

Perspective

I κ B kinase: Beginning, not the end

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It is not often that a problem in biology is so well defined and yet so elusive. Just a decade ago, David Baltimore and colleagues reported the identification of a nuclear factor that bound to an enhancer element in the κ light chain gene. They called this factor NF- κ B (1). Unlike other transcription factors, NF- κ B was found to be ensconced in the cytoplasm in association with an inhibitory protein, I κ B. Upon stimulation of the cell with a variety of inducers, such as growth factors, lymphokines, cytokines, UV, pharmacological agents, and stress, the NF- κ B proteins translocated to the nucleus (2). It was quickly recognized that nuclear translocation of NF- κ B was promoted by phosphorylation of I κ B followed by dissociation of the complex (3). In the subsequent years, the seven genes encoding NF- κ B proteins have been molecularly cloned and found to form a family, referred to as the Rel family (4). The Rel protein was first identified as a cellular homologue of viral Rel (v-Rel), the transforming component of avian reticuloendotheliosis virus (REV) (5). Thus, early on, there was an indication that NF- κ B proteins were possible oncogenes.

Similarly, the membership of the inhibitory protein I κ B has been expanded to include six members (4). Three years ago, the consensus was that I κ B α formed a complex with two NF- κ B proteins, p50/p65. In response to external stimuli, I κ B α was phosphorylated and degraded in 10–15 min (6). The resultant free p50/p65 traversed into the nucleus to bind a cognate DNA binding site and induce transcription. One of the first genes induced following NF- κ B activation was I κ B α itself, thereby suggesting a regulatory loop of I κ B α degradation and resynthesis (7–9). The first clues to the mechanism for degradation of I κ B α emerged from the observations of Tom Maniatis and colleagues (10). They showed a role for the ubiquitin–proteasome pathway in processing of an I κ B family member, the NF- κ B1 precursor protein. Subsequently Patrick Beaulieu and colleagues (11) and our research group (6) showed that inhibition of the ubiquitin–proteasome pathway stabilized the phosphorylated form of I κ B α and prevented NF- κ B activation. Thus it became clear that (i) phosphorylation of I κ B α preceded ubiquitination; and (ii) ubiquitination was a signal for I κ B α degradation. I κ B α was shown to be phosphorylated at its C terminus by casein kinase II (CKII) (12). However, this phosphorylation is constitutive and does not promote inducible degradation of I κ B α . Instead, the C-terminal phosphorylation plays a role in basal degradation of I κ B α (13). In early 1995, Ulrich Siebenlist and colleagues (14) made the seminal finding that induced phosphorylation of I κ B α occurs on serine residues 32 and 36 (S32/36). Soon a working model was established where external stimulus led to the phosphorylation of I κ B α at S32/36, followed by ubiquitination of lysine residues 21 and 22 (4). Multiubiquitinated I κ B α remains complexed with p50/p65 but is marked for degradation by the ubiquitin–proteasome pathway (Fig. 1). Degradation proceeds rapidly and leads to activation of NF- κ B. Thus, the signal-induced phosphorylation of I κ B α is the key event that triggers the cascade of events leading to activation of NF- κ B. An I κ B kinase was postulated, and a

number of candidates emerged over the years (15–17). CKII readily phosphorylates I κ B α *in vitro* and *in vivo* (12). However the CKII site is not required for signal-induced degradation. Removal of the CKII sites from I κ B α was required to facilitate identification of the inducible I κ B kinase. The identification of S32/36 phosphorylation sites narrowed the field to kinases capable of phosphorylating these sites. So the search for the I κ B kinase continued.

