ligase activity of TRAF6 (Deng et al. 2000). K63-linked autoubiquitination of TRAF6 was shown to be required for association with and activation of TAK1. The TAK1 complex has previously been proposed to preassemble on IRAK family members, via an interaction between IRAK and TAB2 (Takaesu et al. 2000). More recently it has been argued that the Lys 63 polyubiquitin chain on TRAF6 is bound by the zinc finger domain of TAB2, resulting in activation of TAK1 (Chen 2004; Sun et al. 2004). It is not known if there is a DUB responsible for the negative regulation of this signaling pathway in a manner analogous to that observed for CYLD.

The small ubiquitin-like modifier SUMO-1 has also been suggested to play a role in NF- κ B signaling. Sumoilation of I κ B α occurs in multiple cell types and is not affected by stimulation (Desterro et al. 1998). I κ B α contains a target sequence for sumoilation between amino acids 19 and 25, and can be sumolated by Ubch9 in vitro, primarily on Lys 21. Overexpression of SUMO inhibits NF- κ B activation by multiple stimuli, indicating that SUMO modification serves to prevent ubiquitination of a subfraction of I κ B α . Regulation of SUMO levels may, therefore, dictate the amount of NF- κ B that can be activated. A more recent report (already discussed above) implicated sumoilation of NEMO in the response to genotoxic stress. Sumoilation was suggested to cause NEMO nuclear localization, and the nuclear NEMO was then subjected to ubiquitination. It is currently unknown, however, how genotoxic stress leads to this modification of NEMO.

Regulation of NF- κ B transcriptional activity

The activity of NF- κ B is also regulated through covalent modifications that alter the ability of the NF- κ B dimers to bind DNA, recruit coactivators to the enhancer region, and interact with I κ B. Stimulus-dependent phosphorylation of p65 was the first regulatory event that was recognized to occur downstream from I κ B degradation (Naumann and Scheidereit 1994; Neumann et al. 1995). Both p65 and c-Rel have a consensus protein kinase A (PKA) site at Ser 276, located ~25 residues N-terminal to their NLS, and overexpression of PKA leads

to increased DNA binding activity (Fig. 4A; Mosialos and Gilmore 1993; Neumann et al. 1995). The involvement of PKA in regulation of NF- κ B was further enhanced with the finding that the catalytic subunit of PKA exists in a complex with cytosolic NF- κ B:I κ B complexes (Zhong et al. 1997). Degradation of I κ B upon stimulation activates this associated PKAc, which then phosphorylates p65 on Ser 276. However, it has also been reported that in certain cells the cytosolic p65 is already phosphorylated on Ser 276 before stimulation, suggesting that phosphorylation might occur concomitant with the assembly of the cytosolic NF- κ B-I κ B-PKAc complex (Anrather et al. 1999). The role of this phosphorylation

Figure 4. A model for how phosphorylation of p65 regulates its transcriptional activity through recruitment of CBP/p300. (A) The different phosphorylation sites on p65, and the protein kinases implicated in phosphorylating those sites. The domain within the C-terminal TAD of p65 that interacts with CBP/p300 (amino acids 477–504) is shown. (B) In unstimulated cells, both the phosphorylation-dependent site of interaction (Ser 276) and the TAD are masked by an intramolecular interaction between the N- and C-terminal domains of p65. This interaction also blocks the DNA-binding capability of p65. Upon phosphorylation, probably at any of the proposed sites, this intramolecular interaction is weakened, thereby making the CBP/p300 domains accessible for interaction. Involvement of GSK3 β and TBK1 has been deduced from knockout studies, but it is unclear whether they directly phosphorylate p65.

appears to be twofold. First, the phosphorylation is necessary for enhancing the efficiency of DNA binding by p65, and second, it provides an additional interaction site for the transcriptional coactivator CBP/p300 (Fig. 4B). In unphosphorylated p65, the C-terminal region of the protein interacts with the Rel-homology domain, thereby interfering with DNA binding as well as association with CBP/p300. Phosphorylation of Ser 276 prevents this intramolecular association between the N- and C-terminal domains of p65 and therefore facilitates both DNA binding and transcriptional activity of NF- κ B (Zhong et al. 1998, 2002). Recently the protein kinases MSK1 and MSK2 (mitogen and stress activated protein kinase), which possess substrate specificity identical to PKA, were also shown to phosphorylate Ser 276 and stimulate NF- κ B transcriptional activity (Vermeulen et al. 2003). Because MSK1/2 and PKA have identical substrate specificity, the only difference appears to be in their subcellular localization. MSK1/2 are nuclear, whereas the NF- κ B:I κ B-associated PKAc is believed to be in the cytosol. Because the *MSK1*^{-/-} *MSK2*^{-/-} MEFs seem to demonstrate significantly diminished transcriptional activity in response to TNF, it appears that the MSK kinases contribute to the phosphorylation of Ser 276, although how these nuclear kinases synergize with cytosolic PKA in phosphorylating nuclear NF- κ B remains to be clarified (Vermeulen et al. 2003).

Additional p65 phosphorylation events have been described, although their significance remains unclear. IKK is one of the many kinases implicated in the direct phosphorylation of p65 at Ser 536 (Sakurai et al. 2003). A recent study showed that IKK- α was responsible for phosphorylation of p65 at Ser 536, and that this event was required for transcriptional activation following introduction of the HTLV Tax gene into MEFs (O'Mahony et al. 2004). A role for IKK α in regulating the transcriptional activity of NF- κ B by phosphorylating p65 was also suggested in a previous study that examined activation of NF- κ B in response to PI-3K/Akt pathway, although the actual sites for phosphorylation were not tested (Sizemore et al. 2002). Surprisingly, another recent study demonstrated that IKK β , but not IKK α , was responsible for phosphorylating p65 at this same site following LPS stimulation (Yang et al. 2003). This confusion also extends to potential phosphorylation at another serine residue at position 529. It has been reported that this site is phosphorylated by CK2 following IL-1 or TNF- α stimulation (Bird et al. 1997; Wang and Baldwin 1998; Wang et al. 2000), although there is less evidence that it regulates the NF- κ B transcriptional response (Yang et al. 2003). It is even less clear whether such phosphorylation events can be generalized to other members of the Rel family. Indeed, it is likely that each member of the Rel family of proteins has distinct requirements for modification that affect transcriptional responses, adding more complexity and specificity to post-I κ B regulation of NF- κ B pathways.

TNF α signaling to NF- κ B also regulates the transcriptional activity of NF- κ B through direct modification of p65. Akt and members of the atypical protein kinase C (PKC) family have been implicated in this process, as

have the IKKs. RIP1 has been proposed to link TNFR1 to PKC ζ via p62 (Sanz et al. 1999, 2000), and multiple studies have reported the ability of PKC ζ to phosphorylate p65 at Ser 311 (Anrather et al. 1999; Leitges et al. 2001). Furthermore, NF- κ B DNA binding is drastically decreased despite normal IKK activation in *PKC ζ* ^{-/-} MEFs. The link between PKC ζ and p65 phosphorylation requires further characterization.

Besides the protein kinases that are capable of phosphorylating specific sites on p65 such as PKA, PKC ζ , CK2, and IKK α / β , two other kinases have been implicated in NF- κ B regulation through genetic studies, although it is unknown whether they directly phosphorylate p65. The kinases, TBK1 (T2K, NAK), and GSK3 β were first suspected of being involved in NF- κ B activation because their knockout led to embryonic lethality at approximately E15 from massive hepatocyte apoptosis. Subsequent analysis with knockout MEFs showed that although degradation of I κ B and nuclear translocation and DNA binding by NF- κ B was normal in these cells, they were dramatically deficient in NF- κ B-dependent transcriptional activity (Bonnard et al. 2000; Hoefflich et al. 2000). Similar results were obtained using LiCl as a GSK3 β inhibitor in wild-type hepatocytes (Schwabe and Brenner 2002). However, a recent report was unable to reproduce the defect in transcription using TBK1 knockout MEFs, and therefore it is unclear what role, if any, TBK1 plays in regulating the transcriptional activity of NF- κ B (McWhirter et al. 2004).

Recently an additional phosphorylation event on Thr 254 of p65 has been suggested to play an important role in regulation of nuclear NF- κ B function (Ryo et al. 2003). This phosphorylation event is reported to be the target of postphosphorylation modification by the peptidyl-prolyl isomerase PIN-1 (Ryo et al. 2003). The authors posit that PIN-1 modifies p65 down-regulation by preventing binding of SOCS-1 (suppressor of cytokine signaling 1), and subsequent SOCS-1-mediated ubiquitination and proteasomal degradation of p65. However, as discussed by others, mice deficient in either Pin-1 or SOCS-1 do not exhibit phenotypes that would be consistent with such a prominent role in NF- κ B signaling (Ben-Neriah 2003). Yet another nuclear p65 interaction that merits further study is peroxisome proliferator-activated receptor- γ (PPAR γ), a nuclear hormone receptor and transcription factor. PPAR γ is reported to negatively regulate NF- κ B signaling at the level of p65 as well as upstream of I κ B, a role that is supported by increased NF- κ B activity in *PPAR*^{+/-} heterozygous mice (Setoguchi et al. 2001; Kelly et al. 2004). PPAR γ and NF- κ B are proposed to inhibit each other in a manner that results in export of both to the cytoplasm (Kelly et al. 2004).

It is probably fair to say that it is difficult to reconcile all the published data concerning the different sites of phosphorylation, and the different protein kinases, into a unified model. Interestingly, among the four proposed sites of phosphorylation, two are in the dimerization domain of the Rel-homology domain (Ser 276 and Ser 311), whereas the remaining two sites are in the C-terminal, TAD region (Ser 529 and Ser 536). It is possible that

phosphorylation on at least one site among either group is necessary for transactivation. As reported earlier, phosphorylation at Ser 276 was shown to decrease the intramolecular interaction between the C-terminal and the N-terminal domains of p65 (Fig. 4B). If this interaction is regulated through phosphorylation, then phosphorylation at any of the sites would contribute to the unfolding of the C terminus of p65 from the N terminus, thereby unmasking the domains that interact with CBP/p300. Hence, phosphorylation at all four sites would probably be most efficient in promoting transactivation. However, this model appears inconsistent with published studies where reconstitution of p65-deficient MEFs with single-site mutants at positions 276, 311, 529, and 536 all abolish transcriptional activity. Also depending on the laboratory, cells lacking MSK1/2, PKC ζ , and IKK α (cells lacking PKA and CK2 are unavailable because of early embryonic lethality) all appear to contribute equally to the transcriptional activation of NF- κ B (Leitges et al. 2001; Sizemore et al. 2002; Vermeulen et al. 2003). Clearly, further analysis will be required to resolve which phosphorylation sites are critical for NF- κ B activity, and if multiple phosphorylation events all contribute to the final outcome, then it will be important to understand how the cell sorts out the contribution of the different phosphorylations. One concern that remains about techniques in which knockout MEFs are reconstituted with mutants is that the site of integration of the expression vector or retrovirus into the genome might affect some regulatory gene that contributes to NF- κ B activity. Therefore, the best way to address such concerns would be to introduce mutant forms of p65 into the genome using a "knock-in strategy," although it is unclear what would be the interpretation if such approaches also showed equivalent importance for all the proposed phosphorylation sites.

The role of direct acetylation of p65 in the regulation of transcriptional activity is even more controversial. Acetylation of p65 by CBP/p300 was initially reported to prolong NF- κ B DNA binding by preventing the interaction with I κ B (Chen et al. 2001). This model posits that p65 must then be deacetylated by HDAC-3, which directly interacts with p65, before being bound by I κ B and exported to the cytosol. However, a subsequent report suggested that acetylation of p65 by both CBP and p300/CBP-associated factor (PCAF) on Lys 122/123 did not change the affinity of p65 for I κ B α (Kiernan et al. 2003). Finally, it has also been suggested that acetylation at Lys 221 increases affinity of p65 for the κ B site and inhibits I κ B binding, whereas acetylation of Lys 310 enhances transcriptional activity without altering binding to DNA or I κ B (L.F. Chen et al. 2002). Although these data provide an intriguing twist to p65 regulation, which is consistent with mechanisms that regulate the activity of other transcription factors such as p53 (Gu and Roeder 1997), the physiological relevance of these findings in NF- κ B remains to be established using targeted genetic approaches.

NF- κ B-independent regulation of transcription by components of the NF- κ B signaling pathway is also

emerging as a novel regulatory step in NF- κ B activation. Nuclear IKK α was recently shown to mediate the phosphorylation of histone H3. Chromatin immunoprecipitation (ChIP) experiments clearly show accumulation of IKK α , and perhaps IKK β , at the promoter sites of NF- κ B-responsive genes (Anest et al. 2003; Y. Yamamoto et al. 2003). Furthermore, in the absence of IKK- α , gene transcription diminishes despite normal recruitment of p65 to the κ B enhancer. Analysis of the histone status indicates that induced phosphorylation of H3 does not occur in the absence of IKK α but is maintained without IKK β . It remains unclear whether IKK α catalyzes histone phosphorylation directly or through a downstream kinase and what the biological relevance of this finding is, given that classical NF- κ B activity remains largely intact in IKK α ^{-/-} cells.

