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Cell

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Review

Shared Principles in NF-κB Signaling

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DOI 10.1016/j.cell.2008.01.020

The transcription factor NF-κB has served as a standard for inducible transcription factors for more than 20 years. The numerous stimuli that activate NF-κB, and the large number of genes regulated by NF-κB, ensure that this transcription factor is still the subject of intense research. Here, we attempt to synthesize some of the basic principles that have emerged from studies of NF-κB, and we aim to generate a more unified view of NF-κB regulation.

Introduction

Twenty years following the identification of nuclear factor-κB (NF-κB) as a regulator of expression of the κB light chain in B cells, research into the function and regulation of the NF-κB family continues at a blistering pace. Advances in understanding how the immune system senses pathogens and processes this information through the activation of NF-κB, as well as an ever-expanding list of diseases in which dysregulation of NF-κB has been implicated, have continued to invite broad interest into the regulation of this inducible transcription factor. A PubMed search reveals close to 30,000 papers and, staggeringly, nearly 3,000 reviews related to NF-κB. Why then, one more? Part of the answer lies in these numbers themselves. The sheer volume of work in this area has allowed the field of NF-κB research to be both a source of signaling paradigms that have been broadly applied to other systems, as well as a melting pot in which ideas from disparate fields have merged, been modified, and matured into new concepts. Here we attempt to unify some of the recent advances into a cohesive framework encompassing key principles governing NF-κB signaling that illustrate this give and take.

The inducible regulation of gene expression is a central element of normal physiology and is the key to the ability of multicellular organisms to adapt to environmental, mechanical, chemical, and microbiological stresses. Owing to its amenability to experimentation and its importance in disease, NF-κB has served as a model of cell, tissue, and organism level responses that are orchestrated through inducible transcription factors. NF-κB plays its most important and evolutionarily conserved role in the immune system, regulating the expression of inducers and effectors at many points in the expansive networks that define responses to pathogens. Consequently much of our understanding of NF-κB is derived from studying immunologically relevant signaling pathways. The reach of NF-κB, however, extends to transcriptional regulation beyond the confines of the immune response, acting broadly to influence gene expression events that impact cell survival, differentiation, and proliferation.

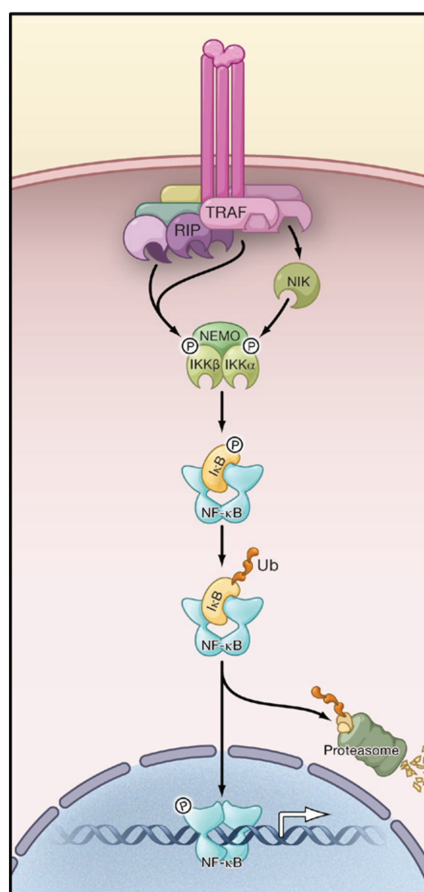

Figure 1. NF-κB Signaling Pathways
Following receptor ligation and recruitment of receptor proximal adaptor proteins, signaling to IKK proceeds through TRAF/RIP complexes, generally in conjunction with TAK1, leading to canonical NF-κB signaling, or through TRAFs and NIK leading to the noncanonical NF-κB pathway. IKK activation results in IκB phosphorylation and degradation in the canonical pathway or p100 processing to p52 in the noncanonical pathway. Phosphorylated NF-κB dimers bind to κB DNA elements and induce transcription of target genes.

The diversity of biological roles fulfilled by NF-κB raises several intriguing questions about how a limited set of signal transduction molecules regulates signaling to NF-κB in all pathways and, conversely, how discrete inputs create transcriptional responses tailored to particular tissues and organs with the same limited set of regulators. One corollary of so many pathways coalescing on one transcription factor, however, is that dysregulation of its function can have broad deleterious consequences. Indeed, the literature is filled with reports implicating dysregulation of NF-κB in various pathological situations (Courtois and Gilmore, 2006; Karin, 2006).

The progressively increasing size of the NF-κB literature has made writing a comprehensive review on NF-κB a daunting, if not untenable, undertaking. Instead, in the current Review we have attempted to highlight aspects of NF-κB regulation and function that reflect the broad physiologi-

cal and medical significance of this transcription factor and in particular focus on those areas that we feel are undergoing significant progress, e.g., regulatory mechanisms that illustrate shared principles that are likely to extend to pathways beyond those affecting NF-κB. We have organized this Review around the core components of the NF-κB pathway, namely the IKK complex, the inhibitory IκB proteins, and the transcription factor NF-κB itself. Thus the first section will deal with commonalities in signaling to IKK; the second with the organization and activation of IKK; the third with the emerging dualistic roles of IκB proteins; and the fourth with the interface between NF-κB and chromatin in the regulation of transcription.

Background

The basic scheme of NF-κB signaling consists of a series of positive and negative regulatory elements. Inducing stimuli trigger IKK activation leading to phosphorylation, ubiquitination, and degradation of IκB proteins (Figure 1). Released NF-κB dimers are further activated through various posttranslational modifications and translocate to the nucleus where they bind to specific DNA sequences and promote transcription of target genes. In its most basic form, therefore, the pathway consists of receptor and receptor proximal signaling adaptor molecules; the IKK complex; IκB proteins; and NF-κB dimers.

The NF-κB family of transcription factors consists of five members, p50, p52, p65 (RelA), c-Rel, and RelB, encoded by *NFKB1*, *NFKB2*, *RELA*, *REL*, and *RELB*, respectively, which share an N-terminal Rel homology domain (RHD) responsible for DNA binding and homo- and heterodimerization (Figure 2). NF-κB dimers bind to κB sites within the promoters/enhancers of target genes and regulate transcription through the recruitment of coactivators and corepressors. The transcription activation domain (TAD) necessary for the positive regulation of gene expression is present only in p65, c-Rel, and RelB. As they lack TADs, p50 and p52 may repress transcription unless associated with a TAD-containing NF-κB family member or other proteins capable of coactivator recruitment. Constitutive binding of p50 or p52 homodimers to κB sites on NF-κB-responsive promoters may thus act to check NF-κB transactivation until displaced by transcriptionally competent NF-κB dimers.

There is considerable structural information about NF-κB dimers in both its inactive IκB-bound form and active DNA bound state. Crystal structures of NF-κB dimers bound to κB sites reveal how the immunoglobulin-like domains that comprise the RHD contact DNA. The NH_2-terminal Ig-like domain confers selectivity for certain types of κB sites, whereas the hydrophobic residues within the C-terminal domain provide the dimerization interface between NF-κB subunits (Hoffmann et al., 2006). Unfortunately the three-dimensional structure of the C-terminal TAD has not been determined yet, most likely due to the disordered nature of the protein in this region. Although RHD and TAD function are typically considered independent, both domains undergo posttranslational modifications that can affect NF-κB transcriptional activity as well as DNA binding (Figure 2).

In its inactive state, NF-κB dimers are associated with one of three typical IκB proteins, IκBα (*NFKBIA*), IκBβ (*NFKBIB*), or IκBε (*NFKBIE*), or the precursor proteins p100 (*NFKB2*)

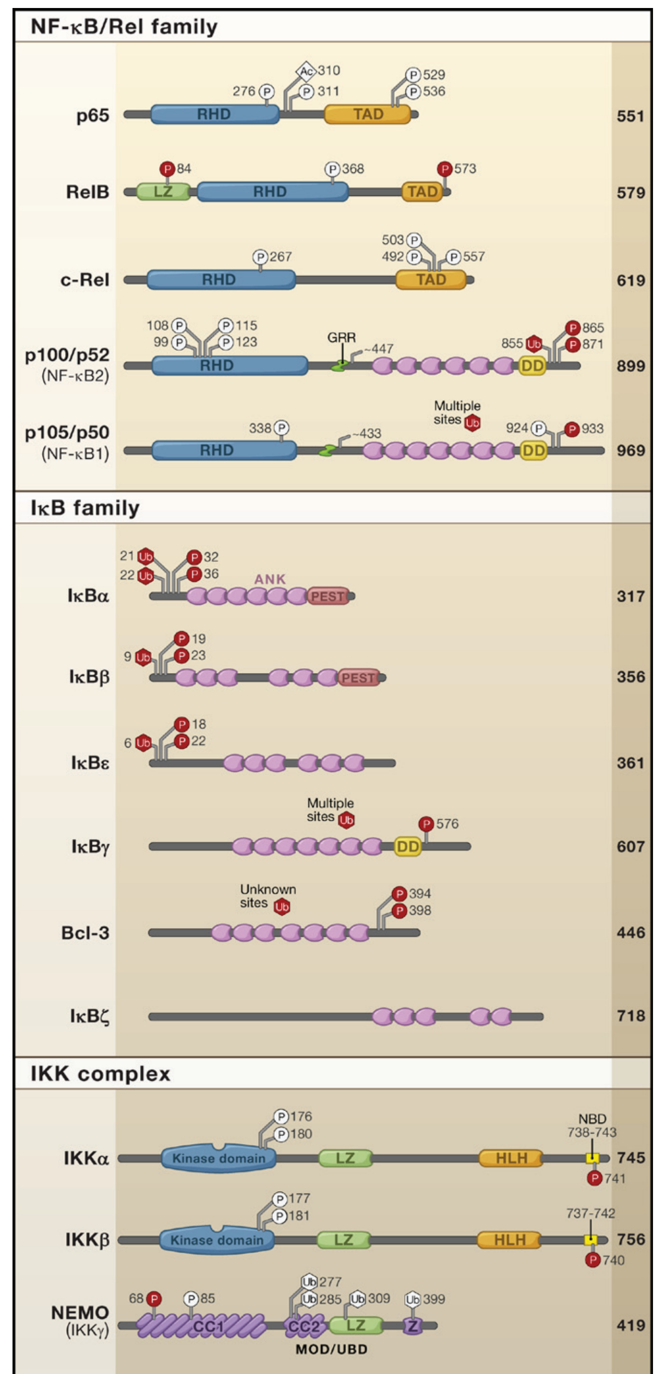

Figure 2. The NF-κB, IκB, and IKK Protein Families

Members of the NF-κB, IκB, and IKK proteins are shown. The number of amino acids in each human protein is indicated on the right. Posttranslational modifications that influence IKK activity or transcriptional activation are indicated with P, U, or Ac for phosphorylation, ubiquitination, or acetylation, respectively. Inhibitory events and phosphorylation and ubiquitination sites on p100, p105, and IκB proteins that mediate proteasomal degradation are indicated with red Ps and Us, respectively. RHD, Rel homology domain; TAD, transactivation domain; LZ, leucine zipper domain; GRR, glycine-rich region; HLH, helix-loop-helix domain; Z, zinc finger domain; CC1/2, coiled-coil domains; NBD, NEMO-binding domain; MOD/UBD, minimal oligomerization domain and ubiquitin-binding domain; and DD, death domain.