A tantalizing observation was made by the Maniatis group (18). They identified a 700-kDa complex from unstimulated HeLa cell extracts that could be activated by either MEKK-1 or ubiquitin-conjugating enzymes or the phosphatase inhibitor okadaic acid (18, 19). This observation suggested there was a unique kinase that required either or both ubiquitination and phosphatase inhibition for activity. However, the lack of activation of this complex upon stimulation by proinflammatory cytokines such as tumor necrosis factor (TNF) led others to look further. Two groups identified another large complex. One group, led by Michael Karin, systematically fractionated activity from the complex that phosphorylated I κ B α at S32/36. The fraction with the highest activity was enriched with polypeptides of 85, 87, and 64 kDa. Microsequence analysis of the 85-kDa polypeptide followed by partial cloning of the cDNA revealed that it was a previously identified serine/threonine kinase of unknown function called CHUK, a conserved helix–loop–helix ubiquitously kinase (20, 21). Another group, led by Frank Mercurio at Signal Pharmaceuticals, found that in HeLa cells stimulated by TNF, I κ B α was recruited into a high molecular mass complex (\approx 700 kDa) and phosphorylated at S32/36. This fraction also contained RelA, I κ B β , two phosphotyrosine proteins, MEKK-1, and a protein of 50 kDa that cross-reacted with antibody to mitogen-activated protein (MAP) kinase phosphatase 1 (MKP-1) but was not MKP-1. This group used cell fractionation and MKP-1 antibody to purify I κ B kinase activity. They identified two polypeptides, 85 kDa and 87 kDa. Nano-electrospray sequencing and an expressed sequence tag (EST) database search revealed that the 85-kDa protein was CHUK and that the 87-kDa protein was 51% identical to CHUK. Thus there are at least two related I κ B kinases in this 700-kDa complex (22).

Another kinase involved in NF- κ B signaling was identified in early 1997 by a group led by David Wallach (Weizmann Institute of Science, Israel). They termed this kinase NIK (NF- κ B-inducible kinase) (23). NIK shares sequence homology with several MAPKK kinases (mitogen-activated protein kinases, also called MAPK3). NIK binds to TRAF2 (a component of the TNF α signaling pathway) but does not appear to directly phosphorylate I κ B α . Instead, NIK acts upstream of an I κ B kinase. NIK was the object of study for David Goeddel, Mike Rothe, and a team of investigators at Tularik, Inc. This group has a long-term interest in TNF signaling and so, using a yeast two-hybrid screen, they began to identify proteins that

Abbreviations: NF- κ B, nuclear factor κ B; I κ B, inhibitor of NF- κ B; TNF, tumor necrosis factor; MAP, mitogen-activated protein; NIK, NF- κ B-inducible kinase; IKK, I κ B kinase.