Perspective and summary

The progress in understanding the regulatory mechanisms that operate in the NF- κ B signaling pathway, and understanding how NF- κ B functions in normal biology and disease, has been tremendous. A search of PubMed reveals nearly 15,000 papers reporting on some facet of NF- κ B, clearly a reflection of the pleiotropic nature of this transcription factor that makes it the subject of inquiry for many different laboratories with diverse interests. Although we continue to gain insight from parallel research efforts in different systems, it is fair to say that there is much that remains to be learned. Nevertheless, certain themes are clearly emerging. That IKK activation occurs through oligomerization, either of the IKK complex itself or upstream scaffolds, that ubiquitination plays a role in the activation of IKK, and that phosphorylation of p65 plays an important role are ideas that continue to gain support. However, the mechanistic details are lacking in many of these areas. One significant hurdle continues to be the lack of structural information about the IKKs, although progress continues to be made in illuminating the functional significance of structural motifs of the IKK components.

Another area of rapid progress has been in understanding the relationship between NF- κ B and the pathophysiology of various diseases. Abnormal NF- κ B signaling is the subject of particularly intensive research focusing on cancer, inflammatory diseases, and neurodegenerative disorders. However, it remains to be seen whether specific inhibition of only NF- κ B will be sufficient for treatment of these diseases or will help increase the efficacy of current treatments as an adjuvant therapeutic. Either way, the promise of NF- κ B as a therapeutic target can only be fulfilled through a better understanding of the mechanistic differences that regulate specific NF- κ B signaling pathways, and we are therefore likely to see continued progress at the same pace in the next few years.

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References

- Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., and Akira, S. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* **9**: 143–150.
- Aggarwal, B.B. 2003. Signalling pathways of the TNF superfamily: A double-edged sword. *Nat. Rev. Immunol.* **3**: 745–756.
- Agou, F., Ye, F., Goffinont, S., Courtois, G., Yamaoka, S., Israel, A., and Veron, M. 2002. NEMO trimerizes through its coiled-coil C-terminal domain. *J. Biol. Chem.* **277**: 17464–17475.
- Agou, F., Traincard, F., Vinolo, E., Courtois, G., Yamaoka, S., Israel, A., and Veron, M. 2004. The trimerization domain of NEMO is comprised of the interacting C-terminal CC2 and LZ coiled-coil subdomains. *J. Biol. Chem.* **279**: 27861–27869.
- Akira, S. 2004. Toll-like receptor signaling. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposia, Snowbird Resort, Snowbird, UT.
- Alcama, E., Mizgerd, J.P., Horwitz, B.H., Bronson, R., Beg, A.A., Scott, M., Doerschuk, C.M., Hynes, R.O., and Baltimore, D. 2001. Targeted mutation of TNF receptor I rescues the RelA-deficient mouse and reveals a critical role for NF- κ B in leukocyte recruitment. *J. Immunol.* **167**: 1592–1600.
- Alkalay, I., Yaron, A., Hatzubai, A., Orian, A., Ciechanover, A., and Ben-Neriah, Y. 1995. Stimulation-dependent I κ B α phosphorylation marks the NF- κ B inhibitor for degradation via the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci.* **92**: 10599–10603.
- Amir, R.E., Iwai, K., and Ciechanover, A. 2002. The NEDD8 pathway is essential for SCF(β -TrCP)-mediated ubiquitination and processing of the NF- κ B precursor p105. *J. Biol. Chem.* **277**: 23253–23259.
- Amir, R.E., Haecker, H., Karin, M., and Ciechanover, A. 2004. Mechanism of processing of the NF- κ B2 p100 precursor: Identification of the specific polyubiquitin chain-anchoring lysine residue and analysis of the role of NEDD8-modification on the SCF(β -TrCP) ubiquitin ligase. *Oncogene* **23**: 2540–2547.
- Anest, V., Hanson, J.L., Cogswell, P.C., Steinbrecher, K.A., Strahl, B.D., and Baldwin, A.S. 2003. A nucleosomal function for I κ B kinase- α in NF- κ B-dependent gene expression. *Nature* **423**: 659–663.
- Anrather, J., Csizmadia, V., Soares, M.P., and Winkler, H. 1999. Regulation of NF- κ B RelA phosphorylation and transcriptional activity by p21^{ras} and protein kinase C ζ in primary endothelial cells. *J. Biol. Chem.* **274**: 13594–13603.
- Ashikawa, K., Shishodia, S., Fokt, I., Priebe, W., and Aggarwal, B.B. 2004. Evidence that activation of nuclear factor- κ B is essential for the cytotoxic effects of doxorubicin and its analogues. *Biochem. Pharmacol.* **67**: 353–364.
- Barroga, C.F., Stevenson, J.K., Schwarz, E.M., and Verma, I.M. 1995. Constitutive phosphorylation of I κ B α by casein kinase II. *Proc. Natl. Acad. Sci.* **92**: 7637–7641.
- Barton, G.M. and Medzhitov, R. 2003. Toll-like receptor signaling pathways. *Science* **300**: 1524–1525.
- Beg, A.A., Sha, W.C., Bronson, R.T., and Baltimore, D. 1995a. Constitutive NF- κ B activation, enhanced granulopoiesis, and neonatal lethality in I κ B α -deficient mice. *Genes & Dev.* **9**: 2736–2746.
- Beg, A.A., Sha, W.C., Bronson, R.T., Ghosh, S., and Baltimore, D. 1995b. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF- κ B. *Nature* **376**: 167–170.
- Beinke, S., Deka, J., Lang, V., Belich, M.P., Walker, P.A., Howell, S., Smerdon, S.J., Gamblin, S.J., and Ley, S.C. 2003. NF- κ B1 p105 negatively regulates TPL-2 MEK kinase activity. *Mol. Cell. Biol.* **23**: 4739–4752.
- Belich, M.P., Salmeron, A., Johnston, L.H., and Ley, S.C. 1999. TPL-2 kinase regulates the proteolysis of the NF- κ B-inhibitory protein NF- κ B1 p105. *Nature* **397**: 363–368.
- Ben-Neriah, Y. 2002. Regulatory functions of ubiquitination in the immune system. *Nat. Immunol.* **3**: 20–26.
- . 2003. Pinning NF- κ B to the nucleus. *Mol. Cell* **12**: 1344–1345.
- Ben-Neriah, Y. and Schmitz, M.L. 2004. Of mice and men. *EMBO Rep.* **5**: 668–673.
- Bertin, J., Wang, L., Guo, Y., Jacobson, M.D., Poyet, J.L., Srinivasula, S.M., Merriam, S., DiStefano, P.S., and Alnemri, E.S. 2001. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF- κ B. *J. Biol. Chem.* **276**: 11877–11882.
- Bird, T.A., Schooley, K., Dower, S.K., Hagen, H., and Virca, G.D. 1997. Activation of nuclear transcription factor NF- κ B by interleukin-1 is accompanied by casein kinase II-mediated phosphorylation of the p65 subunit. *J. Biol. Chem.* **272**: 32606–32612.
- Bonizzi, G. and Karin, M. 2004. The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **25**: 280–288.
- Bonnard, M., Mirtsos, C., Suzuki, S., Graham, K., Huang, J., Ng, M., Itie, A., Wakeham, A., Shahinian, A., Henzel, W.J., et al. 2000. Deficiency of T2K leads to apoptotic liver degeneration and impaired NF- κ B-dependent gene transcription. *EMBO J.* **19**: 4976–4985.
- Bonnet, M.C., Weil, R., Dam, E., Hovanessian, A.G., and Meurs, E.F. 2000. PKR stimulates NF- κ B irrespective of its kinase function by interacting with the I κ B kinase complex. *Mol. Cell. Biol.* **20**: 4532–4542.
- Bours, V., Franzoso, G., Azarenko, V., Park, S., Kanno, T., Brown, K., and Siebenlist, U. 1993. The oncoprotein Bcl-3 directly transactivates through κ B motifs via association with DNA-binding p50B homodimers. *Cell* **72**: 729–739.
- Bouwmeester, T., Bauch, A., Ruffner, H., Angrand, P.O., Bergamini, G., Croughton, K., Cruciat, C., Eberhard, D., Gagneur, J., Ghidelli, S., et al. 2004. A physical and functional map of the human TNF- α /NF- κ B signal transduction pathway. *Nat. Cell Biol.* **6**: 97–105.
- Brach, M.A., Hass, R., Sherman, M.L., Gunji, H., Weichselbaum, R., and Kufe, D. 1991. Ionizing radiation induces expression and binding activity of the nuclear factor κ B. *J. Clin. Invest.* **88**: 691–695.
- Bradley, J.R. and Pober, J.S. 2001. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* **20**: 6482–6491.
- Brasier, A.R., Lu, M., Hai, T., Lu, Y., and Boldogh, I. 2001. NF- κ B-inducible BCL-3 expression is an autoregulatory loop controlling nuclear p50/NF- κ B1 residence. *J. Biol. Chem.* **276**: 32080–32093.
- Brummelkamp, T.R., Nijman, S.M., Dirac, A.M., and Bernards, R. 2003. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF- κ B. *Nature* **424**: 797–801.
- Bulut, Y., Faure, E., Thomas, L., Equils, O., and Arditi, M. 2001. Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and *Borrelia burgdorferi*