and p105 (*NFKB1*). These IκBs maintain NF-κB dimers in the cytoplasm and are crucial for signal responsiveness. There are two inducibly expressed, atypical IκB proteins, Bcl-3 (*BCL3*) and IκBζ (*NFKBZ*), that function quite differently in the regulation of NF-κB (discussed below). Lastly an alternative transcript of the *NFKB1* gene in mouse encodes an IκB molecule, IκBγ, whose biological role remains unclear. All IκB proteins are characterized by the presence of multiple ankyrin repeat domains (Figure 2). The prototypical and most extensively studied member of the family is IκBα. IκBα is rapidly degraded during activation of canonical NF-κB signaling pathways leading to the release of multiple NF-κB dimers, although the p65:p50 heterodimer is likely the primary target of IκBα. The established model of IκB function posits that IκBα retains NF-κB dimers in the cytoplasm, thereby preventing their nuclear translocation and subsequent DNA binding; however, the situation is actually more complex. The crystal structure of IκBα bound to the p65/p50 heterodimer reveals that the IκBα protein masks only the nuclear localization sequence (NLS) of p65, whereas the NLS of p50 remains exposed. The exposed NLS of p50 coupled with nuclear export sequences (NES) in IκBα and p65 leads to constant shuttling of IκBα/NF-κB complexes between the nucleus and the cytoplasm, despite steady-state localization that appears almost exclusively cytosolic (Ghosh and Karin, 2002). Degradation of IκBα drastically alters the dynamic balance between cytosolic and nuclear localization signals to favor nuclear localization of NF-κB. The noncanonical or alternative NF-κB pathway, however, proceeds through proteasomal processing, rather than degradation, of p100 to p52, thereby liberating p52 containing NF-κB dimers that drive a transcriptional response that is distinct from that induced by the canonical, IκBα-regulated pathway. In part because IκBα degradation and p100 processing regulate different populations of NF-κB dimers, canonical and noncanonical NF-κB pathways regulate distinct sets of target genes.

Degradation of IκB is a rapidly induced signaling event that is initiated upon specific phosphorylation of these molecules by activated IKK. The IKK complex contains two highly homologous kinase subunits, IKKα/IKK1 (*CHUK*) and IKKβ/IKK2 (*IKBKB*), and a regulatory subunit NEMO (NF-κB essential modulator)/IKKγ (*IKBKG*) (Hacker and Karin, 2006). Although they are generally found in a heteromeric kinase complex, IKKα and IKKβ are somewhat selectively required for specific NF-κB signaling pathways. In most canonical NF-κB signaling, e.g., downstream of TNFR1, IKKβ is both necessary and sufficient for phosphorylation of IκBα on Ser32 and Ser36 and of IκBβ on Ser19 and Ser23. While not generally required for IκBα phosphorylation and degradation in canonical signaling pathways, IKKα can mediate IκBα phosphorylation and appears to play a critical role in canonical NF-κB-dependent transcriptional responses (discussed below). The noncanonical pathway, conversely, depends only on the IKKα subunit, which functions by phosphorylating p100 and causing its inducible processing to p52. The noncanonical pathway is activated by a subset of TNFR superfamily members, while the canonical pathway is activated by a broader and overlapping array of receptors.

Phosphorylation of the conserved serine residues (DS*GXXS*) in IκB proteins results in their K48-linked polyubiquitination by βTrCP containing Skp1-Culin-Roc1/Rbx1/Hrt-1-F-box (SCF) E3 ubiquitin ligase complexes ($SCF^{\beta TrCP}$) coordinately with the E2 UbcH5 (Perkins, 2006). The released NF-κB dimers bind promoter and enhancer regions containing κB consensus sequences 5′ GGGRNWYYCC 3′ (N—any base; R—purine; W—adenine or thymine; and Y—pyrimidine) (Hoffmann et al., 2006). The degenerate nature of the κB site sequence, which shows far greater sequence variability than the consensus sequence given here, combined with the varied binding preferences of NF-κB dimers yields the large list of NF-κB-regulated genes (Gilmore, 2008). Transcription of target genes is further regulated through posttranslational modifications of NF-κB that affect the ability of NF-κB dimers to interact with transcriptional coactivators. NF-κB-dependent transcription of IκB proteins as well as additional mechanisms targeting DNA-bound NF-κB dimers terminate the response.

Signaling to IKK

A remarkable diversity of stimuli lead to activation of NF-κB. These include both endogenous and exogenous ligands as well as a plethora of physical and chemical stresses (Gilmore, 2008). There has been great progress in identifying the protein components of pathways that culminate in IKK activation and many of the remaining gaps in our knowledge are being rapidly filled in. However, while the identities of these molecules provide critical clues, much remains to be done to understand the mechanism(s) of IKK activation.

Strikingly, work in numerous signaling pathways leading to NF-κB has demonstrated that many of the signaling intermediates, especially those just upstream of the IKK complex, are shared. Signaling to NF-κB proceeds through intracellular adaptor proteins that provide modularity to NF-κB activation pathways and allows their incorporation into various receptor-induced signaling events. Thus diverse signaling pathways can utilize several shared components for both activating and inhibitory pathways. In particular, RIP and TRAF families of proteins play similar roles in most pathways that lead to IKK activation (Figure 1). Although there are exceptions, some of which are noted below, it appears that both canonical and noncanonical pathways utilize TRAF family members for activation, while only canonical, NEMO-dependent signaling to typical IκBs additionally requires RIP proteins.

The core elements of NF-κB signaling pathways are generally several steps removed from the receptor itself. The intervening steps between receptor and IKK form links to parallel signaling pathways. For example, IL-1R and RIG-I, which both signal through TRAF6 to IKK, do so through distinct receptor proximal adaptor components MyD88 and MAVS, respectively (Hacker and Karin, 2006). Thus when IL-1R binds IL-1 or RIG-I binds cytoplasmic dsRNA they activate overlapping but unique signaling pathways due to these differences in upstream signaling components, and hence they induce distinct transcriptional programs. It is important to bear in mind that although diverse upstream events may mediate IKK activation through a common mechanism, parallel signaling pathways emanating from nonredundant receptor proximal sig-

naling components frequently produce crosstalk that *shapes* the NF-κB response in ways that are unique to individual signaling pathways.

One striking area of growth in the field of NF-κB signaling has been in the characterization of the role of K63-linked, or regulatory, ubiquitination. This area of research began with the biochemical characterization of TRAF6 as an E3 ligase that with the E2 ligase Ubc13/Uev1A could catalyze the formation of regulatory ubiquitin chains and induce IKK activation in vitro (Deng et al., 2000). Subsequently, several signaling components have been shown to be modified by K63-linked ubiquitin moieties following stimulation (Chen et al., 2006). While there are numerous reports demonstrating K63 ubiquitination of various signaling proteins, what continues to be lacking is an understanding of how regulatory ubiquitination functions during signaling. The delayed kinetics of regulatory ubiquitination and the observation that it often targets a very small fraction of any given protein, even within signaling complexes, suggest that K63 ubiquitination may be a consequence of certain adapters with E3 ligase activity aggregating with other proteins during NF-κB signaling. Even for particularly robustly and rapidly ubiquitinated adapters such as IRAK1 and MALT1, it is unclear whether K63-linked ubiquitination occurs before IKK activation and IκBα degradation (Oeckinghaus et al., 2007; Windheim et al., 2008). Because K63-linked ubiquitination has not been clearly shown to precede IKK activation, it remains to be determined whether these events are intrinsic to the act of signaling or necessary for the competence of the signaling pathways in which they occur. One piece of evidence that strongly suggests that K63 ubiquitination does play an active role in signaling is the existence of several deubiquitinases (DUBs), most notably A20, that provide negative feedback in NF-κB signaling pathways (Chen et al., 2006). More genetic evidence and mechanistic insight are still needed for the centrality of regulatory ubiquitination in NF-κB signaling to be unequivocally accepted.

TRAFs—Adapters in Most NF-κB Pathways

TRAFs are key intermediates in nearly all NF-κB signaling pathways; the DNA-damage response appears to be the only notable exception (Hacker and Karin, 2006; Scheidereit, 2006). There are seven TRAF proteins that share a C-terminal TRAF domain, consisting of a coiled-coil domain that mediates both homo- and heterotypic protein-protein interactions. In addition, TRAFs 2–7 have N-terminal RING finger domains that may function as E3 ubiquitin ligases by catalyzing the transfer of ubiquitin to target proteins—a function that has been demonstrated most clearly for TRAFs 2 and 6. Among the TRAF proteins, TRAF2, TRAF5, and TRAF6 have been most extensively characterized as positive regulators of signaling to NF-κB. Research focused on the role of K63-linked ubiquitination in NF-κB signaling over the past 7 years has established an important role for TRAF E3 ligase activity in the activation of the IKK complex leading to both canonical and noncanonical NF-κB pathways and demonstrated the existence of K63 ubiquitination of multiple pathway components (Chen et al., 2006). In addition to NF-κB, TRAF proteins are necessary in several other pathways, e.g., AP-1, and therefore serve as branch points downstream of multiple receptors. The highly studied TNFR1 and Toll/IL-1R signaling pathways have provided the clearest evidence of the function of TRAF proteins in IKK activation (Hacker and Karin, 2006; Hayden et al., 2006).

Following binding of TNFα, TRAF2 is recruited to TNFR1 through its interaction with TRADD (Hsu et al., 1996). However, despite deficiencies in AP-1 activation, TRAF2-deficient cells have relatively intact TNF signaling to NF-κB (Yeh et al., 1997). TRAF5 was also shown to interact with the TNFR1 signaling complex, yet TRAF5 knockouts also exhibit normal NF-κB activation by TNF; TRAF2/5 double knockout cells, however, are defective in IKK activation (Nakano et al., 1999; Tada et al., 2001; Yeh et al., 1997). While the E3 ligase activity of TRAF2 is thought to be required for IKK activation there are several caveats to this assumption. First, deletion of the RING finger domain abrogates the ability of TRAF2 to recruit IKK to the receptor complex making it difficult to assess the importance of TRAF2 E3-ligase activity independent of adaptor function in NF-κB activation (Devin et al., 2000). Second, knockdown of the K63-specific E2 ligase Ubc13 blocks TRAF2 autoubiquitination but not activation of NF-κB, while *ubc13*$^{-/-}$ macrophages show a similar lack of effect on NF-κB activation (Habelhah et al., 2004; Yamamoto et al., 2006a). Intriguingly, another group did observe partial defects in TNF signaling to NF-κB in *ubc13*$^{+/-}$ heterozygous macrophages and splenocytes (Fukushima et al., 2007). Therefore, in TNFR1 signaling the function of TRAF2 ubiquitin ligase activity remains to be more definitively established. It is, however, safe to conclude that TRAF2 and TRAF5 are together required for NF-κB activation by TNFR1.