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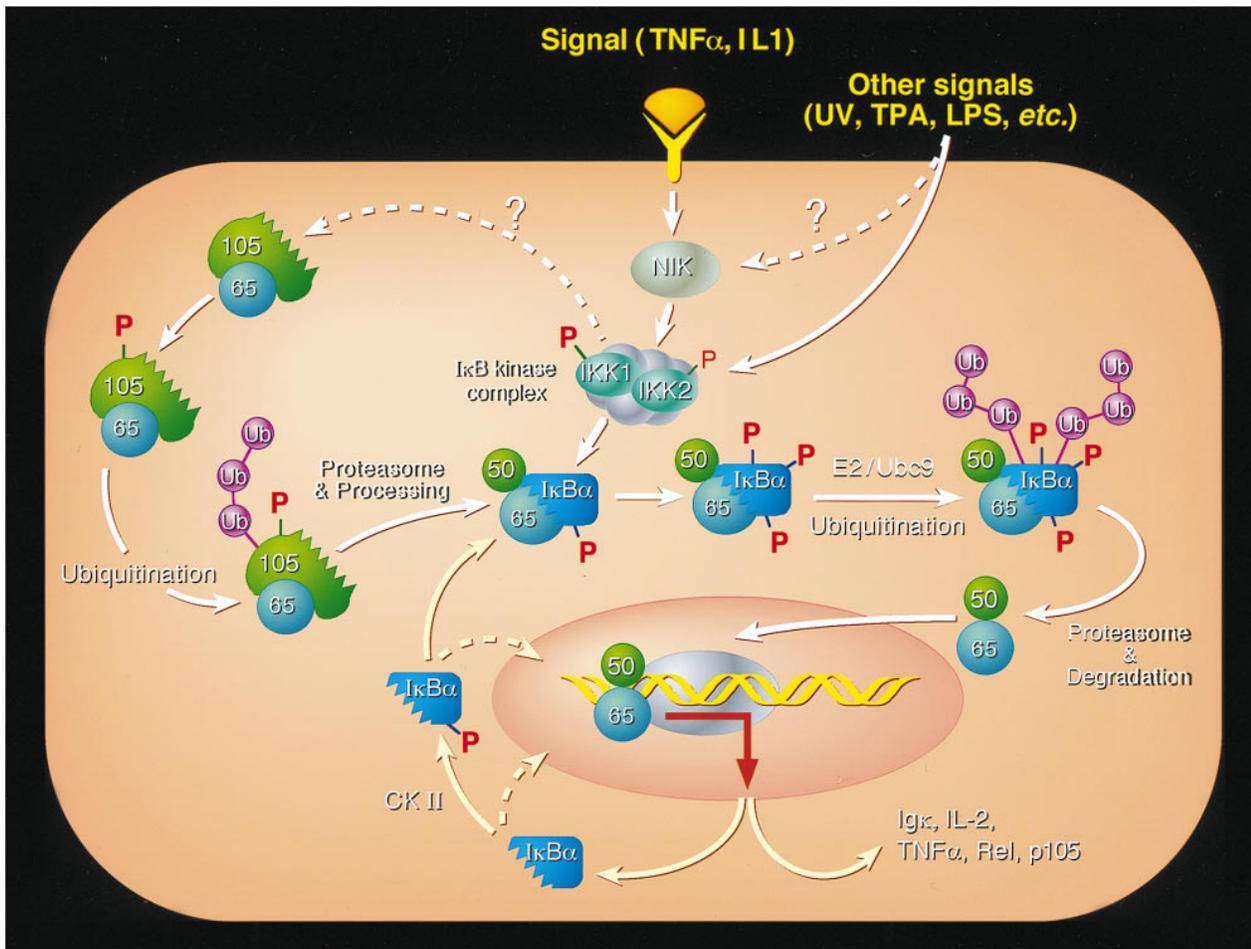


FIG. 1. Schematic representation of the molecular mechanism of NF- κ B activation. Following signal [tumor necrosis factor α (TNF α), interleukin 1 (IL-1)] induction, NF- κ B-inducible kinase (NIK) is activated, which in turn phosphorylates IKK-1 (and perhaps IKK-2) in the I κ B kinase complex. Other signals, such as UV, phorbol 12-tetradecanoate 13-acetate (TPA), lipopolysaccharide (LPS), etc., may also activate NIK or directly activate I κ B kinase complex. The I κ B kinase may also phosphorylate p105/p65 complex in the cell. Following association of p50/p65-I κ B α complex with I κ B kinase complex, I κ B α is phosphorylated at S32/36, followed by ubiquitination, degradation, and release of p50/p65 to the nucleus. Once in the nucleus, p50/p65 induces transcription of many genes, including I κ B α . The newly synthesized I κ B α can then bind to newly processed p50/p65 in the cytoplasm and await the next signal. Some newly synthesized I κ B can also traverse to the nucleus. The processing of p105/p65, the precursor of p50, is also indicated.

interact with NIK. One of the cDNA clones that interacted with NIK in the two-hybrid screen happened to be CHUK (24).

Three independent groups with different methodologies implicated the same kinase in I κ B α phosphorylation. Ironically, this kinase was known but functionally orphaned. Additionally, subsequent to the signal group's identification of IKK-2, the other two groups have also identified this second related kinase (D. Goeddel and M. Karin, personal communication). Mercifully, all groups have settled on the same nomenclature, I κ B kinase (IKK). The two related kinases are referred to as IKK-1 and IKK-2.