- outer surface protein A lipoprotein: Role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. *J. Immunol.* **167**: 987–994.
- Burkly, L., Hession, C., Ogata, L., Reilly, C., Marconi, L.A., Olson, D., Tizard, R., Cate, R., and Lo, D. 1995. Expression of relB is required for the development of thymic medulla and dendritic cells. *Nature* **373**: 531–536.
- Burns, K., Clatworthy, J., Martin, L., Martinon, F., Plumpton, C., Maschera, B., Lewis, A., Ray, K., Tschopp, J., and Volpe, F. 2000. Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nat. Cell Biol.* **2**: 346–351.
- Burns, K., Janssens, S., Brissoni, B., Olivos, N., Beyaert, R., and Tschopp, J. 2003. Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J. Exp. Med.* **197**: 263–268.
- Caamano, J.H., Perez, P., Lira, S.A., and Bravo, R. 1996. Constitutive expression of Bcl-3 in thymocytes increases the DNA binding of NF- κ B (p50) homodimers in vivo. *Mol. Cell. Biol.* **16**: 1342–1348.
- Caamano, J.H., Rizzo, C.A., Durham, S.K., Barton, D.S., Rantos-Suarez, C., Snapper, C.M., and Bravo, R. 1998. Nuclear factor (NF)- κ B2 (p100/p52) is required for normal splenic microarchitecture and B cell-mediated immune responses. *J. Exp. Med.* **187**: 185–196.
- Campbell, K.J., Rocha, S., and Perkins, N.D. 2004. Active repression of antiapoptotic gene expression by RelA(p65) NF- κ B. *Mol. Cell Biol.* **24**: 853–865.
- Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., and Goeddel, D.V. 1996. TRAF6 is a signal transducer for interleukin-1. *Nature* **383**: 443–446.
- Cao, Y., Bonizzi, G., Seagroves, T.N., Greten, F.R., Johnson, R., Schmidt, E.V., and Karin, M. 2001. IKK α provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. *Cell* **107**: 763–775.
- Carrasco, D., Cheng, J., Lewin, A., Warr, G., Yang, H., Rizzo, C., Rosas, F., Snapper, C., and Bravo, R. 1998. Multiple hematopoietic defects and lymphoid hyperplasia in mice lacking the transcriptional activation domain of the c-Rel protein. *J. Exp. Med.* **187**: 973–984.
- Chen, Z.J. 2004. Ubiquitin signaling in the NF- κ B pathway. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposia, Snowbird Resort, Snowbird, UT.
- Chen, L.F. and Greene, W.C. 2004. Shaping the nuclear action of NF- κ B. *Nat. Rev. Mol. Cell Biol.* **5**: 392–401.
- Chen, Z.J., Parent, L., and Maniatis, T. 1996. Site-specific phosphorylation of I κ B α by a novel ubiquitination-dependent protein kinase activity. *Cell* **84**: 853–862.
- Chen, F.E., Huang, D.B., Chen, Y.Q., and Ghosh, G. 1998. Crystal structure of p50/p65 heterodimer of transcription factor NF- κ B bound to DNA. *Nature* **391**: 410–413.
- Chen, Y.Q., Ghosh, S., and Ghosh, G. 1998. A novel DNA recognition mode by the NF- κ B p65 homodimer. *Nat. Struct. Biol.* **5**: 67–73.
- Chen, L., Fischle, W., Verdin, E., and Greene, W.C. 2001. Duration of nuclear NF- κ B action regulated by reversible acetylation. *Science* **293**: 1653–1657.
- Chen, G., Cao, P., and Goeddel, D.V. 2002. TNF-induced recruitment and activation of the IKK complex require Cdc37 and Hsp90. *Mol. Cell* **9**: 401–410.
- Chen, L.F., Mu, Y., and Greene, W.C. 2002. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF- κ B. *EMBO J.* **21**: 6539–6548.
- Cheng, J.D., Ryseck, R.P., Attar, R.M., Dambach, D., and Bravo, R. 1998. Functional redundancy of the nuclear factor κ B inhibitors I κ B α and I κ B β . *J. Exp. Med.* **188**: 1055–1062.
- Chu, W.M., Ostertag, D., Li, Z.W., Chang, L., Chen, Y., Hu, Y., Williams, B., Perrault, J., and Karin, M. 1999. JNK2 and IKK β are required for activating the innate response to viral infection. *Immunity* **11**: 721–731.
- Chung, J.Y., Park, Y.C., Ye, H., and Wu, H. 2002. All TRAFs are not created equal: Common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J. Cell Sci.* **115**: 679–688.
- Claudio E., Brown, K., Park, S., Wang, H., and Siebenlist, U. 2002. BAFF-induced NEMO-independent processing of NF- κ B2 in maturing B cells. *Nat. Immunol.* **3**: 958–965.
- Cohen, L., Henzel, W.J., and Baeuerle, P.A. 1998. IKAP is a scaffold protein of the I κ B kinase complex. *Nature* **395**: 292–296.
- Cohen, S., Orian, A., and Ciechanover, A. 2001. Processing of p105 is inhibited by docking of p50 active subunits to the ankyrin repeat domain, and inhibition is alleviated by signaling via the carboxyl-terminal phosphorylation/ubiquitin-ligase binding domain. *J. Biol. Chem.* **276**: 26769–26776.
- Cohen, S., Achbert-Weiner, H., and Ciechanover, A. 2004. Dual effects of I κ B kinase β -mediated phosphorylation on p105 Fate: SCF(β -TrCP)-dependent degradation and SCF(β -TrCP)-independent processing. *Mol. Cell Biol.* **24**: 475–486.
- Connelly, M.A. and Marcu, K.B. 1995. CHUK, a new member of the helix-loop-helix and leucine zipper families of interacting proteins, contains a serine-threonine kinase catalytic domain. *Cell Mol. Biol. Res.* **41**: 537–549.
- Cooper, J.T., Stroka, D.M., Brostjan, C., Palmetshofer, A., Bach, F.H., and Ferran, C. 1996. A20 blocks endothelial cell activation through a NF- κ B-dependent mechanism. *J. Biol. Chem.* **271**: 18068–18073.
- D'Acquisto, F. and Ghosh, S. 2001. PACT and PKR: Turning on NF- κ B in the absence of virus. *Sci. STKE* **2001**: RE1.
- Davis, M., Hatzubai, A., Andersen, J.S., Ben-Shushan, E., Fisher, G.Z., Yaron, A., Bauskin, A., Mercurio, F., Mann, M., and Ben-Neriah, Y. 2002. Pseudosubstrate regulation of the SCF(β -TrCP) ubiquitin ligase by hnRNP-U. *Genes & Dev.* **16**: 439–451.
- Dejardin, E., Droin, N.M., Delhase, M., Haas, E., Cao, Y., Makris, C., Li, Z.W., Karin, M., Ware, C.F., and Green, D.R. 2002. The lymphotoxin- β receptor induces different patterns of gene expression via two NF- κ B pathways. *Immunity* **17**: 525–535.
- Delhase, M. and Karin, M. 1999. The I κ B kinase: A master regulator of NF- κ B, innate immunity, and epidermal differentiation. *Cold Spring Harb. Symp. Quant. Biol.* **64**: 491–503.
- Delhase, M., Hayakawa, M., Chen, Y., and Karin, M. 1999. Positive and negative regulation of I κ B kinase activity through IKK β subunit phosphorylation. *Science* **284**: 309–313.
- Dempsey, P.W., Doyle, S.E., He, J.Q., and Cheng, G. 2003. The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev.* **14**: 193–209.
- Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., and Chen, Z.J. 2000. Activation of the I κ B kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* **103**: 351–361.
- De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D.U., Jin, R., Jones, J., Cong, R., and Franzoso, G. 2001. Induction of gadd45 β by NF- κ B downregulates pro-apoptotic JNK signaling. *Nature* **414**: 308–313.
- Destero, J.M., Rodriguez, M.S., and Hay, R.T. 1998. SUMO-1 modification of I κ B α inhibits NF- κ B activation. *Mol. Cell* **2**: 233–239.

- Devary, Y., Rosette, C., DiDonato, J.A., and Karin, M. 1993. NF- κ B activation by ultraviolet light not dependent on a nuclear signal. *Science* **261**: 1442–1445.
- Devin, A., Cook, A., Lin, Y., Rodriguez, Y., Kelliher, M., and Liu, Z. 2000. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* **12**: 419–429.
- Devin, A., Lin, Y., Yamaoka, S., Li, Z., Karin, M., and Liu, Z. 2001. The α and β subunits of I κ B kinase (IKK) mediate TRAF2-dependent IKK recruitment to tumor necrosis factor (TNF) receptor 1 in response to TNF. *Mol. Cell. Biol.* **21**: 3986–3994.
- DiDonato, J., Mercurio, F., Rosette, C., Wu-Li, J., Suyang, H., Ghosh, S., and Karin, M. 1996. Mapping of the inducible I κ B phosphorylation sites that signal its ubiquitination and degradation. *Mol. Cell. Biol.* **16**: 1295–1304.
- DiDonato, J.A., Hayakawa, M., Rothwarf, D.M., Zandi, E., and Karin, M. 1997. A cytokine-responsive I κ B kinase that activates the transcription factor NF- κ B. *Nature* **388**: 548–554.
- Dobrzanski, P., Ryseck, R.P., and Bravo, R. 1993. Both N- and C-terminal domains of RelB are required for full transactivation: Role of the N-terminal leucine zipper-like motif. *Mol. Cell. Biol.* **13**: 1572–1582.
- . 1994. Differential interactions of Rel-NF- κ B complexes with I κ B α determine pools of constitutive and inducible NF- κ B activity. *EMBO J.* **13**: 4608–4616.
- Doi, T.S., Takahashi, T., Taguchi, O., Azuma, T., and Obata, Y. 1997. NF- κ B RelA-deficient lymphocytes: Normal development of T cells and B cells, impaired production of IgA and IgG1 and reduced proliferative responses. *J. Exp. Med.* **185**: 953–961.
- Doi, T.S., Marino, M.W., Takahashi, T., Yoshida, T., Sakakura, T., Old, L.J., and Obata, Y. 1999. Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. *Proc. Natl. Acad. Sci.* **96**: 2994–2999.
- Dumitru, C.D., Ceci, J.D., Tsatsanis, C., Kontoyiannis, D., Stamatidis, K., Lin, J.H., Patriotis, C., Jenkins, N.A., Copeland, N.G., Kollias, G., et al. 2000. TNF- α induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. *Cell* **103**: 1071–1083.
- Dunne, A. and O'Neill, L.A. 2003. The interleukin-1 receptor/Toll-like receptor superfamily: Signal transduction during inflammation and host defense. *Sci. STKE* **2003**: re3.
- Duran, A., Diaz-Meco, M.T., and Moscat, J. 2003. Essential role of RelA Ser311 phosphorylation by zetaPKC in NF- κ B transcriptional activation. *EMBO J.* **22**: 3910–3918.
- Duran, A., Serrano, M., Leitges, M., Flores, J.M., Picard, S., Brown, J.P., Moscat, J., and Diaz-Meco, M.T. 2004. The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. *Dev. Cell* **6**: 303–309.
- Eliopoulos, A.G., Dumitru, C.D., Wang, C.C., Cho, J., and Tschlis, P.N. 2002. Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals. *EMBO J.* **21**: 4831–4840.
- Endres, R., Hacker, G., Brosch, I., and Pfeffer, K. 2003. Apparently normal tumor necrosis factor receptor 1 signaling in the absence of the silencer of death domains. *Mol. Cell. Biol.* **23**: 6609–6617.
- Eto, A., Muta, T., Yamazaki, S., and Takeshige, K. 2003. Essential roles for NF- κ B and a Toll/IL-1 receptor domain-specific signal(s) in the induction of I κ B- ζ . *Biochem. Biophys. Res. Commun.* **301**: 495–501.
- Evans, P.C., Ovaa, H., Hamon, M., Kilshaw, P.J., Hamm, S., Bauer, S., Ploegh, H.L., and Smith, T.S. 2004. Zinc-finger protein A20, a regulator of inflammation and cell survival, has de-ubiquitinating activity. *Biochem. J.* **378**: 727–734.
- Fan, C.M. and Maniatis, T. 1991. Generation of p50 subunit of NF- κ B by processing of p105 through an ATP-dependent pathway. *Nature* **354**: 395–398.
- Fisher, D.L., Mandart, E., and Doree, M. 2000. Hsp90 is required for c-Mos activation and biphasic MAP kinase activation in *Xenopus* oocytes. *EMBO J.* **19**: 1516–1524.
- Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., Caffrey, D.R., Visintin, A., Latz, E., Monks, B., Pitha, P.M., and Golenbock, D.T. 2003. LPS-TLR4 signaling to IRF-3/7 and NF- κ B involves the toll adapters TRAM and TRIF. *J. Exp. Med.* **198**: 1043–1055.
- Fong, A. and Sun, S.C. 2002. Genetic evidence for the essential role of β -transducin repeat-containing protein in the inducible processing of NF- κ B2/p100. *J. Biol. Chem.* **277**: 22111–22114.
- Franzoso, G., Bours, V., Azarenko, V., Park, S., Tomita-Yamaguchi, M., Kanno, T., Brown, K., and Siebenlist, U. 1993. The oncoprotein Bcl-3 can facilitate NF- κ B-mediated transactivation by removing inhibiting p50 homodimers from select κ B sites. *EMBO J.* **12**: 3893–3901.
- Franzoso, G., Carlson, L., Scharton-Kersten, T., Shores, E.W., Epstein, S., Grinberg, A., Tran, T., Shacter, E., Leonardi, A., Anver, M., et al. 1997. Critical roles for the Bcl-3 oncoprotein in T cell-mediated immunity, splenic microarchitecture, and germinal center reactions. *Immunity* **6**: 479–490.
- Franzoso, G., Carlson, L., Poljak, L., Shores, E.W., Epstein, S., Leonardi, A., Grinberg, A., Tran, T., Scharton-Kersten, T., Anver, M., et al. 1998. Mice deficient in nuclear factor (NF)- κ B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture. *J. Exp. Med.* **187**: 147–159.
- Fuchs, S.Y., Chen, A., Xiong, Y., Pan, Z.Q., and Ronai, Z. 1999. HOS, a human homolog of Slimb, forms an SCF complex with Skp1 and Cullin1 and targets the phosphorylation-dependent degradation of I κ B and β -catenin. *Oncogene* **18**: 2039–2046.
- Fujimoto, K., Yasuda, H., Sato, Y., and Yamamoto, K. 1995. A role for phosphorylation in the proteolytic processing of the human NF- κ B1 precursor. *Gene* **165**: 183–189.
- Fujita, T., Nolan, G.P., Liou, H.C., Scott, M.L., and Baltimore, D. 1993. The candidate proto-oncogene bcl-3 encodes a transcriptional coactivator that activates through NF- κ B p50 homodimers. *Genes & Dev.* **7**: 1354–1363.
- Gaide, O., Martinon, F., Mischeau, O., Bonnet, D., Thome, M., and Tschopp, J. 2001. Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF- κ B activation. *FEBS Lett.* **496**: 121–127.
- Gaide, O., Favier, B., Legler, D.F., Bonnet, D., Brissoni, B., Valitutti, S., Bron, C., Tschopp, J., and Thome, M. 2002. CARMA1 is a critical lipid raft-associated regulator of TCR-induced NF- κ B activation. *Nat. Immunol.* **3**: 836–843.
- Gerondakis, S., Morrice, N., Richardson, I.B., Wettenhall, R., Fecondo, J., and Grumont, R.J. 1993. The activity of a 70 kilodalton I κ B molecule identical to the carboxyl terminus of the p105 NF- κ B precursor is modulated by protein kinase A. *Cell Growth Differ.* **4**: 617–627.
- Ghosh, G. 2004. NF- κ B biology at the interface of structure and biochemistry. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposia, Snowbird Resort, Snowbird, UT.
- Ghosh, S. and Karin, M. 2002. Missing pieces in the NF- κ B puzzle. *Cell* **109** Suppl: S81–S96.
- Ghosh, G., van Duyn, G., Ghosh, S., and Sigler, P.B. 1995. Structure of NF- κ B p50 homodimer bound to a κ B site. *Nature* **373**: 303–310.
- Ghosh, S., May, M.J., and Kopp, E.B. 1998. NF- κ B and Rel pro-