In Toll/IL-1 signaling TRAF6 is recruited to the receptor complex and is necessary for MyD88-dependent activation of NF-κB by IL-1 and ligands of TLR4 (Hacker and Karin, 2006; Hayden et al., 2006). However, like TRAF2, the importance of the E3-ligase activity of TRAF6 remains controversial. Reconstitution of TRAF6-deficient cells with a TRAF6 mutant lacking the signature motif of E3 RING-finger ligases—that is, the RING finger itself—completely restored IL-1-induced activation of NF-κB but not activation of JNK (Kobayashi et al., 2001). More recently, however, it has been shown that a ring finger point mutation of TRAF6 is unable to restore NF-κB activation in TRAF6 knockout cells (Lamothe et al., 2007). Therefore, the role of the TRAF6 E3 activity is yet to be definitively established. Deletion of Ubc13, likewise, yields conflicting results. Ubc13 knockouts failed to show significant defects in TRAF6-mediated activation of NF-κB downstream of LPS, IL-1, CD40, or BAFF despite impaired MAPK activation (Yamamoto et al., 2006a). Heterozygous splenocytes and macrophages, however, showed a mild defect in LPS-induced IκBα degradation, less severe than the loss of p38 phosphorylation, and in these mice BCR signaling to NF-κB appeared normal (Fukushima et al., 2007). In T cell receptor signaling, conditional ablation of Ubc13 resulted in a partial, but significant, reduction in NF-κB activation, although activation of JNK and TAK1 was more severely impaired (Yamamoto et al., 2006b). Therefore, it is probably too early to draw a definitive conclusion about the general role of TRAF/Ubc13-mediated ubiquitination in IKK activation.

In addition to TRAF2 and TRAF6, the ubiquitin ligase activity of TRAF3 has been reported to regulate NF-κB signaling pathways. The noncanonical NF-κB pathway is characterized

by processing of p100 to p52 and by its independence from IKKβ and NEMO. Instead the alternative pathway relies on the activation of IKKα by the NF-κB-inducing kinase (NIK) (Hacker and Karin, 2006; Scheidereit, 2006). TRAF3, which interacts with receptors that trigger the alternative pathway (Hauer et al., 2005), also interacts with NIK and it now appears that the activation of NIK is negatively regulated by TRAF3. In the resting state, TRAF3 induces NIK ubiquitination and degradation, but upon stimulation TRAF3 undergoes signal-dependent degradation, mediated by other TRAF family members, resulting in the accumulation and activation of NIK and consequent activation of the noncanonical pathway (Liao et al., 2004). Recently this negative role of TRAF3 has been demonstrated genetically by rescuing the lethality of TRAF3-deficient mice by deleting the p100 gene (He et al., 2006). It remains unclear how alternative pathway signaling through degradation of TRAF3 might affect the ability of TRAF3 to fulfill its additional role as a key mediator of TLR-induced type I interferon responses (Hacker et al., 2006; Oganesyan et al., 2006).

Thus, TRAF proteins seem to play a crucial role in receptor-induced IKK activation in both canonical and noncanonical pathways. It remains unclear whether they act primarily by catalyzing K63-linked ubiquitination or as adaptor proteins. TRAF proteins may directly recruit the IKK complex through IKKα or IKKβ binding (Devin et al., 2001), although in most signaling pathways additional IKK recruitment mechanisms have been reported. For example, in antigen receptor signaling IKK can be recruited through an interaction with PKC family members while in TNFR1 signaling, IKK may be recruited to the receptor complex through the RIP1 kinase. The key remaining questions are to address the mechanism(s) by which TRAF proteins contribute to IKK activation, particularly in conjunction with RIP proteins, and their contributions to NF-κB pathways in which their role has not yet been definitively established.

RIPs—Key Adapters in Canonical NF-κB Signaling

Receptor-interacting proteins (RIPs) appear to act both upstream of and with TRAF proteins to activate IKK. RIP proteins act as true adapters in NF-κB signaling pathways by interacting with upstream signaling cassettes through well-characterized protein-binding domains and recruiting the IKK complex through NEMO binding. RIP family members have been implicated in most TRAF-dependent pathways, e.g., signaling from TNFR superfamily and Toll/IL-1R. Additionally, there are also several pathways in which RIP family members are important, even though the requirement for TRAFs is less clear. These RIP-dependent/TRAF-independent IKKβ activation pathways may include antigen receptor signaling as well as signaling in response to DNA damage.

There are seven RIP family kinases that are characterized by their conserved serine/threonine kinase domains (Meylan and Tschopp, 2005). RIP1 and RIP3 also share the RIP homotypic interaction motif (RHIM). RIP1 possesses a death domain (DD), which mediates interaction with other death domain-containing adapters and receptors. RIP3 was thought to function coordinately with RIP1, through RHIM-mediated interactions; however most NF-κB signaling pathways are normal in the absence of RIP3 (Newton et al., 2004). Instead, RIP3 may interact with and repress RIP1-induced NF-κB activation in specific instances (Meylan et al., 2004). RIP2 contains a C-terminal caspase activation and recruitment domain (CARD) that likewise mediates interactions with certain receptors and adaptors. RIP family members have also been implicated in canonical NF-κB signaling pathways where they appear to function in the TRAF-independent recruitment of the IKK complex by directly interacting with NEMO. Because the kinase activity of RIP1 and RIP2 has been shown to be dispensable for some NF-κB-activating pathways, it is thought that RIPs may act as adapters and scaffolds in facilitating TRAF-induced IKK activation (Lee et al., 2004; McCarthy et al., 1998). Similar to TRAFs, the TNFR1 signaling pathway has served as a model of RIP function in NF-κB activation.

RIP1 binds to NEMO and is essential for TNFα-induced IKK and NF-κB activation (Hsu et al., 1996; Kelliher et al., 1998; Ting et al., 1996; Zhang et al., 2000). In the absence of RIP1, IKK recruitment occurs through TRAF2 but does not lead to IKK activation (Devin et al., 2001). Thus RIP1 has a role that extends beyond the simple recruitment of the IKK complex. Although a typical phosphorylation cascade might have provided an appealing mechanism for RIP action, surprisingly RIP1 kinase activity was shown to be dispensable for IKK activation (Lee et al., 2004). RIP1 may instead nucleate the assembly of a signaling complex that induces IKK activation through oligomerization of NEMO and subsequent autophosphorylation of IKK (Delhase et al., 1999). Recent reports have indicated that this event might serve as a paradigm for the role of regulatory ubiquitination in signaling. RIP1 is inducibly ubiquitinated by TRAF2 following TNFα stimulation, and it has been reported that the mutation of acceptor lysines on RIP1 abrogates NEMO binding and IKK activation (Ea et al., 2006; Li et al., 2006a). A careful analysis of these data, however, suggests that the RIP1 lysine acceptor mutants that fail to be ubiquitinated also do not associate with TNFR1, thereby leaving the role of RIP1 ubiquitination unclear (Ea et al., 2006). Furthermore, while TNFα-induced RIP1 ubiquitination appears to be completely abolished in *traf2*$^{-/-}$ cells, TNFα-induced IKK activation remains partly intact (Lee et al., 2004). In TNFR1 signaling, therefore, there is a clear requirement for RIP1 acting as an adaptor protein mediating IKK recruitment and activation, although the mechanism by which activation is achieved remains unclear.

In addition to TNFR1 signaling, and IKK activation via other death domain-containing TNFR family members, RIP1 has also been reported to be required for TRIF-dependent NF-κB activation via TLR3 and TLR4, as well as for NF-κB activation via RIG-I (Cusson-Hermance et al., 2005; Meylan et al., 2004). Furthermore, RIP1 is necessary for DNA-damage-induced activation of IKK via the PIDDosome (Hur et al., 2003; Janssens et al., 2005). Interestingly, RIP1 is not required for activation of the noncanonical pathway through CD40 or LTβR (Vivarelli et al., 2004). Therefore, RIP1 appears to be required for activation of a subset of canonical, but not noncanonical, NF-κB signaling pathways. Regulation of NIK directly by TRAFs and the binding of NEMO by RIP1 are consistent with RIP proteins not being involved in noncanonical signaling.

In addition to RIP1, RIP2 has been widely implicated in signaling pathways to NF-κB. RIP2 contains a C-terminal CARD, a homotypic interaction motif that allows RIP2 to function in a

distinct set of NF-κB signaling pathways. The antigen receptor signaling pathways have shed significant light on the role of CARDs in the activation of NF-κB. Both BCL10 and CARD11 (CARMA1) are CARD-containing proteins that are crucial for IKK activation downstream of either the T cell or B cell antigen receptors. The most notable feature of the BCL10, CARD11, MALT1 (CBM) complex is its induced oligomerization following signaling, which is thought to be a key event in IKK activation downstream of these CARD-containing adaptor proteins (Schulze-Luehrmann and Ghosh, 2006). RIP2 seems to be required for BCL10-mediated NF-κB signaling (Ruefli-Brasse et al., 2004). Whether antigen receptors also act through TRAF family proteins to signal to NF-κB has yet to be shown conclusively. TRAF6, perhaps partially complemented by TRAF2, is the most likely TRAF downstream of the CBM complex; however this remains controversial (Scheidereit, 2006; Schulze-Luehrmann and Ghosh, 2006).

Members of the NOD-LRR family of intracellular pattern recognition receptors are CARD-containing proteins that can activate IKKβ via RIP2. RIP2 binds to NEMO and is believed to directly mediate activation of the IKK complex by proximity-induced mechanisms (Inohara et al., 2000). However, more recent results suggest that RIP2 acts with TAK1 and TRAFs to induce NEMO ubiquitination and downstream signaling pathways (Abbott et al., 2007; Kim et al., 2008). Similar to RIP1, the kinase activity of RIP2 does not appear to be required, and RIP2 is also ubiquitinated through the action of Ubc13/TRAF6 (Hasegawa et al., 2007; Park et al., 2007; Yang et al., 2007). The role of TRAF proteins has not been clearly delineated in NOD-LRR-mediated NF-κB activation. It is possible that confusion on this issue may be the result of different TRAF family members, TRAF2, 5, and 6, serving redundant or compensatory functions, similar to the situation seen for TRAF2 and TRAF5 in TNFR1 signaling (Abbott et al., 2007; Hasegawa et al., 2007).

Thus, RIP proteins, RIP1 as a DD to NEMO adaptor and RIP2 as a CARD to NEMO adaptor, seem to play an analogous role in several canonical NF-κB activation pathways. RIP proteins recruit the IKK complex through binding to NEMO and may mediate activation of the complex through direct oligomerization or ubiquitin-dependent mechanisms. Whether TRAF proteins directly contribute to this RIP function through ubiquitination or do so indirectly is unclear, although the field may have moved significantly closer with the identification of the key role of TAK1 in most RIP-dependent NF-κB pathways.