What are the properties of IKK? (i) IKK-1 is a 745-aa protein with an N-terminal serine/threonine kinase catalytic domain, a leucine zipper-like amphipathic helix, followed by a C-terminal helix-loop-helix domain. IKK-2 is a 756-aa protein with the same overall topology as IKK-1 plus an additional 11 aa at the C terminus (22). (ii) Expression of both IKK-1 and IKK-2 in transfected HeLa or 293 cells leads to phosphorylation of S32/36 of I κ B α and serines 19 and 23 of I κ B β . (iii) Overexpression of IKK-1 leads to transactivation of κ B-linked reporter genes. This transactivation is augmented by the addition of TNF. Thus expression of IKK-1 alone is not sufficient to achieve maximal NF- κ B activation. Maximal activation may require modification to IKK-1 or recruitment of additional proteins such as IKK-2. (iv) IKK-1 is autophospho-

rylated and its activity is inducible. IKK-2 is not autophosphorylated but when over-expressed is constitutively active. (v) The activity of IKK-1 is enhanced by wild-type NIK but not by a kinase mutant of NIK and is decreased upon treatment with phosphatase PP2A (20, 24). Thus phosphorylation of IKK-1 is important for its activity. (vi) Kinase mutant IKK-1 or IKK-2 (K44A) or antisense IKK-1 blocks induction of NF- κ B activity. (vii) IKK-1 physically associates with NIK and I κ B α alone or I κ B α in complex with p50/p65. The IKK-1 kinase mutant (K44A) still associates with I κ B α , suggesting that other domains may be involved in protein-protein interaction. (viii) Both IKK-1 and IKK-2 have canonical sequences in their N termini that can be phosphorylated by the MAP kinase NIK/MEKK-1. An inactive mutant IKK-2 is created when the putative MAP kinase phosphorylation sites serine-177 and -181 (S177/181) are changed to alanine. Alternatively, when S177/181 are mutated to glutamic acid, IKK-2 is constitutively active. This Ser-to-Glu mutant induces NF- κ B activity to the same extent as TNF. (ix) Finally, both IKK-1 and IKK-2 can form heterodimers when translated *in vitro* or cotransfected *in vivo*.

Has the true I κ B kinase been found? The accumulated evidence from the three papers is convincing. The association of IKK-1/2 with I κ B α , and the parallel kinetics of activation of IKK-1 and I κ B α phosphorylation favor the idea that

IKK-1/2 are I κ B kinases. However, no one has yet shown that bacterially expressed IKK-1 or IKK-2 can phosphorylate I κ B α *in vitro*. It is formally possible that IKK-1/2 are upstream of the true I κ B kinase. Additionally, why are there two functionally similar kinases in the complex? IKK-1 is expressed ubiquitously, whereas the expression pattern of IKK-2 is not yet known. Both kinases can independently phosphorylate I κ B α or I κ B β ; however it is not known if they have synergistic effects on NF- κ B activity. Does either IKK-1 or IKK-2 function in the processing of p105? The p105 C terminus is phosphorylated, ubiquitinated, and degraded in a manner analogous to I κ B α (10).

The 700-kDa complex containing IKK-1/IKK-2 has a number of additional unidentified proteins. Clearly, the biochemical characterization of these components is warranted. In this regard the leucine zipper domain and helix-loop-helix domain of IKK-1/2 will provide clues for identifying interacting proteins. One of the many questions to be answered is whether IKK-1 or IKK-2 interacts with proteins from the ubiquitination-inducible I κ B kinase complex (18). Because the I κ B kinase is unrelated to MAP or Jun kinases, IKK represents a completely novel kinase pathway. The work to clearly define the components of this pathway begins again.

Soon researchers will "knock-out" IKK-1/2 genes by homologous recombination. Others will establish the chromosomal location of IKK-1/2 to identify any IKK-related diseases. Finally, IKK-1/2 will be excellent drug targets for treatment of inflammatory diseases, cancer, rheumatoid arthritis, and a host of other diseases. The identification of the components of I κ B kinase is the initial thread that will allow us to unravel the complex and versatile regulation of κ B proteins.

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