- teins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**: 225–260.
- Goes, F.S. and Martin, J. 2001. Hsp90 chaperone complexes are required for the activity and stability of yeast protein kinases Mik1, Wee1 and Swe1. *Eur. J. Biochem.* **268**: 2281–2289.
- Grumont, R.J. and Gerondakis, S. 1994. Alternative splicing of RNA transcripts encoded by the murine p105 NF- κ B gene generates I κ B γ isoforms with different inhibitory activities. *Proc. Natl. Acad. Sci.* **91**: 4367–4371.
- Gu, W. and Roeder, R.G. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**: 595–606.
- Guo, Y.L., Baysal, K., Kang, B., Yang, L.J., and Williamson, J.R. 1998. Correlation between sustained c-Jun N-terminal protein kinase activation and apoptosis induced by tumor necrosis factor- α in rat mesangial cells. *J. Biol. Chem.* **273**: 4027–4034.
- Guo, B., Su, T.T., and Rawlings, D.J. 2004. Protein kinase C family functions in B-cell activation. *Curr. Opin. Immunol.* **16**: 367–373.
- Hara, H., Wada, T., Bakal, C., Kozieradzki, I., Suzuki, S., Suzuki, N., Nghiem, M., Griffiths, E.K., Krawczyk, C., Bauer, B., et al. 2003. The MAGUK family protein CARD11 is essential for lymphocyte activation. *Immunity* **18**: 763–775.
- Hardy, M.P., McGettrick, A.F., and O'Neill, L.A. 2004. Transcriptional regulation of the human TRIF (TIR domain-containing adaptor protein inducing interferon β) gene. *Biochem. J.* **380**: 83–93.
- Harhaj, E.W., Maggirwar, S.B., and Sun, S.C. 1996. Inhibition of p105 processing by NF- κ B proteins in transiently transfected cells. *Oncogene* **12**: 2385–2392.
- Haruta, H., Kato, A., and Todokoro, K. 2001. Isolation of a novel interleukin-1-inducible nuclear protein bearing ankyrin-repeat motifs. *J. Biol. Chem.* **276**: 12485–12488.
- Hatada, E.N., Nieters, A., Wulczyn, F.G., Naumann, M., Meyer, R., Nucifora, G., McKeithan, T.W., and Scheidereit, C. 1992. The ankyrin repeat domains of the NF- κ B precursor p105 and the protooncogene bcl-3 act as specific inhibitors of NF- κ B DNA binding. *Proc. Natl. Acad. Sci.* **89**: 2489–2493.
- Hatakeyama, S., Kitagawa, M., Nakayama, K., Shirane, M., Matsumoto, M., Hattori, K., Higashi, H., Nakano, H., Okumura, K., Onoe, K., et al. 1999. Ubiquitin-dependent degradation of I κ B α is mediated by a ubiquitin ligase Skp1/Cul1/F-box protein FWD1. *Proc. Natl. Acad. Sci.* **96**: 3859–3863.
- Heilker, R., Freuler, F., Pulfer, R., Di Padova, F., and Eder, J. 1999. All three I κ B isoforms and most Rel family members are stably associated with the I κ B kinase 1/2 complex. *Eur. J. Biochem.* **259**: 253–261.
- Heissmeyer, V., Krappmann, D., Wulczyn, F.G., and Scheidereit, C. 1999. NF- κ B p105 is a target of I κ B kinases and controls signal induction of Bcl-3-p50 complexes. *EMBO J.* **18**: 4766–4778.
- Heissmeyer, V., Krappmann, D., Hatada, E.N., and Scheidereit, C. 2001. Shared pathways of I κ B kinase-induced SCF(β TrCP)-mediated ubiquitination and degradation for the NF- κ B precursor p105 and I κ B α . *Mol. Cell. Biol.* **21**: 1024–1035.
- Hemmi, H., Takeuchi, O., Sato, S., Yamamoto, M., Kaisho, T., Sanjo, H., Kawai, T., Hoshino, K., Takeda, K., and Akira, S. 2004. The roles of two I κ B kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J. Exp. Med.* **199**: 1641–1650.
- Heusch, M., Lin, L., Gelezianas, R., and Greene, W.C. 1999. The generation of nfkb2 p52: Mechanism and efficiency. *Oncogene* **18**: 6201–6208.
- Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O., and Woodgett, J.R. 2000. Requirement for glycogen synthase kinase-3 β in cell survival and NF- κ B activation. *Nature* **406**: 86–90.
- Hoffmann, A., Levchenko, A., Scott, M.L., and Baltimore, D. 2002. The I κ B-NF- κ B signaling module: Temporal control and selective gene activation. *Science* **298**: 1241–1245.
- Hoffmann, A., Leung, T.H., and Baltimore, D. 2003. Genetic analysis of NF- κ B/Rel transcription factors defines functional specificities. *EMBO J.* **22**: 5530–5539.
- Hornig, T., Barton, G.M., Flavell, R.A., and Medzhitov, R. 2002. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* **420**: 329–333.
- Hsu, H., Shu, H.B., Pan, M.G., and Goeddel, D.V. 1996. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **84**: 299–308.
- Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. 1999. Abnormal morphogenesis but intact IKK activation in mice lacking the IKK α subunit of I κ B kinase. *Science* **284**: 316–320.
- Hu, Y., Baud, V., Oga, T., Kim, K.I., Yoshida, K., and Karin, M. 2001. IKK α controls formation of the epidermis independently of NF- κ B. *Nature* **410**: 710–714.
- Huang, T.T., Kudo, N., Yoshida, M., and Miyamoto, S. 2000. A nuclear export signal in the N-terminal regulatory domain of I κ B α controls cytoplasmic localization of inactive NF- κ B/I κ B α complexes. *Proc. Natl. Acad. Sci.* **97**: 1014–1019.
- Huang, D.B., Chen, Y.Q., Ruetsche, M., Phelps, C.B., and Ghosh, G. 2001. X-Ray crystal structure of proto-oncogene product c-Rel bound to the CD28 response element of IL-2. *Structure (Camb.)* **9**: 669–678.
- Huang, T.T., Feinberg, S.L., Suryanarayanan, S., and Miyamoto, S. 2002. The zinc finger domain of NEMO is selectively required for NF- κ B activation by UV radiation and topoisomerase inhibitors. *Mol. Cell. Biol.* **22**: 5813–5825.
- Huang, T.T., Wuerzberger-Davis, S.M., Wu, Z.H., and Miyamoto, S. 2003. Sequential modification of NEMO/IKK γ by SUMO-1 and ubiquitin mediates NF- κ B activation by genotoxic stress. *Cell* **115**: 565–576.
- Huang, Q., Yang, J., Lin, Y., Walker, C., Cheng, J., Liu, Z.G., and Su, B. 2004. Differential regulation of interleukin 1 receptor and Toll-like receptor signaling by MEKK3. *Nat. Immunol.* **5**: 98–103.
- Hueber, A.O. 2003. Role of membrane microdomain rafts in TNFR-mediated signal transduction. *Cell Death Differ.* **10**: 7–9.
- Hur, G.M., Lewis, J., Yang, Q., Lin, Y., Nakano, H., Nedospasov, S., and Liu, Z.G. 2003. The death domain kinase RIP has an essential role in DNA damage-induced NF- κ B activation. *Genes & Dev.* **17**: 873–882.
- Huxford, T., Huang, D.B., Malek, S., and Ghosh, G. 1998. The crystal structure of the I κ B α /NF- κ B complex reveals mechanisms of NF- κ B inactivation. *Cell* **95**: 759–770.
- Huynh, Q.K., Boddupalli, H., Rouw, S.A., Koboldt, C.M., Hall, T., Sommers, C., Hauser, S.D., Pierce, J.L., Combs, R.G., Reitz, B.A., et al. 2000. Characterization of the recombinant IKK1/IKK2 heterodimer. Mechanisms regulating kinase activity. *J. Biol. Chem.* **275**: 25883–25891.
- Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P.C., Chen, F.F., Ogura, Y., and Nunez, G. 2000. An induced proximity model for NF- κ B activation in the Nod1/RICK and RIP signaling pathways. *J. Biol. Chem.* **275**: 27823–27831.
- Inoue, J., Kerr, L.D., Kakizuka, A., and Verma, I.M. 1992. I κ B γ , a 70 kD protein identical to the C-terminal half of p110 NF- κ B: A new member of the I κ B family. *Cell* **68**: 1109–1120.

- Ishii, T., Kwon, H., Hiscott, J., Mosialos, G., and Koromilas, A.E. 2001. Activation of the I κ B α kinase (IKK) complex by double-stranded RNA-binding defective and catalytic inactive mutants of the interferon-inducible protein kinase PKR. *Oncogene* **20**: 1900–1912.
- Israel, A. 2004. Biochemical and genetic analysis of the NF- κ B signaling pathway. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposium, Snowbird Resort, Snowbird, UT.
- Jaattela, M., Mouritzen, H., Elling, F., and Bastholm, L. 1996. A20 zinc finger protein inhibits TNF and IL-1 signaling. *J. Immunol.* **156**: 1166–1173.
- Jacobs, M.D. and Harrison, S.C. 1998. Structure of an I κ B α /NF- κ B complex. *Cell* **95**: 749–758.
- Janeway Jr., C.A. and Medzhitov, R. 2002. Innate immune recognition. *Annu. Rev. Immunol.* **20**: 197–216.
- Janssens, S. and Beyaert, R. 2003. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol. Cell* **11**: 293–302.
- Janssens, S., Burns, K., Tschopp, J., and Beyaert, R. 2002. Regulation of interleukin-1- and lipopolysaccharide-induced NF- κ B activation by alternative splicing of MyD88. *Curr. Biol.* **12**: 467–471.
- Janssens, S., Burns, K., Vercammen, E., Tschopp, J., and Beyaert, R. 2003. MyD88S, a splice variant of MyD88, differentially modulates NF- κ B- and AP-1-dependent gene expression. *FEBS Lett.* **548**: 103–107.
- Jiang, Y., Woronicz, J.D., Liu, W., and Goeddel, D.V. 1999. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* **283**: 543–546.
- Jin, R., De Smaele, E., Zazzeroni, F., Nguyen, D.U., Papa, S., Jones, J., Cox, C., Gelinas, C., and Franzoso, G. 2002. Regulation of the gadd45 β promoter by NF- κ B. *DNA Cell Biol.* **21**: 491–503.
- Johnson, C., Van Antwerp, D., and Hope, T.J. 1999. An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of I κ B α . *EMBO J.* **18**: 6682–6693.
- Kanamori, M., Kai, C., Hayashizaki, Y., and Suzuki, H. 2002. NF- κ B activator Act1 associates with IL-1/Toll pathway adaptor molecule TRAF6. *FEBS Lett.* **532**: 241–246.
- Kane, L.P., Mollenauer, M.N., Xu, Z., Turck, C.W., and Weiss, A. 2002. Akt-dependent phosphorylation specifically regulates Cot induction of NF- κ B-dependent transcription. *Mol. Cell Biol.* **22**: 5962–5974.
- Karin, M. and Ben-Neriah, Y. 2000. Phosphorylation meets ubiquitination: The control of NF- κ B activity. *Annu. Rev. Immunol.* **18**: 621–663.
- Karin, M., Yamamoto, Y., and Wang, Q.M. 2004. The IKK NF- κ B system: A treasure trove for drug development. *Nat. Rev. Drug Discov.* **3**: 17–26.
- Kato Jr., T., Delhase, M., Hoffmann, A., and Karin, M. 2003. CK2 is a C-terminal I κ B kinase responsible for NF- κ B activation during the UV response. *Mol. Cell* **12**: 829–839.
- Kawai, T., Adachi, O., Ogawa, T., Takeda, K., and Akira, S. 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* **11**: 115–122.
- Kelliher, M.A., Grimm, S., Ishida, Y., Kuo, F., Stanger, B.Z., and Leder, P. 1998. The death domain kinase RIP mediates the TNF-induced NF- κ B signal. *Immunity* **8**: 297–303.
- Kelly, D., Campbell, J.I., King, T.P., Grant, G., Jansson, E.A., Coutts, A.G., Pettersson, S., and Conway, S. 2004. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR- γ and RelA. *Nat. Immunol.* **5**: 104–112.
- Kerr, L.D., Duckett, C.S., Wamsley, P., Zhang, Q., Chiao, P., Nabel, G., McKeithan, T.W., Baeuerle, P.A., and Verma, I.M. 1992. The proto-oncogene bcl-3 encodes an I κ B protein. *Genes & Dev.* **6**: 2352–2363.
- Khoshnan, A., Kempik, S.J., Bennett, B.L., Bae, D., Xu, W., Manning, A.M., June, C.H., and Nel, A.E. 1999. Primary human CD4⁺ T cells contain heterogeneous I κ B kinase complexes: Role in activation of the IL-2 promoter. *J. Immunol.* **163**: 5444–5452.
- Khoshnan, A., Bae, D., Tindell, C.A., and Nel, A.E. 2000. The physical association of protein kinase C θ with a lipid raft-associated inhibitor of κ B factor kinase (IKK) complex plays a role in the activation of the NF- κ B cascade by TCR and CD28. *J. Immunol.* **165**: 6933–6940.
- Kiernan, R., Bres, V., Ng, R.W., Coudart, M.P., El Messaoudi, S., Sardet, C., Jin, D.Y., Emiliani, S., and Benkirane, M. 2003. Post-activation turn-off of NF- κ B-dependent transcription is regulated by acetylation of p65. *J. Biol. Chem.* **278**: 2758–2766.
- Kitamura, H., Kanehira, K., Okita, K., Morimatsu, M., and Saito, M. 2000. MAIL, a novel nuclear I κ B protein that potentiates LPS-induced IL-6 production. *FEBS Lett.* **485**: 53–56.
- Klement, J.F., Rice, N.R., Car, B.D., Abbondanzo, S.J., Powers, G.D., Bhatt, P.H., Chen, C.H., Rosen, C.A., and Stewart, C.L. 1996. I κ B α deficiency results in a sustained NF- κ B response and severe widespread dermatitis in mice. *Mol. Cell Biol.* **16**: 2341–2349.
- Knop, J. and Martin, M.U. 1999. Effects of IL-1 receptor-associated kinase (IRAK) expression on IL-1 signaling are independent of its kinase activity. *FEBS Lett.* **448**: 81–85.
- Kobayashi, K., Hernandez, L.D., Galan, J.E., Janeway Jr., C.A., Medzhitov, R., and Flavell, R.A. 2002. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* **110**: 191–202.
- Komatsu, Y., Shibuya, H., Takeda, N., Ninomiya-Tsuji, J., Yasui, T., Miyado, K., Sekimoto, T., Ueno, N., Matsumoto, K., and Yamada, G. 2002. Targeted disruption of the Tab1 gene causes embryonic lethality and defects in cardiovascular and lung morphogenesis. *Mech. Dev.* **119**: 239–249.
- Kontgen, F., Grumont, R.J., Strasser, A., Metcalf, D., Li, R., Tarlinton, D., and Gerondakis, S. 1995. Mice lacking the c-rel proto-oncogene exhibit defects in lymphocyte proliferation, humoral immunity, and interleukin-2 expression. *Genes & Dev.* **9**: 1965–1977.
- Kopp, E. and Medzhitov, R. 2003. Recognition of microbial infection by Toll-like receptors. *Curr. Opin. Immunol.* **15**: 396–401.
- Kopp, E., Medzhitov, R., Carothers, J., Xiao, C., Douglas, I., Janeway, C.A., and Ghosh, S. 1999. ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. *Genes & Dev.* **13**: 2059–2071.
- Kovalenko, A., Chable-Bessia, C., Cantarella, G., Israel, A., Wallach, D., and Courtois, G. 2003. The tumour suppressor CYLD negatively regulates NF- κ B signalling by deubiquitination. *Nature* **424**: 801–805.
- Krappmann, D., Hatada, E.N., Tegethoff, S., Li, J., Klippel, A., Giese, K., Baeuerle, P.A., and Scheidereit, C. 2000. The I κ B kinase (IKK) complex is tripartite and contains IKK γ but not IKAP as a regular component. *J. Biol. Chem.* **275**: 29779–29787.
- Kravchenko, V.V., Mathison, J.C., Schwamborn, K., Mercurio, F., and Ulevitch, R.J. 2003. IKKi/IKK ϵ plays a key role in integrating signals induced by pro-inflammatory stimuli. *J. Biol. Chem.* **278**: 26612–26619.
- Kreuz, S., Siegmund, D., Scheurich, P., and Wajant, H. 2001. NF- κ B inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. *Mol. Cell Biol.* **21**: 3964–3973.
- Krikos, A., Laherty, C.D., and Dixit, V.M. 1992. Transcriptional