TAK1/NIK—IKK Kinases?

Signaling to IKK downstream of RIPs and TRAFs depends on several kinases that have been implicated in NF-κB signaling pathways. In the case of canonical NF-κB pathways this role is largely fulfilled by TAK1 (TGFβ-activated kinase-1) (Sato et al., 2005; Shim et al., 2005). In noncanonical pathways NIK is instead required for IKKα activation and p100 phosphorylation (Senftleben et al., 2001; Xiao et al., 2001). Whether NIK and TAK1 function analogously to one another, that is as putative IKK kinases (IKK-Ks), is a matter of some debate. Despite the clear requirement for TAK1 in multiple signaling pathways to IKK, the mechanism of action of TAK1 in signaling to NF-κB remains unclear. First, genetic ablation of TAK1 reveals variable NF-κB activation deficiencies in different canonical signaling pathways. TNFR1 signaling to NF-κB appears completely abolished, while the effect on antigen receptor signaling is more controversial (Liu et al., 2006; Sato et al., 2005; Wan et al., 2006). Second, whether TAK1 directly serves as an IKK-K or mediates activation through an intermediary kinase, MEKK3 for example, is not yet clear (Blonska et al., 2005; Li et al., 2006a). Third, even though the pathways triggered by LTβR lead to IKK through many of the same signaling intermediates, this pathway does not depend on TAK1 (Shim et al., 2005). Instead it seems that TAK1 generally functions in pathways that also require RIP for the activation of IKK. Indeed, RIP may be responsible for the recruitment of TAK1 (Blonska et al., 2005). TAK1 has been implicated in both antigen receptor and NOD signaling pathways, neither of which has yet been shown conclusively to depend on TRAF proteins, although there are indications that this is likely to be the case (Abbott et al., 2007; Hasegawa et al., 2007; Sun et al., 2004). Alternatively, it may be that some pathways that signal independently of RIPs, which to date includes mainly noncanonical pathways, may activate IKK without TAK1. Conversely, NIK can activate the noncanonical pathway in the absence of RIP proteins.

NIK directly phosphorylates and activates IKKα and this model is supported by analyses of *NIK*$^{-/-}$ and *aly/aly* mice, which bear an inactivating point mutation in the NIK kinase (Hacker and Karin, 2006). Regulation of NIK is regulated by the combined action of TRAF proteins, as discussed above. In addition to TRAF3, cIAP1 and cIAP2 have been implicated as E3 ligases responsible for regulating constitutive NIK levels (Petersen et al., 2007; Varfolomeev et al., 2007; Vince et al., 2007). Degradation of cIAP downstream of noncanonical stimuli may function, like degradation of TRAF3, in leading to the accumulation of NIK and IKKα activation (Varfolomeev et al., 2007). However, the mechanism of cIAP regulation following receptor ligation needs to be further characterized.

In summary, a few common signaling components mediate activation of IKK under most circumstances. In the noncanonical pathway, TRAF and NIK are sufficient to activate IKKα in a NEMO-independent manner. However, the canonical pathway appears more complex. Generally canonical signaling relies on both TRAF and RIP proteins, as well as the kinase TAK1, although in certain pathways other proteins, e.g., IRAK1, may function analogously to RIP. It seems that the key to this difference is likely to lie in binding of RIP, or analogous proteins, to NEMO, as both of these components may be universally required for canonical NF-κB activation. Whether this interaction allows TAK1 or another kinase to phosphorylate IKK or promotes IKK transautophosphorylation remains a matter of continuing debate. If there is no IKK-K in canonical pathways, however, then the exact function of TAK1 in IKK activation remains to be discovered.

Organization and Activation of the IKK Complex

Although exceptions have been reported, it is generally accepted that activation of NF-κB requires activation of either IKKα or IKKβ. It therefore follows that understanding the regulation of IKK activity is central to understanding the activation of NF-κB. Despite the significance of IKK, major gaps remain in our knowledge of the biochemistry of the IKK complex.

However, several recent advances suggest that the field is on the verge of significant breakthrough into understanding the mechanism of IKK activation.

The IκB kinase was first purified as a basally active, high-molecular-weight complex capable of phosphorylating serines 32 and 36 of IκBα (Chen et al., 1996). A stimulus-dependent kinase activity was subsequently identified by several groups and found to be composed of the two catalytic kinase subunits, IKKα (IKK1) and IKKβ (IKK2), and a regulatory subunit NEMO (IKKγ) (DiDonato et al., 1997; Mercurio et al., 1997; Rothwarf et al., 1998; Woronicz et al., 1997; Yamaoka et al., 1998; Zandi et al., 1997; Regnier et al., 1997). IKKα and IKKβ, along with IKKi (IKKε) and TBK1, comprise the IKK family of proteins. IKKα and IKKβ share 52% overall sequence identity, with a greater degree of similarity in the catalytic domain (65%). NEMO is a 48 kDa protein that is not related to IKKα and IKKβ and contains a C-terminal Zn finger-like domain, a leucine zipper, and N-terminal and C-terminal coiled-coil domains (Figure 2).

Targeted disruption of each of the IKK genes as well as transgenic and conditional knockout animals have been generated and extensively analyzed and reviewed recently (Gerondakis et al., 2006; Pasparakis et al., 2006). Initially the similarity between the IKKβ knockout and p65 knockout phenotypes argued for a central role for IKKβ in activation of p65 dimers via IκBα phosphorylation. Mice deficient in IKKα survive embryonic development but die perinatally due to multiple morphological defects, in particular aborted epidermal and skeletal development. While initially it appeared that IKKα was dispensable for classical NF-κB activation, subsequent reports revealed the requirement for IKKα in multiple noncanonical NF-κB signaling pathways and perhaps some canonical signaling pathways as well (Solt et al., 2007; Takaesu et al., 2003). Furthermore, as discussed below, while not required for IκBα degradation in all pathways, IKKα likely plays an important role in NF-κB-dependent gene expression in canonical signaling pathways. NEMO is required for signaling in all canonical NF-κB pathways and NEMO-deficient mice also die embryonically of massive hepatocyte apoptosis. NEMO-deficient cells exhibit a more severe and broader loss of NF-κB activation than do IKKβ knockout cells, demonstrating that some canonical pathways are intact in the absence of IKKβ (Schmidt-Supprian et al., 2000; Solt et al., 2007). Therefore, it is more appropriate to categorize NF-κB pathways as canonical or noncanonical based on the requirement for NEMO or on the specific IκB protein that is phosphorylated and degraded/processed, e.g., IκBα, IκBβ, and IκBε for canonical and p100 for noncanonical, rather than on the requirement for IKKα or IKKβ.

The IKK Complex

Multiple lines of evidence point toward an IKK kinase complex that is composed of only IKKα, IKKβ, and NEMO. Recombinant NEMO plus either IKKα or IKKβ assembles into a complex with an apparent molecular weight that is similar to the purified complex (Krappmann et al., 2000; Miller and Zandi, 2001). NEMO has a large Stoke's radius in vitro and consequently NEMO trimers with a predicted molecular weight of 150,000 elute at an apparent molecular weight of 550 kDa upon gel filtration (Agou et al., 2004). However, it remains to be seen whether NEMO assembled with IKK in vivo exhibits a similar discrepancy between predicted and actual molecular weights. Alternatively, this discrepancy may simply reflect an IKKα/β/NEMO complex of higher-order stoichiometry. Recombinant NEMO and IKKβ appear to associate in a 2:2 molar ratio and the minimum interaction domains form a dimer of dimers (tetramer) that can further assemble into octamers and dodecamers (Drew et al., 2007). These higher-ordered assemblies easily reach the molecular weight of the endogenous complex. Therefore, additional subunits are most likely not present in the active IKK complex, nor are they required to explain the large apparent molecular weight of the complex. Instead, based on existing biochemical evidence, and our own unpublished observations, it would appear that proteins reported to associate with the IKK complex do so in a transient and/or substoichiometric manner and do not reflect additional bona fide components of the IKK complex. Therefore, we confine our discussion of the IKK complex largely to the core IKK components IKKα, IKKβ, and NEMO.

IKKα and IKKβ dimerize through the leucine zipper domain, which is also required for kinase activity (Mercurio et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). It appears that IKKα and IKKβ preferentially form heterodimers in vivo, and in vitro studies indicate that IKKα/IKKβ heterodimers have higher catalytic efficiency than either homodimer (Huynh et al., 2000). While both IKKα and IKKβ homodimerize in cells lacking IKKβ or IKKα, respectively, there is little evidence that homodimers form under normal conditions. The exceptions are descriptions of IKKβ/IKKβ/NEMO complexes in T cells and an IKKβ-only complex in TNFα-stimulated HeLa cells (Khoshnan et al., 1999; Mercurio et al., 1999). Interestingly, T cells have been reported to express truncated IKKα isoforms lacking the leucine zipper that would presumably promote IKKβ homodimer formation (McKenzie et al., 2000). In addition, despite the lack of hard evidence, it is still commonly believed that IKKα homodimers, which are not bound to NEMO, exist in vivo because the noncanonical pathway can function in the absence of IKKβ or NEMO (Senftleben et al., 2001).

IKKα and IKKβ bind NEMO through the C-terminal hexapeptide NEMO-binding domain (NBD) (Leu-Asp-Trp-Ser-Trp-Leu) (May et al., 2000, 2002) (Figure 2). NEMO binding to IKKs requires at a minimum residues 47–80, located within the first coiled-coil motif (Drew et al., 2007; Marienfeld et al., 2006; May et al., 2002). Competition experiments and biophysical analyses using the NBD peptide indicate that IKKβ binds to NEMO with considerably higher affinity than IKKα (May et al., 2002 and unpublished data). Furthermore, IKKα is less sensitive to mutation of two residues within the NBD (Asp749 and Trp742) that abolish IKKβ binding to NEMO, indicating that the binding of IKKα is less stringent. Differences in NEMO-binding affinity may be crucial to understanding differences in IKKα and IKKβ function in vivo because swapping the IKKα and IKKβ C termini produces an IKKα that exhibits IKKβ-like behavior (Kwak et al., 2000). Furthermore, while IKKα lacking the leucine zipper, HLH domain and NBD is active upon overexpression, the same deletions abrogate IKKβ activity (McKenzie et al., 2000). Therefore the C-terminal region of IKK, which mediates both NEMO binding and dimerization, affects kinase activity and selection.

