PERSPECTIVES: SIGNAL TRANSDUCTION

IκB Kinases: Kinsmen with Different Crafts

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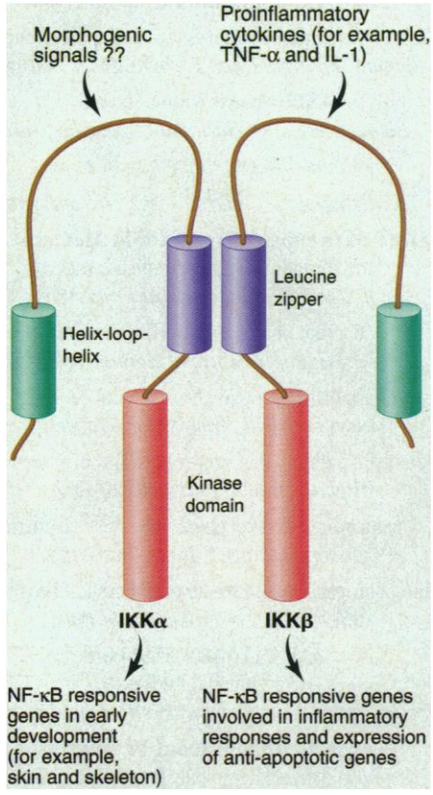
One of the most intensely studied regulators of gene expression is the transcription factor NF-κB. NF-κB activates groups of genes in response to a wide array of extracellular factors, including those that promote inflammation and apoptosis (*1*). Determining the molecular mechanisms that regulate the activation of NF-κB is crucial to understanding how multiple intracellular signaling pathways converge to activate a single transcription factor. Four reports in this issue of *Science* shed light on the activities of a pair of kinases that, although united in the same heterodimeric complex, activate NF-κB separately and under different conditions (*2–5*).

In most cell types, NF-κB is sequestered in the cytoplasm through its association with members of a family of inhibitory proteins known as IκBs (*1*). Seminal work from a number of laboratories has determined a sequence of biochemical events that cause the degradation of IκB proteins, leading to the release of NF-κB, which is then free to move to the nucleus and switch on the expression of certain genes. After activation of cells by, for example, the binding of certain cytokines to their surface receptors, IκB proteins are rapidly phosphorylated on two critical amino-terminal serine residues (Ser^{32} and Ser^{36} in IκBα, Ser^{19} and Ser^{23} in IκBβ); this modification facilitates their interaction with a recently described protein (containing WD- and F-box motifs) called β-TrCP (*6*). Interaction with β-TrCP triggers the formation of a ubiquitin-ligase complex that adds ubiquitin groups to IκBα (and probably IκBβ) on nearby lysine residues. These ubiquitinated forms of the IκB proteins are then targeted to the 26*S* proteasome and degraded. Clearly a key player in this cascade of events is the kinase responsible for phosphorylating the IκBs, and in 1997 the identity of this crucial intermediate in the NF-κB signaling pathway was revealed (*7*).

The activity of IκB kinase (IKK) was found to reside in a high molecular weight (>600 kD) complex that contained two kinase subunits (*7*). These two kinases, IKKα and IKKβ, share significant sequence homology and contain identical structural domains (see the figure). Through their leucine-zipper domains, IKKα and IKKβ interact to form hetero- and homodimers in vitro, although only heterodimers are found in vivo. A third protein in the IKK complex, IKKγ (also called NEMO or IKKAP), has been identified (*8, 9*). IKKγ, which does not contain a catalytic kinase domain, preferentially associates with IKKβ and is required for the activation of IKKα-IKKβ heterodimers in response to the proinflammatory cytokines tumor necrosis factor–α (TNF)-α and interleukin-1 (IL-1). Another protein called IKAP has been found to associate with both IKKs and may function as a scaffold for the formation of a functional IKK complex (*10*). A number of in vitro experimental approaches have culminated in a model in which both IKKα and IKKβ are involved in NF-κB activation after binding of TNF-α or IL-1 to the cell surface (*1*, *7*). However, the presence of two catalytic subunits with apparently the same target raises intriguing questions about the roles played by the two kinases in the IKK complex. The four *Science* reports now reveal entirely unpredicted features about the differential function and regulation of IKKα and IKKβ (*2–5*).