- activation of the tumor necrosis factor α -inducible zinc finger protein, A20, is mediated by κ B elements. *J. Biol. Chem.* **267**: 17971–17976.
- Kroll, M., Margottin, F., Kohl, A., Renard, P., Durand, H., Concorde, J.P., Bachellerie, F., Arenzana-Seisdedos, F., and Benarous, R. 1999. Inducible degradation of I κ B α by the proteasome requires interaction with the F-box protein h- β TrCP. *J. Biol. Chem.* **274**: 7941–7945.
- Kucharczak, J., Simmons, M.J., Fan, Y., and Gelinas, C. 2003. To be, or not to be: NF- κ B is the answer—Role of Rel/NF- κ B in the regulation of apoptosis. *Oncogene* **22**: 8961–8982.
- Kumar, A., Haque, J., Lacoste, J., Hiscott, J., and Williams, B.R. 1994. Double-stranded RNA-dependent protein kinase activates transcription factor NF- κ B by phosphorylating I κ B. *Proc. Natl. Acad. Sci.* **91**: 6288–6292.
- Kuwata, H., Watanabe, Y., Miyoshi, H., Yamamoto, M., Kaisho, T., Takeda, K., and Akira, S. 2003. IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF- α production in macrophages. *Blood* **102**: 4123–4129.
- Kwak, Y.T., Guo, J., Shen, J., and Gaynor, R.B. 2000. Analysis of domains in the IKK α and IKK β proteins that regulate their kinase activity. *J. Biol. Chem.* **275**: 14752–14759.
- Laherty, C.D., Hu, H.M., Opiari, A.W., Wang, F., and Dixit, V.M. 1992. The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor κ B. *J. Biol. Chem.* **267**: 24157–24160.
- Lallena, M.J., Diaz-Meco, M.T., Bren, G., Paya, C.V., and Moscat, J. 1999. Activation of I κ B kinase β by protein kinase C isoforms. *Mol. Cell. Biol.* **19**: 2180–2188.
- Lamb, J.A., Ventura, J.J., Hess, P., Flavell, R.A., and Davis, R.J. 2003. JunD mediates survival signaling by the JNK signal transduction pathway. *Mol. Cell* **11**: 1479–1489.
- Lang, V., Janzen, J., Fischer, G.Z., Soneji, Y., Beinke, S., Salmeron, A., Allen, H., Hay, R.T., Ben-Neriah, Y., and Ley, S.C. 2003. β TrCP-mediated proteolysis of NF- κ B1 p105 requires phosphorylation of p105 serines 927 and 932. *Mol. Cell. Biol.* **23**: 402–413.
- Lee, S.H. and Hannink, M. 2002. Characterization of the nuclear import and export functions of I κ B ϵ . *J. Biol. Chem.* **277**: 23358–23366.
- Lee, F.S., Hagler, J., Chen, Z.J., and Maniatis, T. 1997. Activation of the I κ B α kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* **88**: 213–222.
- Lee, E.G., Boone, D.L., Chai, S., Libby, S.L., Chien, M., Lodolce, J.P., and Ma, A. 2000. Failure to regulate TNF-induced NF- κ B and cell death responses in A20-deficient mice. *Science* **289**: 2350–2354.
- Legler, D.F., Micheau, O., Doucey, M.A., Tschopp, J., and Bron, C. 2003. Recruitment of TNF receptor 1 to lipid rafts is essential for TNF α -mediated NF- κ B activation. *Immunity* **18**: 655–664.
- Leitges, M., Sanz, L., Martin, P., Duran, A., Braun, U., Garcia, J.F., Camacho, F., Diaz-Meco, M.T., Rennert, P.D., and Moscat, J. 2001. Targeted disruption of the ζ PKC gene results in the impairment of the NF- κ B pathway. *Mol. Cell* **8**: 771–780.
- Lernbecher, T., Kistler, B., and Wirth, T. 1994. Two distinct mechanisms contribute to the constitutive activation of RelB in lymphoid cells. *EMBO J.* **13**: 4060–4069.
- Li, X. 2004. Act1, a negative regulator in B cell survival. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposia, Snowbird Resort, Snowbird, UT.
- Li, Z. and Nabel, G.J. 1997. A new member of the I κ B protein family, I κ B ϵ , inhibits RelA (p65)-mediated NF- κ B transcription. *Mol. Cell. Biol.* **17**: 6184–6190.
- Li, N. and Karin, M. 1998. Ionizing radiation and short wavelength UV activate NF- κ B through two distinct mechanisms. *Proc. Natl. Acad. Sci.* **95**: 13012–13017.
- Li, Q. and Verma, I.M. 2002. NF- κ B regulation in the immune system. *Nat. Rev. Immunol.* **2**: 725–734.
- Li, Q., Lu, Q., Hwang, J.Y., Buscher, D., Lee, K.F., Izpisua-Belmonte, J.C., and Verma, I.M. 1999a. IKK1-deficient mice exhibit abnormal development of skin and skeleton. *Genes & Dev.* **13**: 1322–1328.
- Li, Q., Van Antwerp, D., Mercurio, F., Lee, K.F., and Verma, I.M. 1999b. Severe liver degeneration in mice lacking the I κ B kinase 2 gene. *Science* **284**: 321–325.
- Li, X., Commane, M., Burns, C., Vithalani, K., Cao, Z., and Stark, G.R. 1999. Mutant cells that do not respond to interleukin-1 (IL-1) reveal a novel role for IL-1 receptor-associated kinase. *Mol. Cell. Biol.* **19**: 4643–4652.
- Li, Y., Kang, J., Friedman, J., Tarassishin, L., Ye, J., Kovalenko, A., Wallach, D., and Horwitz, M.S. 1999. Identification of a cell protein (FIP-3) as a modulator of NF- κ B activity and as a target of an adenovirus inhibitor of tumor necrosis factor α -induced apoptosis. *Proc. Natl. Acad. Sci.* **96**: 1042–1047.
- Li, Z.W., Chu, W., Hu, Y., Delhase, M., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. 1999. The IKK β subunit of I κ B kinase (IKK) is essential for nuclear factor κ B activation and prevention of apoptosis. *J. Exp. Med.* **189**: 1839–1845.
- Li, Q., Estepa, G., Memet, S., Israel, A., and Verma, I.M. 2000. Complete lack of NF- κ B activity in IKK1 and IKK2 double-deficient mice: Additional defect in neurulation. *Genes & Dev.* **14**: 1729–1733.
- Li, X., Commane, M., Nie, H., Hua, X., Chatterjee-Kishore, M., Wald, D., Haag, M., and Stark, G.R. 2000. Act1, an NF- κ B-activating protein. *Proc. Natl. Acad. Sci.* **97**: 10489–10493.
- Li, N., Banin, S., Ouyang, H., Li, G.C., Courtois, G., Shiloh, Y., Karin, M., and Rotman, G. 2001. ATM is required for I κ B kinase (IKK) activation in response to DNA double strand breaks. *J. Biol. Chem.* **276**: 8898–8903.
- Liao, G. and Sun, S.C. 2003. Regulation of NF- κ B2/p100 processing by its nuclear shuttling. *Oncogene* **22**: 4868–4874.
- Lin, L. and Ghosh, S. 1996. A glycine-rich region in NF- κ B p105 functions as a processing signal for the generation of the p50 subunit. *Mol. Cell. Biol.* **16**: 2248–2254.
- Lin, R., Beaulieu, P., Makris, C., Meloche, S., and Hiscott, J. 1996. Phosphorylation of I κ B α in the C-terminal PEST domain by casein kinase II affects intrinsic protein stability. *Mol. Cell. Biol.* **16**: 1401–1409.
- Lin, L., DeMartino, G.N., and Greene, W.C. 1998. Cotranslational biogenesis of NF- κ B p50 by the 26S proteasome. *Cell* **92**: 819–828.
- Lin, X., Mu, Y., Cunningham Jr., E.T., Marcu, K.B., Geleziunas, R., and Greene, W.C. 1998. Molecular determinants of NF- κ B-inducing kinase action. *Mol. Cell. Biol.* **18**: 5899–5907.
- Lin, X., Cunningham Jr., E.T., Mu, Y., Geleziunas, R., and Greene, W.C. 1999. The proto-oncogene Cot kinase participates in CD3/CD28 induction of NF- κ B acting through the NF- κ B-inducing kinase and I κ B kinases. *Immunity* **10**: 271–280.
- Ling, L., Cao, Z., and Goeddel, D.V. 1998. NF- κ B-inducing kinase activates IKK- α by phosphorylation of Ser-176. *Proc. Natl. Acad. Sci.* **95**: 3792–3797.
- Lomaga, M.A., Yeh, W.C., Sarosi, I., Duncan, G.S., Furlonger, C., Ho, A., Morony, S., Capparelli, C., Van, G., Kaufman, S., et al. 1999. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes & Dev.* **13**: 1015–1024.
- Lucas, P.C., McAllister-Lucas, L.M., and Nunez, G. 2004. NF- κ B signaling in lymphocytes: A new cast of characters. *J. Cell Sci.* **117**: 31–39.