Although the core IKK complex most likely consists of only IKKα/IKKβ/NEMO, there are two well-characterized and highly cited interactions that deserve some discussion. The first is the kinase chaperone HSP-90/Cdc37 that has been reported to constitutively associate with the IKK complex. The HSP-90 inhibitor geldanamycin has also been shown to inhibit activation of IKK by TNF-α (Chen et al., 2002a). However, HSP-90 also associates with multiple kinases that are involved in the NF-κB pathway (Ghosh and Karin, 2002). It has recently been shown that rather than being an IKK complex component per se, HSP-90 is instead recruited by Cdc37 to IKK where it functions as a chaperone during assembly of the IKK complex, or during its reconstitution following signaling (Hinz et al., 2007). The second interacting protein that requires mentioning is ELKS, which has been proposed to be a regulatory component of the IKK complex in addition to NEMO (Ducut Sigala et al., 2004). While RNAi knockdown studies support a role for ELKS in IKK activation and immunodepletion analyses indicate that ELKS is a stoichiometric component of the IKK complex, the importance of ELKS in IKK function has not been established genetically, and its regulation remains to be characterized in detail (Ducut Sigala et al., 2004).

Activation of IKK

Activation of the IKK complex requires phosphorylation of T loop serines of at least one of the IKK subunits. However, the mechanism through which this phosphorylation occurs remains unclear. Active IKKβ is phosphorylated on two serines, Ser177 and Ser181, within the activation loop of the kinase domain, and IKKα is similarly phosphorylated on activation loop serine residues 176 and 180. Mutation of the activation loop serines to glutamic acid yields constitutively active IKK, while mutation to alanines abrogates signal responsiveness (Hacker and Karin, 2006). How enzymatic activity is regulated by phosphorylation of these serines can be surmised from comparison with other kinases but ultimately awaits a crystal structure of the IKK complex that, to date, has been elusive. Regardless of the precise conformational change induced by these key phosphorylation events, the fundamental question remains whether IKK phosphorylation occurs by transautophosphorylation or through phosphorylation by an upstream kinase (IKK-K).

There are several lines of evidence that can be used to support either IKK-K or transautophosphorylation mechanisms: in fact, to some extent, the same evidence can be used to support both models. One common element in IKK activation is the requirement for TRAF family members and the induced oligomerization of TRAFs following signaling. This commonality underscores the importance of the assembly of complex signalosomes for the activation of IKK. Further, oligomerization is universal even where TRAFs may not be required. In AgR signaling the CBM complex also assembles into a higher-order oligomer following signaling (Schulze-Luehrmann and Ghosh, 2006). Thus it appears that canonical NF-κB signaling pathways all lead to the assembly of large clusters of signaling components immediately upstream of IKK. Oligomerization of RIP proteins, RIP1 downstream of TRAFs and RIP2 downstream of CARD-containing adapters, may also provide a scaffold for IKK complex oligomerization (Inohara et al., 2000). Assembly of these signaling complexes suggests that IKK activation might occur through an induced proximity model of transautophosphorylation; however it is also possible that these same signalosomes could just as easily serve to position IKK near an IKK-K.

The C-terminal region of NEMO mediates activation of IKK and interaction with upstream signaling adapters, whereas it is the NEMO N terminus that is responsible for interaction with IKKs (Makris et al., 2002; Rothwarf et al., 1998). Inducible oligomerization of NEMO mediated by RIP1 has been speculated to activate IKK, and artificially enforced NEMO oligomerization leading to IKK activation further supports this hypothesis (Inohara et al., 2000; Poyet et al., 2000). As discussed above, NEMO can form tetramers in vitro and also is reported to oligomerize in vivo, although the stoichiometry of endogenous NEMO is debatable (Agou et al., 2004; Drew et al., 2007; Tegethoff et al., 2003). Nevertheless, mutation of sequences required for the observed oligomerization results in a loss of IKK activity, as does overexpression of the oligomerization domain alone (Agou et al., 2004; Tegethoff et al., 2003). Without structural information it is difficult to know how NEMO oligomerization might bring about IKK activation; however, three recent publications may shed some light on the mechanism of IKK activation (Marienfeld et al., 2006; Palkowitsch et al., 2008; Schomer-Miller et al., 2006).

It is possible that oligomerization of NEMO is required for transautophosphorylation of the IKK T loop serines or even to expose T loop serines to an IKK-K. Indeed, the kinase activity of wild-type IKKβ expressed in yeast is increased by coexpression of NEMO; however, the activity of IKK in which the T loop serines have been mutated to glutamic acid is high and unchanged by coexpression of NEMO (Schomer-Miller et al., 2006). Therefore, augmentation of IKK kinase activity by NEMO most likely occurs through facilitation of IKK T loop phosphorylation. The ability to recreate this process in yeast strongly suggests that transautophosphorylation occurs as a result of the formation of spontaneous higher-order structures of NEMO and IKK that display apparent molecular weights similar to that of activated IKK (Miller and Zandi, 2001). Transautophosphorylation is also consistent with the known dependence on IKK dimerization for IKK activation. Abrogation of both homo- and heterodimer formation by deletion or mutation of the IKK leucine zipper domain also blocks activation of overexpressed IKKs (McKenzie et al., 2000; Tang et al., 2003; Zandi et al., 1997). However, kinase activity can be restored by forced oligomerization of IKK using an unrelated dimerization interface (Tang et al., 2003). In addition, kinase-active IKKβ is capable of transautophosphorylation of kinase-dead IKKβ, and this activation requires leucine zipper-dependent dimerization or artificially enforced oligomerization (Tang et al., 2003). Intriguingly, phosphorylation of the IKK NBD serine residues (Ser740) inhibits IKK activity and prevents augmentation of recombinant IKK activity by coexpression with NEMO (May et al., 2002; Schomer-Miller et al., 2006). IKK lacking the NBD, on the other hand, exhibits constitutive activity in the absence of stimulation (May et al., 2002). Because IKK containing Ser740 mutated to Ala continues to interact with NEMO, these data suggest that the nature of the NBD/NEMO interaction is important for NEMO-mediated IKK activation (May et al., 2002; Schomer-

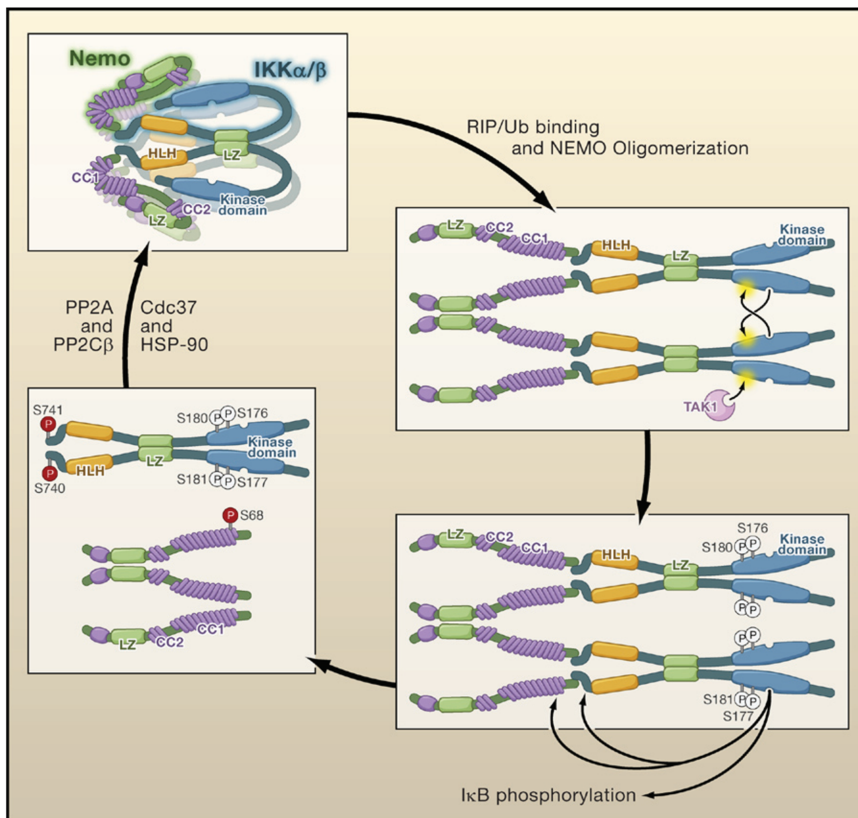

Figure 3. A Putative Model for IKK Activation
In the resting state, IKKα and IKKβ bind to NEMO such that activation is prevented. Conformational changes in the IKK complex induced by binding of NEMO to RIP, and/or ubiquitination of NEMO, lead to the exposure of IKK kinase domain and T loop serines and consequent transautophosphorylation or phosphorylation by an IKK-K such as TAK1. The active IKK then phosphorylates downstream substrates, including serine 740 within the IKK NBD and serine 68 in NEMO. NEMO phosphorylation results in the separation of stable NEMO dimers and NEMO binding to IKK. Dephosphorylation of the IKK T loop results in kinase inactivation, whereas phosphorylation of the IKK NBD and NEMO serine 68 prevents reactivation of the kinase. Cdc37/HSP-90-mediated chaperone activity and PP2A and PP2Cβ phosphatase activity may then mediate regeneration of the IKK complex.

Miller et al., 2006). In addition to undergoing stimulus-induced oligomerization, NEMO constitutively forms stable dimers, and both dimerization and IKK binding are independently required for activation of NF-κB (Marienfeld et al., 2006). While overexpression of the NEMO dimerization and IKK interaction domain alone (aa 1–197) can activate IKK, similar to the effect of a C-terminal portion containing the minimal oligomerization domain (MOD) (179–419), neither constructs facilitate IKK activation in the absence of full-length (FL) NEMO (Marienfeld et al., 2006).

However this interaction may be an important mechanism of negative feedback regulation. NEMO becomes phosphorylated at Ser68 within the IKK-binding domain following stimulation. This phosphorylation occurs in a stimulus-dependent manner and induces the disruption of NEMO dimers and interaction between IKK and NEMO, thereby terminating signaling (Palkowitsch et al., 2008). This separation of IKK and NEMO is further augmented by phosphorylation of the IKK NBD (Palkowitsch et al., 2008). These induced conformational changes may then allow recognition by the HSP-90 kinase chaperone complex or proposed IKK complex phosphatases, resulting in reconstitution of a signaling-competent IKK complex through either HSP-90 chaperone function or dephosphorylation of NEMO at Ser68.

Based on these findings the following model for IKK activation can be proposed (Figure 3). In the resting state, in $(\mathrm{NEMO}_2\mathrm{IKK}\alpha_1\mathrm{IKK}\beta_1)_2$ complexes, the IKKs are held inactive through their interaction with NEMO. Upon stimulation, NEMO binds to a RIP protein, perhaps in an ubiquitin-dependent manner, which induces a conformational change that allows transautophosphorylation or IKK-K phosphorylation of the IKK T loop serine residues. Active IKK then phosphorylates both downstream substrates such as IκBs, as well as Ser740 in IKKβ and Ser68 in NEMO. These phosphorylation events open up the complex allowing access to phosphatases that by dephosphorylating IKK T loop serines cause termination of kinase activity. The chaperone activity of HSP-90 and dephosphorylation of Ser740 of IKKβ may be required to overcome the high affinity of the IKKβ NBD-NEMO interaction. The lower affinity of the IKKα NBD for NEMO may allow active IKKα to escape from Ser68-phosphorylated NEMO/IKK complexes and further augment NF-κB activation through the phosphorylation of additional downstream targets.