Twin kinases get busy. The kinases IKKα and IKKβ activate the transcription factor NF-κB. They do this by targeting IκB, a protein that binds to and inhibits NF-κB, for destruction. IKKα and IKKβ associate together in a heterodimeric complex, but they respond to different biological inducers of NF-κB activation. IKKβ is activated, and in turn activates NF-κB, in response to proinflammatory cytokines such as TNF-α, whereas IKKα responds to unknown morphogenic signals and is crucial for NF-κB activation during embryonic development of the skin and skeletal systems.

To specifically address the in vivo role of IKKα, two separate groups used gene targeting to disrupt the *Ikkα* locus and generate $Ikk\alpha^{-/-}$ mice (*2, 3*). These animals survive to term but die shortly after birth. The mice demonstrate striking developmental abnormalities: In place of normal limbs, they display rotund protrusions; their heads and snouts are truncated; they lack external ears; and instead of the wrinkled, folded skin of wild-type mice, the skin of $Ikk\alpha^{-/-}$ animals is shiny and tight. The stunted limbs are somewhat reminiscent of individuals exposed in utero to the drug thalidomide. However, the two phenotypes are probably not related; thalidomide blocks limb bud development, whereas autopsy reveals that the limbs of $Ikk\alpha^{-/-}$ mice develop to near normal size under the skin, although they are shortened and tightly folded. But why do these limbs not emerge? The answer may lie in the skin phenotype of the $Ikk\alpha^{-/-}$ mice: In these animals, differentiation of skin epidermal cells (keratinocytes) is blocked and there is significant hyperplasia of the suprabasal layer (stratum spinosum) of the epidermis. This hyperplasia causes a thickening of the skin that Hu *et al.* (page 316) propose results in engulfment of the limbs (*2*). These investigators also observed severe abnormalities in the development of the sternum and vertebrae in $Ikk\alpha^{-/-}$ mice, which, together with the underdeveloped skulls, tails, and shortened limbs, indicate the involvement of IKKα in skeletal development (*2*).

These findings imply that NF-κB is important for embryonic development. This is perhaps not surprising, even though mice lacking individual members of the NF-κB/Rel transcription factor family do not have the $Ikk\alpha^{-/-}$ phenotype (*1*). In *Drosophila*, the NF-κB homolog, dorsal,

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is critically involved in embryonic patterning and regulates the expression of a number of genes important for early development, including *twist* (*twi*) and *decapentaplegic* (*dpp*) (*11*). Defects in the expression of the mammalian homolog of *twi* lead to a phenotype similar to that of the *Ikkα*$^{-/-}$ mice (craniofacial, limb, and skeletal abnormalities) (*12*). Expression of IκBα mutant proteins in developing limb buds of chickens causes an acute block in limb bud development (*13*). The phenotype is even more severe than that seen in the *Ikkα*$^{-/-}$ mice probably because of inhibition of the anti-apoptotic function of NF-κB. Furthermore, transgenic mice expressing mutant IκB proteins in keratinocytes exhibit a thickened stratum spinosum similar to that seen in the *Ikkα*$^{-/-}$ animals (*14*). These results—together with those of Takeda *et al.* (page 313), who report that nuclear translocation of p65 (RelA), a member of the NF-κB family, in cells of the stratum spinosum is impaired in *Ikkα*$^{-/-}$ mice (*3*)—strongly suggest that IKKα-induced NF-κB activation directs key stages in early skin and skeletal development. The signals that control IKKα activity early in development are unknown, but they are unlikely to be IL-1 or TNF-α because both of these proinflammatory cytokines can activate NF-κB in cells from *Ikkα*$^{-/-}$ mice (*2, 3*).