- Lynch, O.T. and Gadina, M. 2004. Ubiquitination for activation: New directions in the NF- κ B roadmap. *Mol. Interv.* **4**: 144–146.
- MacKichan, M.L., Logeat, F., and Israel, A. 1996. Phosphorylation of p105 PEST sequence via a redox-insensitive pathway up-regulates processing of p50 NF- κ B. *J. Biol. Chem.* **271**: 6084–6091.
- Makris, C., Godfrey, V.L., Krahn-Senftleben, G., Takahashi, T., Roberts, J.L., Schwarz, T., Feng, L., Johnson, R.S., and Karin, M. 2000. Female mice heterozygous for IKK γ /NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. *Mol. Cell* **5**: 969–979.
- Makris, C., Roberts, J.L., and Karin, M. 2002. The carboxyl-terminal region of I κ B kinase γ (IKK γ) is required for full IKK activation. *Mol. Cell. Biol.* **22**: 6573–6581.
- Malek, S., Chen, Y., Huxford, T., and Ghosh, G. 2001. I κ B β , but not I κ B α , functions as a classical cytoplasmic inhibitor of NF- κ B dimers by masking both NF- κ B nuclear localization sequences in resting cells. *J. Biol. Chem.* **276**: 45225–45235.
- Malek, S., Huang, D.B., Huxford, T., Ghosh, S., and Ghosh, G. 2003. X-Ray crystal structure of an I κ B β $\times$ NF- κ B p65 homodimer complex. *J. Biol. Chem.* **278**: 23094–23100.
- Mauro, C., Vito, P., Mellone, S., Pacifico, F., Chariot, A., Formisano, S., and Leonardi, A. 2003. Role of the adaptor protein CIKS in the activation of the IKK complex. *Biochem. Biophys. Res. Commun.* **309**: 84–90.
- May, M.J., D'Acquisto, F., Madge, L.A., Glockner, J., Pober, J.S., and Ghosh, S. 2000. Selective inhibition of NF- κ B activation by a peptide that blocks the interaction of NEMO with the I κ B kinase complex. *Science* **289**: 1550–1554.
- May, M.J., Marienfeld, R.B., and Ghosh, S. 2002. Characterization of the I κ B-kinase NEMO binding domain. *J. Biol. Chem.* **277**: 45992–46000.
- McCarthy, J.V., Ni, J., and Dixit, V.M. 1998. RIP2 is a novel NF- κ B-activating and cell death-inducing kinase. *J. Biol. Chem.* **273**: 16968–16975.
- McElhinny, J.A., Trushin, S.A., Bren, G.D., Chester, N., and Paya, C.V. 1996. Casein kinase II phosphorylates I κ B α at S-283, S-289, S-293, and T-291 and is required for its degradation. *Mol. Cell. Biol.* **16**: 899–906.
- McGettrick, A.F. and O'Neill, L.A. 2004. The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction. *Mol. Immunol.* **41**: 577–582.
- McWhirter, S.M., Fitzgerald, K.A., Rosains, J., Rowe, D.C., Golenbock, D.T., and Maniatis, T. 2004. IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. *Proc. Natl. Acad. Sci.* **101**: 233–238.
- Mebius, R.E. 2003. Organogenesis of lymphoid tissues. *Nat. Rev. Immunol.* **3**: 292–303.
- Melmed, G., Thomas, L.S., Lee, N., Tesfay, S.Y., Lukasek, K., Michelsen, K.S., Zhou, Y., Hu, B., Arditi, M., and Abreu, M.T. 2003. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: Implications for host-microbial interactions in the gut. *J. Immunol.* **170**: 1406–1415.
- Mercurio, F. 2004. IKK-related kinases as a target for drug therapy. In *Keystone Symposium on NF- κ B: Biology and pathology* (eds. A.R. Ulrich et al.). Keystone Symposia, Snowbird Resort, Snowbird, UT.
- Mercurio, F., Zhu, H., Murray, B.W., Shevchenko, A., Bennett, B.L., Li, J., Young, D.B., Barbosa, M., Mann, M., Manning, A., et al. 1997. IKK-1 and IKK-2: Cytokine-activated I κ B kinases essential for NF- κ B activation. *Science* **278**: 860–866.
- Mercurio, F., Murray, B.W., Shevchenko, A., Bennett, B.L., Young, D.B., Li, J.W., Pascual, G., Motiwala, A., Zhu, H., Mann, M., et al. 1999. I κ B kinase (IKK)-associated protein 1, a common component of the heterogeneous IKK complex. *Mol. Cell. Biol.* **19**: 1526–1538.
- Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M., and Tschoop, J. 2004. RIP1 is an essential mediator of Toll-like receptor 3-induced NF- κ B activation. *Nat. Immunol.* **5**: 503–507.
- Miller, B.S. and Zandi, E. 2001. Complete reconstitution of human I κ B kinase (IKK) complex in yeast. Assessment of its stoichiometry and the role of IKK γ on the complex activity in the absence of stimulation. *J. Biol. Chem.* **276**: 36320–36326.
- Miskolci, V., Castro-Alcaraz, S., Nguyen, P., Vancura, A., Davidson, D., and Vancurova, I. 2003. Okadaic acid induces sustained activation of NF κ B and degradation of the nuclear I κ B α in human neutrophils. *Arch. Biochem. Biophys.* **417**: 44–52.
- Mohan, N. and Meltz, M.L. 1994. Induction of nuclear factor κ B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway. *Radiat. Res.* **140**: 97–104.
- Mosialos, G. and Gilmore, T.D. 1993. v-Rel and c-Rel are differentially affected by mutations at a consensus protein kinase recognition sequence. *Oncogene* **8**: 721–730.
- Moustakas, A. and Heldin, C.H. 2003. Ecsit-ement on the crossroads of Toll and BMP signal transduction. *Genes & Dev.* **17**: 2855–2859.
- Muller, C.W., Rey, F.A., Sodeoka, M., Verdine, G.L., and Harrison, S.C. 1995. Structure of the NF- κ B p50 homodimer bound to DNA. *Nature* **373**: 311–317.
- Muta, T., Yamazaki, S., Eto, A., Motoyama, M., and Takeshige, K. 2003. I κ B- ζ , a new anti-inflammatory nuclear protein induced by lipopolysaccharide, is a negative regulator for nuclear factor- κ B. *J. Endotoxin Res.* **9**: 187–191.
- Nakano, H., Sakon, S., Koseki, H., Takemori, T., Tada, K., Matsumoto, M., Munechika, E., Sakai, T., Shirasawa, T., Akiba, H., et al. 1999. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc. Natl. Acad. Sci.* **96**: 9803–9808.
- Nakayama, K., Hatakeyama, S., Maruyama, S., Kikuchi, A., Onoe, K., Good, R.A., and Nakayama, K.I. 2003. Impaired degradation of inhibitory subunit of NF- κ B (I κ B) and β -catenin as a result of targeted disruption of the β -TrCP1 gene. *Proc. Natl. Acad. Sci.* **100**: 8752–8757.
- Naumann, M. and Scheidereit, C. 1994. Activation of NF- κ B in vivo is regulated by multiple phosphorylations. *EMBO J.* **13**: 4597–4607.
- Naumann, M., Wulczyn, F.G., and Scheidereit, C. 1993. The NF- κ B precursor p105 and the proto-oncogene product Bcl-3 are I κ B molecules and control nuclear translocation of NF- κ B. *EMBO J.* **12**: 213–222.
- Neumann, M., Grieshammer, T., Chuvpilo, S., Kneitz, B., Lohoff, M., Schimpl, A., Franza Jr., B.R., and Serfling, E. 1995. RelA/p65 is a molecular target for the immunosuppressive action of protein kinase A. *EMBO J.* **14**: 1991–2004.
- Nolan, G.P., Fujita, T., Bhatia, K., Huppi, C., Liou, H.C., Scott, M.L., and Baltimore, D. 1993. The bcl-3 proto-oncogene encodes a nuclear I κ B-like molecule that preferentially interacts with NF- κ B p50 and p52 in a phosphorylation-dependent manner. *Mol. Cell. Biol.* **13**: 3557–3566.
- O'Mahony, A.M., Montano, M., Van Beneden, K., Chen, L.F., and Greene, W.C. 2004. Human T-cell lymphotropic virus type 1 tax induction of biologically active NF- κ B requires I κ B kinase-1-mediated phosphorylation of RelA/p65. *J. Biol. Chem.* **279**: 18137–18145.
- Orian, A., Schwartz, A.L., Israel, A., Whiteside, S., Kahana, C.,

- and Ciechanover, A. 1999. Structural motifs involved in ubiquitin-mediated processing of the NF- κ B precursor p105: Roles of the glycine-rich region and a downstream ubiquitin domain. *Mol. Cell. Biol.* **19**: 3664–3673.
- Orian, A., Gonen, H., Bercovich, B., Fajerman, I., Eytan, E., Israel, A., Mercurio, F., Iwai, K., Schwartz, A.L., and Ciechanover, A. 2000. SCF ^{β -TrCP} ubiquitin ligase-mediated processing of NF- κ B p105 requires phosphorylation of its C-terminus by I κ B kinase. *EMBO J.* **19**: 2580–2591.
- Oshiumi, H., Matsumoto, M., Funami, K., Akazawa, T., and Seya, T. 2003. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon- β induction. *Nat. Immunol.* **4**: 161–167.
- Palombella, V.J., Rando, O.J., Goldberg, A.L., and Maniatis, T. 1994. The ubiquitin-proteasome pathway is required for processing the NF- κ B precursor protein and the activation of NF- κ B. *Cell* **78**: 773–785.
- Papa, S., Zazzeroni, F., Bubici, C., Jayawardena, S., Alvarez, K., Matsuda, S., Nguyen, D.U., Pham, C.G., Nelsbach, A.H., Melis, T., et al. 2004. Gadd45 β mediates the NF- κ B suppression of JNK signalling by targeting MKK7/JNK2. *Nat. Cell Biol.* **6**: 146–153.
- Paxian, S., Merkle, H., Riemann, M., Wilda, M., Adler, G., Hameister, H., Liptay, S., Pfeffer, K., and Schmid, R.M. 2002. Abnormal organogenesis of Peyer's patches in mice deficient for NF- κ B1, NF- κ B2, and Bcl-3. *Gastroenterology* **122**: 1853–1868.
- Piret, B. and Piette, J. 1996. Topoisomerase poisons activate the transcription factor NF- κ B in ACH-2 and CEM cells. *Nucleic Acids Res.* **24**: 4242–4248.
- Poyet, J.L., Srinivasula, S.M., Lin, J.H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P.N., and Alnemri, E.S. 2000. Activation of the I κ B kinases by RIP via IKK γ /NEMO-mediated oligomerization. *J. Biol. Chem.* **275**: 37966–37977.
- Prajapati, S., Verma, U., Yamamoto, Y., Kwak, Y.T., and Gaynor, R.B. 2004. Protein phosphatase 2C β association with the I κ B kinase complex is involved in regulating NF- κ B activity. *J. Biol. Chem.* **279**: 1739–1746.
- Qian, Y., Commare, M., Ninomiya-Tsuji, J., Matsumoto, K., and Li, X. 2001. IRAK-mediated translocation of TRAF6 and TAB2 in the interleukin-1-induced activation of NF κ B. *J. Biol. Chem.* **276**: 41661–41667.
- Qian, Y., Zhao, Z., Jiang, Z., and Li, X. 2002. Role of NF- κ B activator Act1 in CD40-mediated signaling in epithelial cells. *Proc. Natl. Acad. Sci.* **99**: 9386–9391.
- Regnier, C.H., Song, H.Y., Gao, X., Goeddel, D.V., Cao, Z., and Rothe, M. 1997. Identification and characterization of an I κ B kinase. *Cell* **90**: 373–383.
- Rothwarf, D.M. and Karin, M. 1999. The NF- κ B activation pathway: A paradigm in information transfer from membrane to nucleus. *Sci. STKE* **1999**: RE1.
- Rothwarf, D.M., Zandi, E., Natoli, G., and Karin, M. 1998. IKK- γ is an essential regulatory subunit of the I κ B kinase complex. *Nature* **395**: 297–300.
- Rudolph, D., Yeh, W.C., Wakeham, A., Rudolph, B., Nallainathan, D., Potter, J., Elia, A.J., and Mak, T.W. 2000. Severe liver degeneration and lack of NF- κ B activation in NEMO/IKK γ -deficient mice. *Genes & Dev.* **14**: 854–862.
- Ruefli-Brasse, A.A., French, D.M., and Dixit, V.M. 2003. Regulation of NF- κ B-dependent lymphocyte activation and development by paracaspase. *Science* **302**: 1581–1584.
- Ruefli-Brasse, A.A., Lee, W.P., Hurst, S., and Dixit, V.M. 2004. Rip2 participates in Bcl10 signaling and T-cell receptor-mediated NF- κ B activation. *J. Biol. Chem.* **279**: 1570–1574.
- Ruland, J. and Mak, T.W. 2003. Transducing signals from antigen receptors to nuclear factor κ B. *Immunol. Rev.* **193**: 93–100.
- Ruland, J., Duncan, G.S., Elia, A., del Barco Barrantes, I., Nguyen, L., Plyte, S., Millar, D.G., Bouchard, D., Wakeham, A., Ohashi, P.S., et al. 2001. Bcl10 is a positive regulator of antigen receptor-induced activation of NF- κ B and neural tube closure. *Cell* **104**: 33–42.
- Ryo, A., Suizu, F., Yoshida, Y., Perrem, K., Liou, Y.C., Wulf, G., Rottapel, R., Yamaoka, S., and Lu, K.P. 2003. Regulation of NF- κ B signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol. Cell* **12**: 1413–1426.
- Ryseck, R.P., Bull, P., Takamiya, M., Bours, V., Siebenlist, U., Dobrzanski, P., and Bravo, R. 1992. RelB, a new Rel family transcription activator that can interact with p50-NF- κ B. *Mol. Cell. Biol.* **12**: 674–684.
- Sakurai, H., Suzuki, S., Kawasaki, N., Nakano, H., Okazaki, T., Chino, A., Doi, T., and Saiki, I. 2003. Tumor necrosis factor- α -induced IKK phosphorylation of NF- κ B p65 on serine 536 is mediated through the TRAF2, TRAF5, and TAK1 signaling pathway. *J. Biol. Chem.* **278**: 36916–36923.
- Salmeron, A., Janzen, J., Soneji, Y., Bump, N., Kamens, J., Allen, H., and Ley, S.C. 2001. Direct phosphorylation of NF- κ B1 p105 by the I κ B kinase complex on serine 927 is essential for signal-induced p105 proteolysis. *J. Biol. Chem.* **276**: 22215–22222.
- Sanjo, H., Takeda, K., Tsujimura, T., Ninomiya-Tsuji, J., Matsumoto, K., and Akira, S. 2003. TAB2 is essential for prevention of apoptosis in fetal liver but not for interleukin-1 signaling. *Mol. Cell. Biol.* **23**: 1231–1238.
- Sanz, L., Sanchez, P., Lallena, M.J., Diaz-Meco, M.T., and Moscat, J. 1999. The interaction of p62 with RIP links the atypical PKCs to NF- κ B activation. *EMBO J.* **18**: 3044–3053.
- Sanz, L., Diaz-Meco, M.T., Nakano, H., and Moscat, J. 2000. The atypical PKC-interacting protein p62 channels NF- κ B activation by the IL-1-TRAF6 pathway. *EMBO J.* **19**: 1576–1586.
- Sato, S., Fujita, N., and Tsuruo, T. 2000. Modulation of Akt kinase activity by binding to Hsp90. *Proc. Natl. Acad. Sci.* **97**: 10832–10837.
- Scherer, D.C., Brockman, J.A., Chen, Z., Maniatis, T., and Ballard, D.W. 1995. Signal-induced degradation of I κ B α requires site-specific ubiquitination. *Proc. Natl. Acad. Sci.* **92**: 11259–11263.
- Schmidt, C., Peng, B., Li, Z., Sclabas, G.M., Fujioka, S., Niu, J., Schmidt-Suppran, M., Evans, D.B., Abbruzzese, J.L., and Chiao, P.J. 2003. Mechanisms of proinflammatory cytokine-induced biphasic NF- κ B activation. *Mol. Cell* **12**: 1287–1300.
- Schmidt-Suppran, M., Bloch, W., Courtois, G., Addicks, K., Israel, A., Rajewsky, K., and Pasparakis, M. 2000. NEMO/IKK gamma-deficient mice model incontinentia pigmenti. *Mol. Cell* **5**: 981–992.
- Schwabe, R.F. and Brenner, D.A. 2002. Role of glycogen synthase kinase-3 in TNF- α -induced NF- κ B activation and apoptosis in hepatocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* **283**: G204–G211.
- Schwarz, E.M., Krimpenfort, P., Berns, A., and Verma, I.M. 1997. Immunological defects in mice with a targeted disruption in Bcl-3. *Genes & Dev.* **11**: 187–197.
- Senftleben, U., Cao, Y., Xiao, G., Greten, F.R., Krahn, G., Bonizzi, G., Chen, Y., Hu, Y., Fong, A., Sun, S.C., et al. 2001a. Activation by IKK α of a second, evolutionary conserved, NF- κ B signaling pathway. *Science* **293**: 1495–1499.
- Senftleben, U., Li, Z.W., Baud, V., and Karin, M. 2001b. IKK β is essential for protecting T cells from TNF α -induced apoptosis. *Immunity* **14**: 217–230.
- Setoguchi, K., Misaki, Y., Terauchi, Y., Yamauchi, T., Kawahata, K., Kadowaki, T., and Yamamoto, K. 2001. Peroxisome