Oligomerization appears to be a shared theme in signaling to NF-κB. There have been multiple reports describing oligomerization of TRAF proteins in multiple pathways that signal to NF-κB. RIP proteins, which are essential for canonical pathways, also form some degree of higher-order structures most likely as a result of aggregation of receptor and/or adaptor complexes. Furthermore, in certain pathways, such as NOD-LRRs, receptor and RIP oligomerization is particularly well characterized (Inohara and Nunez, 2003). RIP interaction with NEMO would thus be predicted to directly induce clustering of IKK complexes. RIP also becomes rapidly ubiquitinated following TNFα stimulation, and it has been proposed that it is through this posttranslational modification that RIP1 binds to NEMO (Wu et al., 2006). If this is the case then a single RIP molecule or, for that matter, ubiquitinated TRAF, IRAK-1, or MALT1 could provide an oligomeric ubiquitin scaffold mediating oligomerization or conformational changes in the IKK complex. Analysis of the interaction has been complicated by sequence overlap between the NEMO oligomerization domain and the NEMO K63-ubiquitin-binding domain (UBD). In addition to RIP, NEMO is also ubiquitinated in multiple NF-κB pathways. K63-linked NEMO ubiquitination has perhaps been demonstrated most clearly in AgR signaling, where it has been shown that mutation of a single lysine acceptor site prevents

NEMO ubiquitination, although this change did not completely inhibit NF-κB activation (Zhou et al., 2004). NEMO ubiquitination has also been shown to be important in the DNA-damage response, raising the possibility that this phenomenon may be linked to the TRAF independence of these pathways.

While it is clear that a variety of pathways require oligomerization and/or ubiquitination of upstream signaling components for IKK activation, these observations are not inconsistent with the additional requirement for IKK-Ks. These two mechanisms may operate separately in independent NF-κB pathways. In fact the necessity for NIK in activation of IKKα is well established and, as discussed above, appears to be independent of RIP proteins. Therefore in noncanonical signaling pathways, where RIP-mediated oligomerization of NEMO does not occur, IKKα activation may be mediated through direct T loop phosphorylation by NIK. The lower affinity of IKKα for NEMO may facilitate NIK access to the IKKα T loop but not to that of IKKβ, either as part of IKKα-only complexes or due to conformational differences between IKKα and IKKβ when bound to NEMO. As a result IKKα activation can be independent of RIP-mediated NEMO oligomerization, as IKKα is not regulated by NEMO in the same manner as IKKβ.

Activation of IKK activity is a transient event, and therefore IKK must also be subject to negative feedback regulation. There are numerous negative feedback mechanisms that affect upstream signaling components, most notably the deubiquitinases A20 and CYLD, which have been reviewed elsewhere recently (Chen et al., 2006; Hacker and Karin, 2006). However, there is also evidence for IKK intrinsic negative feedback mechanisms (Figure 3). Phosphorylation of the C-terminal NBD of IKK may be an important component of such a negative feedback loop (May et al., 2002; Schomer-Miller et al., 2006). Consistent with this hypothesis, the protein phosphatase 2A (PP2A) has been shown to associate with IKK and potentiate IKK activation in cells (Kray et al., 2005; Li et al., 2006b). Conversely, in vitro PP2A blocks IKK activity by removing T loop phosphorylation (DiDonato et al., 1997). In addition, structural changes induced by NEMO Ser68 and IKK NBD phosphorylation may allow dephosphorylation of the activation loop serines in IKKs as well as N-terminal phosphorylation sites in NEMO by PP2A or PP2Cβ (Kray et al., 2005; Palkowitsch et al., 2008; Prajapati et al., 2004).

IKK Substrates

When overexpressed or assayed in vitro, IKKα and IKKβ are both able to phosphorylate multiple members of the IκB family, although with differing specificities. For example, both IKKα and IKKβ phosphorylate IκBα at Ser32 and Ser36 and IκBβ at Ser19 and Ser23; however, IKKα is less efficient and consequently cannot complement IKKβ knockout cells (Hacker and Karin, 2006; Hayden and Ghosh, 2004). Furthermore, both IKKα and IKKβ prefer IκBα to IκBβ, which is consistent with the difference in IκBα and β degradation kinetics—IκBβ degradation is significantly slower than IκBα in most canonical pathways in which both are degraded (Wu and Ghosh, 2003). IκBα bound to NF-κB is thought to be a preferred substrate to free IκBα (Zandi et al., 1998). This might diminish the degradation of newly synthesized IκBα, thereby allowing this key negative feedback loop to function properly. Phosphorylation of p100 by IKKα occurs at Ser872 as well as at several N-terminal serine residues. While serines are also required for p100 processing, it is thought that these residues primarily function as a docking site for NIK and IKKα (Liang et al., 2006; Xiao et al., 2004). Phosphorylation of other IκB family members is less well characterized, and this is an area where additional research is clearly needed. Finally, IKKs have also been reported to phosphorylate many other proteins besides the IκB family. In particular, the role of IKKα in regulating NF-κB transcriptional responses, by targeting transcriptional cofactors, NF-κB itself, and other targets is discussed in the context of transcriptional regulation.

Expanding Functions for IκBs

Our understanding of how IκB proteins function has undergone significant change. In addition to being inhibitors of NF-κB function, both by preventing nuclear localization and DNA binding, more varied functions for IκBs have now been described. Most importantly, it now appears that multiple IκB family proteins positively regulate transcription by acting as coactivators.

The "Typical" IκBs—IκBα, IκBε, and IκBβ

IκBα is the prototypical member of the IκB family exhibiting the defining traits that characterize IκB proteins. The canonical p65/p50 heterodimer is largely, though not exclusively, found bound to IκBα, and rapid signal-induced IκBα proteasomal degradation is required for nuclear import and DNA binding by NF-κB p50/p65. The nuclear NF-κB drives IκBα expression generating a negative feedback loop. Therefore, in the absence of IκBα the termination of NF-κB activation in response to canonical stimuli such as TNF-α is significantly delayed (Gerondakis et al., 2006; Pasparakis et al., 2006). The duration of the NF-κB response relies heavily on the kinetics of the feedback pathway and, for example, the kinetics of NF-κB inactivation can be grossly restored by placing a different IκB, e.g., IκBβ, under the control of the IκBα promoter (Cheng et al., 1998).

IκBα, IκBβ, and IκBε are considered to function as traditional IκB proteins: that is they sequester NF-κB dimers away from κB elements thus inhibiting transcription. Although IκBβ knocked into the genome replacing IκBα can serve analogously to IκBα, there are enough differences between these inhibitors such that it is unlikely that IκBα and IκBβ are completely interchangeable. Indeed, analyses of NF-κB responses in MEFs lacking one, two, or all three IκB proteins demonstrate that they have unique functions, even within a given signaling pathway. The functional characteristics of IκBα, IκBβ, and IκBε are most likely a result of temporal differences in their degradation and resynthesis (Hoffmann et al., 2002). More recently, using cells deficient in all three traditional IκB proteins, it has been shown that the long-standing model of cytoplasmic sequestration by IκBs is only partially true (Tergaonkar et al., 2005). In particular, cells that lack all three subunits show relatively normal nuclear/cytpolasmic p65 distribution but significantly increased basal NF-κB-dependent gene expression, suggesting that regulation of NF-κB transcriptional activity by IκBs is partly independent of cytoplasmic sequestration. This work also definitively confirmed that stimulus-induced activation of canonical NF-κB requires the three typical IκB proteins (Tergaonkar et al., 2005).

Like IκBα, IκBε is degraded in an IKK-dependent manner and its expression is upregulated by NF-κB. However, IκBε degradation and resynthesis occur with considerably delayed kinetics compared to that of IκBα. The difference in the kinetics of IκBε and IκBα degradation has profound effects on the nature of the transcriptional response to TNF (Kearns et al., 2006). IκBε is primarily expressed in hematopoietic cells, and loss of IκBε results in selective defects in hematopoietic lineages, although it appears that IκBε loss is largely compensated for by IκBα (Goudeau et al., 2003; Samson et al., 2004). The temporal and cell type-specific degradation of IκBε supports the hypothesis that different IκBs can play unique functions in NF-κB responses.

The unique function of IκBβ, however, has been difficult to define. Although IκBβ, like IκBε, undergoes slow degradation and resynthesis, deletion of IκBβ does not dramatically affect the kinetics of the NF-κB responses as seen in the deletion of IκBα or ε (Hoffmann et al., 2002; Kearns et al., 2006). However, both the nuclear/cytoplasmic localization and posttranslational modification of IκBβ seem to be unique, and IκBβ is capable of associating with NF-κB dimers that are bound to DNA (Suyang et al., 1996; Thompson et al., 1995). These data suggest that IκBβ might function to regulate NF-κB dimers bound to κB sites in the nucleus.

The Precursor IκBs—p100 and p105

The IκB/NF-κB precursor protein p105 undergoes processing via the proteasome to yield p50. Multiple reports have demonstrated that IKKβ-dependent phosphorylation of the C-terminal region of p105 at Ser923 and Ser927 (Ser933 in human p105) leads to complete degradation of the protein analogous to IκBα (Hayden and Ghosh, 2004; Perkins, 2006). It was recently demonstrated that SCF$^{\beta TrCP}$ is not responsible for signal-induced processing of p105, and it has been suggested that this event is independent of ubiquitination (Cohen et al., 2004). Constitutive processing of p105 to p50 has been shown to occur cotranslationally, although a recent analysis demonstrates that processing of p105 can occur posttranslationally (Lin et al., 1998, 2000; Moorthy et al., 2006). Normal processing of p105 to p50 in an E1 Ub-activating enzyme-deficient cell line, complemented by in vitro analysis, strongly suggests that p105 processing can occur via the 20S proteasome, independent of ubiquitination, and in a manner that is inconsistent with cotranslational processing (Moorthy et al., 2006). When p105 is bound to NF-κB complexes it appears that processing is inhibited and induced degradation is favored (Cohen et al., 2001; Harhaj et al., 1996). Thus unprocessed p105 acts as an IκB protein that binds NF-κB dimers and can be inducibly degraded upon IKK activation. In addition, the 3′ end of the p105 gene also encodes an independently regulated transcript, IκBγ, which shows separate tissue distribution and may function as an independent IκB protein in murine cells (Hayden and Ghosh, 2004).