Which then is the kinase responsible for cytokine-induced activation of NF-κB? In a previous study, it was demonstrated that IKKβ, and not IKKα, was the target for inhibition by sodium salicylate of TNF-α–induced NF-κB activation (*15*). This apparently dominant role of IKKβ in the inducible activation of NF-κB is now supported by the results of Li *et al.* (page 321) and Delhase *et al.* (page 309) (*4, 5*). In the first of these studies, *Ikkβ*$^{-/-}$ mice were generated to determine the exact function of this kinase (*4*). These mice died between embryonic day 12.5 and day 14 from extensive loss of liver cells (hepatocytes) due to apoptosis, a phenotype similar to that observed for p65 (RelA)–deficient mice (*1*). Hepatocyte apoptosis in the knockout mice appears to be induced by TNF-α because crossing the *Ikkβ*$^{-/-}$ mice with animals lacking the receptor for TNF-α rescues them from embryonic death (*4*). Indeed, embryonic fibroblast cells from *Ikkβ*$^{-/-}$ mice were prone to apoptosis in response to TNF-α. This was presumably due to the absence of a "protective" NF-κB signal, as TNF-α–induced NF-κB activation was severely impaired in these animals. Interestingly, an unanticipated difference between TNF-α– and IL-1–induced NF-κB activation in *Ikkβ*$^{-/-}$ embryonic fibroblast cells was also observed. Although TNF-α–induced degradation of IκBs as well as NF-κB activation was abrogated in these mice, NF-κB activation in response to IL-1 was significantly less affected. But very little IKK activity could be detected in immunoprecipitates of IKKα complexes from IL-1–induced mouse embryonic fibroblast cells, which raises the intriguing possibility that IL-1 might activate an IKKα/β-independent kinase capable of phosphorylating IκB proteins. This intriguing observation will surely be investigated more fully in the future.

Further evidence against the involvement of IKKα in TNF-α and IL-1 signaling pathways is provided by Delhase *et al.* (*5*). Previous in vitro studies have shown that both IKKα and IKKβ can be activated by the structurally related upstream kinases MEKK-1 and NIK, most likely through phosphorylation of specific conserved serine residues within the T-loop (activation domain) in the catalytic domain of each IKK. By mutating these serine residues and rendering the IKKs nonresponsive to NIK- or MEKK-induced phosphorylation, these workers found that mutations in IKKβ profoundly inhibited cytokine- and NIK-induced IKK activity, whereas mutations in IKKα had no effect. This study also provides a clue to potential regulatory mechanisms controlling the duration of cytokine-induced IKKβ activity. In addition to phosphorylation of the T-loop serines, a cluster of serines in the carboxy terminus of IKKβ was found to be phosphorylated after cytokine activation. Kinetic and mutational analysis suggested that this carboxyl-terminal phosphorylation was due to autophosphorylation and was responsible for down-regulation of IKKβ activity. Indeed, mutation of these carboxyl-terminal serines to alanines resulted in a kinase with higher basal activity as well as prolonged activation in response to cytokines. A model proposed by Delhase *et al.* suggests that the helix-loop-helix (HLH) domain of IKKβ physically interacts with the kinase domain and is required for its activation (*5*). When the carboxyl terminus becomes autophosphorylated, the HLH-kinase domain interaction is disrupted and the kinase activity decreases. It will be fascinating to determine whether a similar regulatory mechanism exists for IKKα in development. However, this will require identification of the specific upstream signaling intermediates in this process.

So where do these four reports leave our understanding of the importance of IKKα and IKKβ? We now know that TNF-α and IL-1 activate NF-κB through IKKβ and not IKKα, and that IKKα cannot compensate for the loss of IKKβ in cytokine-induced NF-κB activation. We also know that the two IKKs play crucial but distinct roles in early development. More intriguing still, IKKα has a unique function in early skin and skeletal development and cannot be replaced by IKKβ for this purpose. Potential explanations for this finding might be the presence of IKKα alone in IKK complexes in these developing tissues, or the ability of IKKα, but not IKKβ, to phosphorylate substrates other than IκB (a possibility not accounted for in previous in vitro studies).

The greatest significance of these reports, however, lies in the plethora of questions and avenues for future research that they have generated. Principal among these must surely be the identification of the developmental and biochemical signals that activate IKKα, and the characterization of the target genes of NF-κB in early skin and skeletal morphogenesis. Association of IKKγ with IKKα in IKKβ-deficient animals (*4*) demonstrates that although the formation of IKK complexes is not disrupted, the lack of IKKβ makes these complexes unresponsive to cytokine stimulation. Is this simply because IKKα is not a target for MEKK or NIK (*5*), or is it because IKKβ recruits specific adapter proteins that allow cytokine responsiveness? Finally, as suggested by the differing phenotypes of the IKK knockout mice and supported by a recent study (*9*), might there be multiple complexes containing different combinations of IKKs that perform distinct tasks? Obtaining answers to these and other questions will ensure that the study of NF-κB signaling pathways remains an exciting area of research for some time to come.

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