- proliferator-activated receptor- γ haploinsufficiency enhances B cell proliferative responses and exacerbates experimentally induced arthritis. *J. Clin. Invest.* **108**: 1667–1675.
- Sha, W.C., Liou, H.C., Tuomanen, E.I., and Baltimore, D. 1995. Targeted disruption of the p50 subunit of NF- κ B leads to multifocal defects in immune responses. *Cell* **80**: 321–330.
- Sharma, S., tenOever, B.R., Grandvaux, N., Zhou, G.P., Lin, R., and Hiscott, J. 2003. Triggering the interferon antiviral response through an IKK-related pathway. *Science* **300**: 1148–1151.
- Shinkura, R., Kitada, K., Matsuda, F., Tashiro, K., Ikuta, K., Suzuki, M., Kogishi, K., Serikawa, T., and Honjo, T. 1999. Alymphoplasia is caused by a point mutation in the mouse gene encoding NF- κ B-inducing kinase. *Nat. Genet.* **22**: 74–77.
- Shu, H.B., Takeuchi, M., and Goeddel, D.V. 1996. The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. *Proc. Natl. Acad. Sci.* **93**: 13973–13978.
- Shu, H.B., Halpin, D.R., and Goeddel, D.V. 1997. Casper is a FADD- and caspase-related inducer of apoptosis. *Immunity* **6**: 751–763.
- Sigala, J.L., Bottero, V., Young, D.B., Shevchenko, A., Mercurio, F., and Verma, I.M. 2004. Activation of transcription factor NF- κ B requires ELKS, an I κ B kinase regulatory subunit. *Science* **304**: 1963–1967.
- Sil, A.K., Maeda, S., Sano, Y., Roop, D.R., and Karin, M. 2004. I κ B kinase- α acts in the epidermis to control skeletal and craniofacial morphogenesis. *Nature* **428**: 660–664.
- Silverman, N. and Maniatis, T. 2001. NF- κ B signaling pathways in mammalian and insect innate immunity. *Genes & Dev.* **15**: 2321–2342.
- Simeoni, L., Kliche, S., Lindquist, J., and Schraven, B. 2004. Adaptors and linkers in T and B cells. *Curr. Opin. Immunol.* **16**: 304–313.
- Simeonidis, S., Liang, S., Chen, G., and Thanos, D. 1997. Cloning and functional characterization of mouse I κ B ϵ . *Proc. Natl. Acad. Sci.* **94**: 14372–14377.
- Sizemore, N., Lerner, N., Dombrowski, N., Sakurai, H., and Stark, G.R. 2002. Distinct roles of the I κ B kinase α and β subunits in liberating nuclear factor κ B (NF- κ B) from I κ B and in phosphorylating the p65 subunit of NF- κ B. *J. Biol. Chem.* **277**: 3863–3869.
- Snapper, C.M., Rosas, F.R., Zelazowski, P., Moorman, M.A., Kehry, M.R., Bravo, R., and Weih, F. 1996. B cells lacking RelB are defective in proliferative responses, but undergo normal B cell maturation to Ig secretion and Ig class switching. *J. Exp. Med.* **184**: 1537–1541.
- Solan, N.J., Miyoshi, H., Carmona, E.M., Bren, G.D., and Paya, C.V. 2002. RelB cellular regulation and transcriptional activity are regulated by p100. *J. Biol. Chem.* **277**: 1405–1418.
- Song, H.Y., Rothe, M., and Goeddel, D.V. 1996. The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF- κ B activation. *Proc. Natl. Acad. Sci.* **93**: 6721–6725.
- Spencer, E., Jiang, J., and Chen, Z.J. 1999. Signal-induced ubiquitination of I κ B α by the F-box protein Slimb/ β -TrCP. *Genes & Dev.* **13**: 284–294.
- Sun, Z., Arendt, C.W., Ellmeier, W., Schaeffer, E.M., Sunshine, M.J., Gandhi, L., Annes, J., Petrzilka, D., Kupfer, A., Schwartzberg, P.L., et al. 2000. PKC- θ is required for TCR-induced NF- κ B activation in mature but not immature T lymphocytes. *Nature* **404**: 402–407.
- Sun, L., Deng, L., Ea, C.K., Xia, Z.P., and Chen, Z.J. 2004. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* **14**: 289–301.
- Suzuki, H., Chiba, T., Kobayashi, M., Takeuchi, M., Suzuki, T., Ichiyama, A., Ikenoue, T., Omata, M., Furuichi, K., and Tanaka, K. 1999. I κ B α ubiquitination is catalyzed by an SCF-like complex containing Skp1, cullin-1, and two F-box/WD40-repeat proteins, β TrCP1 and β TrCP2. *Biochem. Biophys. Res. Commun.* **256**: 127–132.
- Suzuki, H., Chiba, T., Suzuki, T., Fujita, T., Ikenoue, T., Omata, M., Furuichi, K., Shikama, H., and Tanaka, K. 2000. Homodimer of two F-box proteins β TrCP1 or β TrCP2 binds to I κ B α for signal-dependent ubiquitination. *J. Biol. Chem.* **275**: 2877–2884.
- Suzuki, N., Suzuki, S., Duncan, G.S., Millar, D.G., Wada, T., Mirtsos, C., Takada, H., Wakeham, A., Itie, A., Li, S., et al. 2002. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature* **416**: 750–756.
- Tada, K., Okazaki, T., Sakon, S., Kobarai, T., Kurosawa, K., Yamaoka, S., Hashimoto, H., Mak, T.W., Yagita, H., Okumura, K., et al. 2001. Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF- κ B activation and protection from cell death. *J. Biol. Chem.* **276**: 36530–36534.
- Takada, H., Chen, N.J., Mirtsos, C., Suzuki, S., Suzuki, N., Wakeham, A., Mak, T.W., and Yeh, W.C. 2003. Role of SODD in regulation of tumor necrosis factor responses. *Mol. Cell. Biol.* **23**: 4026–4033.
- Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Ninomiya-Tsuji, J., and Matsumoto, K. 2000. TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol. Cell* **5**: 649–658.
- Takaesu, G., Ninomiya-Tsuji, J., Kishida, S., Li, X., Stark, G.R., and Matsumoto, K. 2001. Interleukin-1 (IL-1) receptor-associated kinase leads to activation of TAK1 by inducing TAB2 translocation in the IL-1 signaling pathway. *Mol. Cell. Biol.* **21**: 2475–2484.
- Takaesu, G., Surabhi, R.M., Park, K.J., Ninomiya-Tsuji, J., Matsumoto, K., and Gaynor, R.B. 2003. TAK1 is critical for I κ B kinase-mediated activation of the NF- κ B pathway. *J. Mol. Biol.* **326**: 105–115.
- Takeda, K., Takeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N., and Akira, S. 1999. Limb and skin abnormalities in mice lacking IKK α . *Science* **284**: 313–316.
- Takeda, K., Kaisho, T., and Akira, S. 2003. Toll-like receptors. *Annu. Rev. Immunol.* **21**: 335–376.
- Tam, W.F., Lee, L.H., Davis, L., and Sen, R. 2000. Cytoplasmic sequestration of rel proteins by I κ B α requires CRM1-dependent nuclear export. *Mol. Cell. Biol.* **20**: 2269–2284.
- Tanaka, M., Fuentes, M.E., Yamaguchi, K., Durnin, M.H., Dalrymple, S.A., Hardy, K.L., and Goeddel, D.V. 1999. Embryonic lethality, liver degeneration, and impaired NF- κ B activation in IKK- β -deficient mice. *Immunity* **10**: 421–429.
- Tang, G., Minemoto, Y., Dibling, B., Purcell, N.H., Li, Z., Karin, M., and Lin, A. 2001. Inhibition of JNK activation through NF- κ B target genes. *Nature* **414**: 313–317.
- Tang, E.D., Inohara, N., Wang, C.Y., Nunez, G., and Guan, K.L. 2003. Roles for homotypic interactions and transautophosphorylation in I κ B kinase (IKK β) activation. *J. Biol. Chem.* **278**: 38566–38570.
- Tegethoff, S., Behlke, J., and Scheidereit, C. 2003. Tetrameric oligomerization of I κ B kinase γ (IKK γ) is obligatory for IKK complex activity and NF- κ B activation. *Mol. Cell. Biol.* **23**: 2029–2041.
- Tergaonkar, V., Bottero, V., Ikawa, M., Li, Q., and Verma, I.M. 2003. I κ B kinase-independent I κ B α degradation pathway:

- Functional NF- κ B activity and implications for cancer therapy. *Mol. Cell. Biol.* **23**: 8070–8083.
- Thomassen, E., Renshaw, B.R., and Sims, J.E. 1999. Identification and characterization of SIGIRR, a molecule representing a novel subtype of the IL-1R superfamily. *Cytokine* **11**: 389–399.
- Thome, M. 2004. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat. Rev. Immunol.* **4**: 348–359.
- Ting, A.T., Pimentel-Muinos, F.X., and Seed, B. 1996. RIP mediates tumor necrosis factor receptor 1 activation of NF- κ B but not Fas/APO-1-initiated apoptosis. *EMBO J.* **15**: 6189–6196.
- Trompouki, E., Hatzivassiliou, E., Tschritzis, T., Farmer, H., Ashworth, A., and Mosialos, G. 2003. CYLD is a deubiquitinating enzyme that negatively regulates NF- κ B activation by TNFR family members. *Nature* **424**: 793–796.
- Uhlik, M., Good, L., Xiao, G., Harhaj, E.W., Zandi, E., Karin, M., and Sun, S.C. 1998. NF- κ B-inducing kinase and I κ B kinase participate in human T-cell leukemia virus I Tax-mediated NF- κ B activation. *J. Biol. Chem.* **273**: 21132–21136.
- Ventura, J.J., Kennedy, N.J., Lamb, J.A., Flavell, R.A., and Davis, R.J. 2003. c-Jun NH(2)-terminal kinase is essential for the regulation of AP-1 by tumor necrosis factor. *Mol. Cell. Biol.* **23**: 2871–2882.
- Vermeulen, L., De Wilde, G., Van Damme, P., Vanden Berghe, W., and Haegeman, G. 2003. Transcriptional activation of the NF- κ B p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *EMBO J.* **22**: 1313–1324.
- Wajant, H., Pfizenmaier, K., and Scheurich, P. 2003. Tumor necrosis factor signaling. *Cell Death Differ.* **10**: 45–65.
- Wald, D., Qin, J., Zhao, Z., Qian, Y., Naramura, M., Tian, L., Towne, J., Sims, J.E., Stark, G.R., and Li, X. 2003. SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. *Nat. Immunol.* **4**: 920–927.
- Wang, D. and Baldwin Jr., A.S. 1998. 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.
- Wang, C.Y., Mayo, M.W., Korneluk, R.G., Goeddel, D.V., and Baldwin Jr., A.S. 1998. NF- κ B antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**: 1680–1683.
- Wang, D., Westerheide, S.D., Hanson, J.L., and Baldwin Jr., A.S. 2000. Tumor necrosis factor α -induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J. Biol. Chem.* **275**: 32592–32597.
- Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., and Chen, Z.J. 2001. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**: 346–351.
- Waterfield, M.R., Zhang, M., Norman, L.P., and Sun, S.C. 2003. NF- κ B1/p105 regulates lipopolysaccharide-stimulated MAP kinase signaling by governing the stability and function of the Tpl2 kinase. *Mol. Cell* **11**: 685–694.
- Weih, F., Carrasco, D., Durham, S.K., Barton, D.S., Rizzo, C.A., Ryseck, R.P., Lira, S.A., and Bravo, R. 1995. Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF- κ B/Rel family. *Cell* **80**: 331–340.
- Weih, F., Durham, S.K., Barton, D.S., Sha, W.C., Baltimore, D., and Bravo, R. 1997. p50-NF- κ B complexes partially compensate for the absence of RelB: Severely increased pathology in p50^{-/-}relB^{-/-} double-knockout mice. *J. Exp. Med.* **185**: 1359–1370.
- Weil, R., Schwamborn, K., Alcover, A., Bessia, C., Di Bartolo, V., and Israel, A. 2003. Induction of the NF- κ B cascade by recruitment of the scaffold molecule NEMO to the T cell receptor. *Immunity* **18**: 13–26.
- Wesche, H., Henzel, W.J., Shillinglaw, W., Li, S., and Cao, Z. 1997. MyD88: An adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* **7**: 837–847.
- Wesche, H., Gao, X., Li, X., Kirschning, C.J., Stark, G.R., and Cao, Z. 1999. IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. *J. Biol. Chem.* **274**: 19403–19410.
- Whiteside, S.T., Epinat, J.C., Rice, N.R., and Israel, A. 1997. I κ B ϵ , a novel member of the I κ B family, controls RelA and cRel NF- κ B activity. *EMBO J.* **16**: 1413–1426.
- Winston, J.T., Strack, P., Beer-Romero, P., Chu, C.Y., Elledge, S.J., and Harper, J.W. 1999. The SCF β -TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in I κ B α and β -catenin and stimulates I κ B α ubiquitination in vitro. *Genes & Dev.* **13**: 270–283.
- Woronicz, J.D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D.V. 1997. I κ B kinase- β : NF- κ B activation and complex formation with I κ B kinase- α and NIK. *Science* **278**: 866–869.
- Wu, C. and Ghosh, S. 1999. β -TrCP mediates the signal-induced ubiquitination of I κ B β . *J. Biol. Chem.* **274**: 29591–29594.
- Wu, H. and Arron, J.R. 2003. TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *Bioessays* **25**: 1096–1105.
- Wu, C. and Ghosh, S. 2003. Differential phosphorylation of the signal-responsive domain of I κ B α and I κ B β by I κ B kinases. *J. Biol. Chem.* **278**: 31980–31987.
- Wulczyn, F.G., Naumann, M., and Scheidereit, C. 1992. Candidate proto-oncogene bcl-3 encodes a subunit-specific inhibitor of transcription factor NF- κ B. *Nature* **358**: 597–599.
- Xia, Y., Makris, C., Su, B., Li, E., Yang, J., Nemerow, G.R., and Karin, M. 2000. MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. *Proc. Natl. Acad. Sci.* **97**: 5243–5248.
- Xiao, G., Harhaj, E.W., and Sun, S.C. 2001. NF- κ B-inducing kinase regulates the processing of NF- κ B2 p100. *Mol. Cell* **7**: 401–409.
- Xiao, C., Shim, J.H., Kluppel, M., Zhang, S.S., Dong, C., Flavell, R.A., Fu, X.Y., Wrana, J.L., Hogan, B.L., and Ghosh, S. 2003. Ecsit is required for Bmp signaling and mesoderm formation during mouse embryogenesis. *Genes & Dev.* **17**: 2933–2949.
- Xiao, G., Fong, A., and Sun, S.C. 2004. Induction of p100 processing by NF- κ B-inducing kinase involves docking I κ B kinase α (IKK α) to p100 and IKK α -mediated phosphorylation. *J. Biol. Chem.* **279**: 30099–30105.
- Yamamoto, M., Sato, S., Hemmi, H., Sanjo, H., Uematsu, S., Kaisho, T., Hoshino, K., Takeuchi, O., Kobayashi, M., Fujita, T., et al. 2002. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* **420**: 324–329.
- Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., and Akira, S. 2003a. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* **301**: 640–643.
- Yamamoto, M., Sato, S., Hemmi, H., Uematsu, S., Hoshino, K., Kaisho, T., Takeuchi, O., Takeda, K., and Akira, S. 2003b. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat. Immunol.* **4**: 1144–1150.
- Yamamoto, Y., Verma, U.N., Prajapati, S., Kwak, Y.T., and Gaynor, R.B. 2003. Histone H3 phosphorylation by IKK- α is critical for cytokine-induced gene expression. *Nature* **423**: 655–659.
- Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S.T., Weil, R.,

- Agou, F., Kirk, H.E., Kay, R.J., and Israel, A. 1998. Complement cloning of NEMO, a component of the I κ B kinase complex essential for NF- κ B activation. *Cell* **93**: 1231–1240.
- Yamazaki, S., Muta, T., and Takeshige, K. 2001. A novel I κ B protein, I κ B- ζ , induced by proinflammatory stimuli, negatively regulates nuclear factor- κ B in the nuclei. *J. Biol. Chem.* **276**: 27657–27662.
- Yang, J., Lin, Y., Guo, Z., Cheng, J., Huang, J., Deng, L., Liao, W., Chen, Z., Liu, Z., and Su, B. 2001. The essential role of MEKK3 in TNF-induced NF- κ B activation. *Nat. Immunol.* **2**: 620–624.
- Yang, F., Tang, E., Guan, K., and Wang, C.Y. 2003. IKK β plays an essential role in the phosphorylation of RelA/p65 on serine 536 induced by lipopolysaccharide. *J. Immunol.* **170**: 5630–5635.
- Yang, J., Xu, Z.P., Huang, Y., Hamrick, H.E., Duerksen-Hughes, P.J., and Yu, Y.N. 2004. ATM and ATR: Sensing DNA damage. *World J. Gastroenterol.* **10**: 155–160.
- Yaron, A., Gonen, H., Alkalay, I., Hatzubai, A., Jung, S., Beyth, S., Mercurio, F., Manning, A.M., Ciechanover, A., and Ben-Neriah, Y. 1997. Inhibition of NF- κ B cellular function via specific targeting of the I- κ B-ubiquitin ligase. *EMBO J.* **16**: 6486–6494.
- Yaron, A., Hatzubai, A., Davis, M., Lavon, I., Amit, S., Manning, A.M., Andersen, J.S., Mann, M., Mercurio, F., and Ben-Neriah, Y. 1998. Identification of the receptor component of the I κ B α -ubiquitin ligase. *Nature* **396**: 590–594.
- Yeh, W.C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J.L., Ferrick, D., Hum, B., Iscove, N., et al. 1997. Early lethality, functional NF- κ B activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* **7**: 715–725.
- Yilmaz, Z.B., Weih, D.S., Sivakumar, V., and Weih, F. 2003. RelB is required for Peyer's patch development: Differential regulation of p52-RelB by lymphotoxin and TNF. *EMBO J.* **22**: 121–130.
- Yujiri, T., Ware, M., Widmann, C., Oyer, R., Russell, D., Chan, E., Zaitzu, Y., Clarke, P., Tyler, K., Oka, Y., et al. 2000. MEK kinase 1 gene disruption alters cell migration and c-Jun NH2-terminal kinase regulation but does not cause a measurable defect in NF- κ B activation. *Proc. Natl. Acad. Sci.* **97**: 7272–7277.
- Zandi, E., Rothwarf, D.M., Delhase, M., Hayakawa, M., and Karin, M. 1997. 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.
- Zandi, E., Chen, Y., and Karin, M. 1998. Direct phosphorylation of I κ B by IKK α and IKK β : Discrimination between free and NF- κ B-bound substrate. *Science* **281**: 1360–1363.
- Zelazowski, P., Carrasco, D., Rosas, F.R., Moorman, M.A., Bravo, R., and Snapper, C.M. 1997. B cells genetically deficient in the c-Rel transactivation domain have selective defects in germline CH transcription and Ig class switching. *J. Immunol.* **159**: 3133–3139.
- Zetoune, F.S., Murthy, A.R., Shao, Z., Hlaing, T., Zeidler, M.G., Li, Y., and Vincenz, C. 2001. A20 inhibits NF- κ B activation downstream of multiple Map3 kinases and interacts with the I κ B signalosome. *Cytokine* **15**: 282–298.
- Zhang, Y. and Chen, F. 2004. Reactive oxygen species (ROS), troublemakers between nuclear factor- κ B (NF- κ B) and c-Jun NH2-terminal kinase (JNK). *Cancer Res.* **64**: 1902–1905.
- Zhang, G. and Ghosh, S. 2002. Negative regulation of toll-like receptor-mediated signaling by Tollip. *J. Biol. Chem.* **277**: 7059–7065.
- Zhang, S.Q., Kovalenko, A., Cantarella, G., and Wallach, D. 2000. Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKK γ) upon receptor stimulation. *Immunity* **12**: 301–311.
- Zhang, L., Wang, W., Hayashi, Y., Jester, J.V., Birk, D.E., Gao, M., Liu, C.Y., Kao, W.W., Karin, M., and Xia, Y. 2003. A role for MEK kinase 1 in TGF- β /activin-induced epithelium movement and embryonic eyelid closure. *EMBO J.* **22**: 4443–4454.
- Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., and Ghosh, S. 1997. The transcriptional activity of NF- κ B is regulated by the I κ B-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* **89**: 413–424.
- Zhong, H., Voll, R.E., and Ghosh, S. 1998. Phosphorylation of NF- κ B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol. Cell* **1**: 661–671.
- Zhong, H., May, M.J., Jimi, E., and Ghosh, S. 2002. The phosphorylation status of nuclear NF- κ B determines its association with CBP/p300 or HDAC-1. *Mol. Cell* **9**: 625–636.
- Zhou, H., Wertz, I., O'Rourke, K., Ultsch, M., Seshagiri, S., Eby, M., Xiao, W., and Dixit, V.M. 2004. Bcl10 activates the NF- κ B pathway through ubiquitination of NEMO. *Nature* **427**: 167–171.

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