Processing of p100 requires IKKα and is predominantly stimulus dependent (Senftleben et al., 2001). NIK is required for the activation of IKKα and may also be necessary for phosphorylation of p100 by active IKKα (Xiao et al., 2001, 2004). Phosphorylation of p100 at serines 866, 870, and 872 (Ser865, 869, and 871 in human p100) leads to the recruitment of SCF$^{\beta TrCP}$, polyubiquitination of Lys855 in a region with sequence homology to Lys22 of IκBα, and subsequent degradation or processing to p52 (Amir et al., 2004; Fong et al., 2002; Liang et al., 2006). However, it has been suggested that IKKα, although required, may not be responsible for phosphorylation of these key serine residues (Liang et al., 2006; Xiao et al., 2004). Constitutive processing of p100 to p52, which occurs at a low level in a cell type-specific manner, is also dependent on IKKα and NIK (Qing and Xiao, 2005; Xiao et al., 2004). Regulation of RelB by p100 is especially crucial because RelB-containing dimers only associate with p100, and it has been suggested that they require p100 binding for stabilization (Solan et al., 2002). Because p100 undergoes constitutive processing in certain tissues, RelB/p52 heterodimers may exhibit constitutive activation and RelB-deficient mice have decreased baseline NF-κB activity in the thymus and spleen (Gerondakis et al., 2006; Pasparakis et al., 2006). While RelB is regulated exclusively by p100, p100 itself can act more broadly. In addition to IκBα/β/ε, p100 can also act as a traditional IκB regulating p65-containing complexes downstream of IKKα (Basak et al., 2007). Likewise it has been shown that in T cells p100 also limits p65-mediated NF-κB activity in a negative feedback loop set up following T cell activation (Ishimaru et al., 2006). Consequently p100 can act in the induction of canonical and noncanonical NF-κB complexes as well as in the positive and negative regulation of constitutive NF-κB activity. Therefore, p100 displays several key properties that are likely to be important for NF-κB regulation: (1) selective regulation of specific NF-κB complexes such that in its absence a subset of inducible NF-κB responses, i.e., RelB-dependent gene expression, is lost; (2) selective function of IκB subunits downstream of specific signaling pathways; (3) finally, regulation of basal activity of specific NF-κB dimers.

The "Atypical" IκBs—IκBζ and Bcl-3

IκBζ and Bcl-3 are the remaining two IκB family members, and appear to regulate NF-κB signaling by a distinct mechanism. Bcl-3 is unique in that in contains a TAD. Bcl-3 is found in the nucleus associated with p50- and p52-containing homo- and heterodimers. The mechanism of action of Bcl-3 is still not completely understood. Bcl-3 may mediate release of transcriptional repression by removing p50 homodimers from κB sites, thus acting as a traditional IκB but mediating activation by acting on repressive NF-κB dimers and allowing p65:p50 or other TAD-containing dimers access to κB elements (Hayden and Ghosh, 2004; Perkins, 2006). Alternatively, Bcl-3 may also stabilize repressive p50 homodimers and inhibit NF-κB activation by preventing the access of TAD-containing dimers to κB sites (Carmody et al., 2007). As a result, the induction of Bcl-3 expression inhibits subsequent NF-κB activation and may contribute to LPS tolerance in macrophages. Conversely, in the case of p52 homodimers it is thought that Bcl-3 confers transcriptional potential in an inducible manner (Bours et al., 1993). Cyclin D1 is a Bcl-3-regulated gene that is of particular interest owing to its role in cell proliferation and cancer (Rocha et al., 2003; Westerheide et al., 2001). It has been demonstrated recently that the deubiquitinase CYLD, previously shown to function in an NF-κB-driven negative feedback loop, negatively regulates Bcl-3 function by preventing its nuclear accumulation and coactivation with both p50 and p52 homodimers

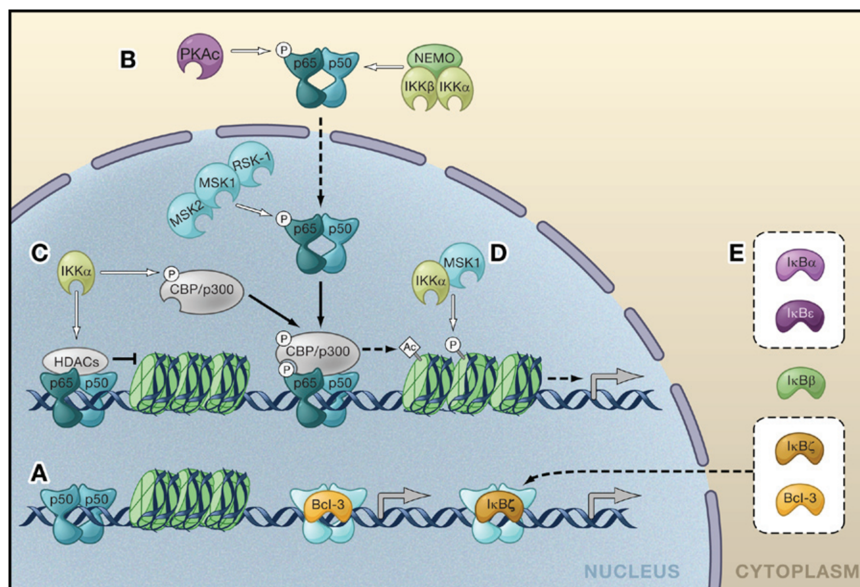

Figure 4. Regulation of NF-κB Transcriptional Activity

(A) In unstimulated cells κB DNA elements may be occupied by p50 or p52 homodimers, which are unable to recruit coactivator complexes. Release of canonical p65:p50 dimers in the absence of cytoplasmic signaling results in DNA binding and HDAC recruitment that may actively repress transcription.

(B) Release of NF-κB dimers through appropriate stimulation is linked to phosphorylation of p65, either in the cytoplasm by IKK or PKAc or in the nucleus by MSK1/2 or RSK-1. Phosphorylated p65 preferentially interacts with CBP/p300 coactivator complexes resulting in histone and p65 acetylation and promotion of target gene transcription.

(C) The exchange of corepressor for coactivator complexes is additionally catalyzed by IKKα, which phosphorylates both CBP/p300-inducing preferential binding to NF-κB and corepressor complexes inducing HDAC release.

(D) IKK and MSK may also promote transcription through direct phosphorylation of histones.

(E) Of the many NF-κB gene products, IκB proteins are critically involved in both positive and negative feedback responses. Bcl-3 and IκBζ interact with p50- and p52-containing complexes and promote transcription of a subset of NF-κB target genes or may stabilize repressive p50 and p52 complexes and repress subsequent rounds of NF-κB activation. IκBα and IκBε exert negative feedback by sequestering NF-κB dimers away from DNA. IκBβ function remains unclear.

(Massoumi et al., 2006; Simonson et al., 2007). Bcl-3 has also recently been implicated in the regulation of p53 by enhancing the transcription of Hdm2 in normal and cancer cells (Kashatus et al., 2006).

IκBζ has relatively weak homology to other IκBs and is more similar to Bcl-3 than the rest of the family. IκBζ is not expressed constitutively but rather is upregulated in response to IL-1 and TLR4 ligands, but not TNF, and upon expression localizes to the nucleus (Hayden and Ghosh, 2004). Most intriguingly in the absence of IκBζ, LPS- or IL-1-induced expression of a subset of NF-κB-regulated genes is lost (Yamamoto et al., 2004). IκBζ is inducibly expressed following NF-κB activation and once expressed associates primarily with p50 homodimers. Furthermore, IκBζ is found associated with p50 on the promoter of IL-6, which is not inducibly expressed in IκBζ knockout cells, and it is, therefore, hypothesized that IκBζ acts as a coactivator for p50 homodimers (Yamamoto et al., 2004). Although IκBζ does not possess a clear TAD or transactivation activity, it does exhibit transactivation potential when coexpressed as a GAL4 fusion protein with GAL4-p50 (Motoyama et al., 2005). IκBζ has also been reported to negatively regulate p65-containing NF-κB complexes, and the slight elevation of NF-κB activity observed in IκBζ knockouts seems consistent with this (Motoyama et al., 2005; Yamamoto et al., 2004). Thus IκBζ, like Bcl-3, may also be capable of selectively inhibiting or activating specific NF-κB dimers.

Based on the recent progress in understanding IκB functions, it is no longer appropriate to generally characterize IκB proteins as inhibitors of NF-κB. IκB proteins instead can also act as NF-κB cofactors that selectively interact with various NF-κB dimers. Through these interactions typical IκBs inhibit NF-κB binding to DNA, while the precursor protein p100 can stabilize the formation of otherwise unstable NF-κB dimers. The atypical IκBs by stabilizing promoter-bound NF-κB dimers can either inhibit or promote NF-κB-driven transcriptional responses (Figure 4).

Regulation of Transcription by NF-κB

The five members of the NF-κB family exhibit unique functions; however, the analysis of these functions is greatly complicated by their propensity to form homo- and heterodimers thus masking their unique roles in knockout animals. Historically, much of what we know about the entire NF-κB pathway has been discovered using model systems, with stimuli and assays that focus on signaling through IκBα to p65-containing complexes. As NF-κB research has expanded into ever more diverse systems, however, specific functions for individual NF-κB subunits and specific dimer pairs are increasingly being described (Hoffmann et al., 2006; Natoli et al., 2005). Rather than focus on these specific functions, we will discuss more general insights into how NF-κB-dependent transcription is initiated and terminated.

There are several steps in addition to the IκB degradation that are required for complete activation of NF-κB-dependent gene expression. Numerous regulatory posttranslational modifications of NF-κB have been reported. These modifications can be directly induced by components of the NF-κB signaling pathway, e.g., IKK, and are also important nodes for crosstalk between diverse signaling pathways. As in other aspects of NF-κB signaling, posttranslational modifications of p65 are best characterized (Figure 2). Therefore, we will focus on several examples of how posttranslational modifications of p65 alter IκB binding, affinity for κB sites, and interaction with transcriptional coactivators/repressors. We will outline how NF-κB signaling alters transcription through affecting coactivator/repressor activity and chromatin modifications before briefly touching upon the termination of NF-κB-dependent transcription.

Regulation of Coactivator Binding

Inducible phosphorylation of p65 by protein kinase A (PKA) was first recognized more than a decade ago and has since been demonstrated to be crucial for NF-κB transcriptional

activity downstream of IκB degradation (Chen and Greene, 2004). PKA exists in a complex with cytosolic NF-κB:IκB complexes and following degradation of IκBα phosphorylates p65 at Ser276, promoting the interaction of p65 with the transcriptional coactivators CBP (CREB-binding protein) and p300 (Zhong et al., 1998). In addition to PKA, other kinases, most notably MSK1 and MSK2 (mitogen- and stress-activated protein kinase), have been reported to phosphorylate Ser276 of p65. MSK1 and MSK2 share substrate specificity with PKA, and *MSK1*$^{-/-}$*MSK2*$^{-/-}$ cells have diminished transcriptional activity in response to TNF (Vermeulen et al., 2003). MSK1/2 are nuclear kinases that are activated by multiple pathways including ERK and p38/MAPK and thus could mediate crosstalk at the level of p65 or c-Rel, which also contains a PKA/MSK1/2 consensus phosphorylation site (Perkins, 2006).

Additional p65 phosphorylation events have been described, although their mechanistic significance is less clear. IKKα and IKKβ have been implicated in the direct phosphorylation of p65 at Ser536 (Chen and Greene, 2004; Perkins, 2006). Mutation of Ser536 to alanine has been reported to abrogate the interaction of p65 with CBP/p300 (Chen et al., 2005). Ser529 of p65 may also be inducibly phosphorylated by CK2 following IL-1 or TNF-α stimulation, although it is unclear whether Ser529 phosphorylation affects transcription (Bird et al., 1997; Wang et al., 2000). PKCζ can phosphorylate p65 at Ser311, and CBP fails to associate with p65 following stimulation of PKCζ-deficient cells (Duran et al., 2003). Among the p65 phosphoacceptor sites discussed, two occur within the dimerization domain of the RHD (serine 276 and serine 311) and two within the C-terminal TAD region (serines 529 and 536). It is possible that each of these phosphorylation events may contribute to complete p65 activation by inducing conformational changes that facilitate CBP/p300 binding.

Acetylation of p65, probably by CBP/p300 and associated HATs, occurs in the nucleus and is associated with increased transcription (Chen and Greene, 2004). Acetylation of multiple lysines in p65 has been demonstrated, but it is acetylation of Lys310 that has most clearly been shown to enhance transcriptional activity without altering binding to DNA or IκB (Chen et al., 2002b). Acetylation of Lys310 is blocked either in the absence of phosphorylation of Ser276 or by overexpressing catalytically inactive PKAc (Chen et al., 2005). Thus p65 phosphorylation is necessary to recruit CBP/p300 allowing acetylation at Lys310. Ser536 phosphorylation, while not completely required, significantly enhanced Lys310 acetylation (Chen et al., 2005). One possibility is that Ser536 phosphorylation alters the interaction of p65 with the SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) corepressor complex such that the level of HDAC3 is decreased and CBP/p300 is increased (Hoberg et al., 2006). In addition to p65 phosphorylation, IKKα also may promote Lys310 acetylation through direct phosphorylation of SMRT, which leads to displacement of HDAC3 from the SMRT corepressor complex (Hoberg et al., 2006). IKKα regulates transcription through several additional nuclear substrates. IKKα is found associated with the κB sites of some NF-κB-responsive genes, and stimulus-induced phosphorylation of histone H3 on serine 10 does not occur in the absence of IKKα (Anest et al., 2003; Yamamoto et al., 2003). In addition to the SMRT and N-CoR (nuclear receptor corepressor) complexes, IKKα also has been shown to phosphorylate the CBP coactivator (Huang et al., 2007).

The p53 protein has long been implicated at multiple levels with the NF-κB pathway. The importance of both NF-κB and p53 in cancer has promoted significant research into the relationship between these two transcription factors. p53 and p65 have been reported to directly compete for CBP/p300 (Webster and Perkins, 1999), which has been suggested to promote their crossrepression. However, CBP/p300 is utilized by a wide variety of transcription factors, leaving it unclear how two opposing factors directly competing for such a crucial resource could account for specific crosstalk between two pathways. Instead, modification of the cofactor itself might be more relevant. It was recently reported that IKKα phosphorylates CBP in a stimulus-dependent manner, inducing upregulation of CBP activity, increased binding to p65, and decreased binding to p53 (Huang et al., 2007). The reciprocal mechanism by which p53 might repress NF-κB is less clear, although an analogous *conditioning* of CBP may occur during p53 signaling that is unfavorable for NF-κB/CBP binding. It would still seem that targeting of CBP by IKK might affect transcription factors too broadly; however, there may be additional elements, such as the conformation of CBP bound with specific transcription factors or the existence of adapters that might limit the effect of such modification to certain groups of transcription factors.

Similar regulation of CBP/p300 might underlie other examples of transcription factor crosstalk with NF-κB. GSK3β-deficient embryonic fibroblasts are deficient in NF-κB-dependent transcriptional activity despite normal nuclear translocation and DNA binding (Bonnard et al., 2000; Hoeflich et al., 2000). While it has been hypothesized that GSK3β might phosphorylate p65, the effect of GSK3β is likely to be more indirect. GSK3β, normally constitutively active, phosphorylates and inactivates CREB disrupting the interaction between CREB and CBP. Thus inactivating GSK3β releases repression of CREB, which binds CBP, reducing its access to NF-κB (Martin et al., 2005). Interestingly in *IKKα*$^{-/-}$ cells, binding of CBP to CREB is increased (Huang et al., 2007). One potential explanation is that CBP bound to CREB is a poor substrate for IKKα phosphorylation, and that CBP phosphorylated by IKKα binds poorly to CREB. In addition to p53 and CREB, there is considerable crosstalk between NF-κB and a wide array of transcription factors. The interplay between nuclear receptors and NF-κB, which is likely to be of considerable physiological significance in understanding regulation of inflammation and cancer, is one area in which there has been much recent progress (De Bosscher et al., 2006).

A surprising aspect of NF-κB-dependent gene expression that has emerged recently is that NF-κB-regulated genes can be divided into distinct groups depending on their requirement for chromatin modification for expression (Natoli et al., 2005). The first report on this phenomenon classified NF-κB-

dependent genes into constitutively and immediately accessible (CIA) promoters that do not require chromatin modification and promoters with regulated and late accessibility (RLA) that are dependent on stimulus-induced chromatin modification (Saccani et al., 2001). This basic classification has been extended to demonstrate that chromatin-remodeling complexes are used differentially for inflammatory gene expression (Ramirez-Carrozzi et al., 2006). The most likely possibility is that the immediate early genes possess an open conformation, although what imprints this state of chromatin modification remains to be determined. It has also been reported recently that mutation of certain phosphorylation sites on p65 affects gene expression in a differential manner. We have recently found in a knockin mouse expressing a S276A mutant form of p65 that expression of a subset of NF-κB-regulated genes is affected (unpublished data). As Ser276 is critical for recruitment of CBP, this suggests that different NF-κB-regulated genes differ in their requirement for histone acetylation, although the basis of this difference remains unclear. While it has been shown that κB site sequence may, in some cases, determine cofactor binding, more general rules governing cofactor binding have yet to be determined (Leung et al., 2004). In addition to traditional coactivator and corepressor complexes, there is a growing list of proteins that interact with NF-κB dimers and affect DNA binding and transcription (Natoli et al., 2005). One recent and intriguing addition to this list is ribosomal protein S3 (RPS3), which seems to be required for the binding of NF-κB at specific κB sites (Wan et al., 2007)

In addition to coactivator requirements, the specific function of individual NF-κB dimers is coming under increased scrutiny (Hoffmann et al., 2003, 2006). For example, while it has been shown that certain dimers show differences in κB binding site affinity in vitro, analyses of promoter binding in vivo suggest that dimer selection cannot be reduced to κB sequence alone (Bonizzi et al., 2004; Britanova et al., 2008; Schreiber et al., 2006). Temporal differences in dimer release, due in part to selective IκB binding; pathway and cell type-specific posttranslation modifications of NF-κB subunits; coordination with other inducible transcription factor pathways; and the complex nature of promoter structures among other factors are probably important. It is likely, however, that exploration of the mechanism by which NF-κB dimers interface with chromatin in gene expression will be a major area of investigation in the future.

Shutting off NF-κB

The mechanisms by which activity of NF-κB is terminated remain poorly understood. To date most efforts in this area have focused on mechanisms that involve IκB proteins and upstream signaling intermediates (Hacker and Karin, 2006; Hayden and Ghosh, 2004). However, none of these mechanisms explain how active, DNA-bound NF-κB is suppressed once signaling is terminated. Therefore, while IκB resynthesis and induction of other gene products impinging on upstream signaling components are clearly critical elements in NF-κB downregulation, there are likely additional mechanisms that function later in the pathway. One possibility is that promoter-bound p65 may be subject to proteasomal degradation (Saccani et al., 2004). In LPS signaling, suppressor of cytokine signaling-1 (SOCS-1), in addition to its effects on upstream JAK-STAT signaling components, decreases NF-κB transcriptional activity (Kinjyo et al., 2002; Nakagawa et al., 2002). Although the exact mechanism of action remains unclear, it has recently been shown that targeting the SOCS-1-containing ubiquitin ligase complex (ECS^{SOCS1}) to p65-containing dimers is carried out by COMMD1 (Copper Metabolism MURR1 Domain containing-1) proteins (Maine et al., 2007). IKKα is also involved in termination of inflammatory transcriptional responses in macrophages, possibly by promoting the nuclear degradation of both p65 and c-Rel (Lawrence et al., 2005; Li et al., 2005). Because IKKα is required for activating NF-κB-dependent transcriptional responses, it seemed unclear whether IKKα acts directly on NF-κB to repress the latter stages of transcription or acts by supporting the expression of gene products that act in negative feedback pathways. However, the recent demonstration that IKKα represses transcription of the tumor suppressor Maspin by acting at the *maspin* promoter strongly supports a direct mechanism of action by IKKα that is independent of NF-κB (Luo et al., 2007). These findings, along with several other recent reports that have focused on negative regulation of nuclear NF-κB, suggest that this aspect of NF-κB regulation will be an area of significant activity in the near future.

Concluding Remarks

As a result of the tremendous progress made over the past 20 years or so, several common themes have emerged as being essential to all NF-κB signaling. Yet, there are still major unanswered questions about the biology and regulation of NF-κB. These include questions about the regulation of different NF-κB dimers, the exact role of ubiquitination as a regulatory event in signaling, the additional roles of different IκB isoforms, and novel roles for IKKs in pathways other than NF-κB. The interaction between chromatin and NF-κB in regulating gene expression programs is also likely to be an area of intense investigation, as are the events that terminate NF-κB signaling. Several of these areas promise to provide significant insight into signaling and transcriptional regulation that will impact our understanding of these processes both within and beyond the field of NF-κB. Although we have not been able to discuss it the current Review, the link between NF-κB and different aspects of physiology keep emerging. For example, a recent report (Adler et al., 2007) has provided evidence that NF-κB plays an important role in aging of the skin. As inflammatory processes are believed to contribute to aging phenomenon in general, it will probably not be surprising that one method of slowing aging may lie in selectively blocking NF-κB-dependent inflammatory processes. Along with the already well-established links between NF-κB and diseases such as cancer, asthma, and muscular dystrophy (Acharyya et al., 2007), it is clear that the major advances in the near future will come from the ability to develop effective inhibitors of the NF-κB pathway that can act selectively, thereby avoiding the risk of undesired side effects. The overlapping roles played by NF-κB pathway components in varied

physiological settings suggest that this task will be both crucial and difficult. However, it is not too far-fetched to imagine a future where we will have a series of NF-κB inhibitors aimed at different diseases, based on their ability to target specific pathways or cells, and therefore we anticipate that interest and excitement in this most versatile of transcription factors will continue at the same pace.

ACKNOWLEDGMENTS

We thank the reviewers for helpful comments on this manuscript. The work in the author's laboratory is supported by grants from the NIH (AI033443, AI066109, AI059440, and AI068977). M.S.H. was also supported by NIH/NIGMS MSTP grant GM07205.

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