

The term NF-kappaB commonly refers specifically to a p50-RelA heterodimer, which is one of the most avidly forming dimers and is the major Rel/NF-kB complex in most cells.

The activity of NF-kB is tightly regulated by interaction with inhibitory IκB proteins. As with the Rel/NF-kB proteins, there are several IκB proteins, which have different affinities for individual Rel/NF-kB complexes, are regulated slightly differently, and are expressed in a tissue-specific manner. The IκB proteins include, at least, p105, p100, IκBa, IκBb, IκBg, IκBe, Bcl-3, and the *Drosophila* Cactus protein.

The best-studied Rel-IκB interaction is that of IκBa with the NF-kB p50-RelA dimer. This interaction blocks the ability of NF-kB to bind to DNA and results in the NF-kB complex being primarily in the cytoplasm due to a strong nuclear export signal in IκBa

In contrast, when IκBb interacts with the NF-kB complex, the complex is retained in the cytoplasm (i.e., does not undergo nucleo-cytoplasmic shuttling). Thus, not all NF-kB-IκB interactions are the same.

In most cells, NF-kB is present as a latent, inactive, IκB-bound complex in the cytoplasm. When a cell receives any of a multitude of extracellular signals, NF-kB rapidly enters the nucleus and activates gene expression.

Therefore, a key step for controlling NF-kB activity is the regulation of the IκB-NF-kB interaction. Many of the molecular details of this control are now understood (Figure 2).

Almost all signals that lead to activation of NF-kB converge on the activation of a high molecular weight complex that contains a serine-specific IκB kinase (IKK). IKK is an unusual kinase in that in most cells IKK contains (at least) three distinct subunits: IKKα, IKKβ and IKKγ. IKKα and IKKβ are related catalytic kinase subunits, and IKKγ is a regulatory subunit that serves as a sensing scaffold for the catalytic subunits.

In the classical or canonical pathway, activation of IKK complex leads to the phosphorylation by IKKβ of two specific serines near the N terminus of IκBa, which targets IκBa for ubiquitination and degradation by the proteasome.

In either pathway, the unmasked Rel/NF-kB complex can then enter the nucleus to activate target gene expression.

In the canonical pathway, one of the target genes activated by NF-kB is that which encodes IκBa. Newly-synthesized IκBa can enter the nucleus, remove NF-kB from DNA, and export the complex back to the cytoplasm to restore the

original latent state. Thus, the activation of the NF- $\kappa$ B pathway is generally a transient process, lasting from 30-60 minutes in most cells.

In some normal cells, such as B cells, some T cells, Sertoli cells and some neurons, NF- $\kappa$ B is constitutively located in the nucleus. In addition, in many cancer cells (including breast cancer, colon cancer, prostate cancer, lymphoid cancers, and probably many others; see Diseases link) NF- $\kappa$ B is constitutively active and located in the nucleus.

In some cancers, this is due to chronic stimulation of the IKK pathway, while in other cases (such as some Hodgkin's and diffuse large B-cell lymphoma cells) the gene encoding I $\kappa$ B $\alpha$  is sometimes mutated and defective. Moreover, several human lymphoid cancer cells have mutations or amplifications of genes encoding Rel/NF- $\kappa$ B transcription factors, which may enable these factors to accumulate in or cycle through the nucleus. It is thought that continuous nuclear Rel/NF- $\kappa$ B activity protects cancer cells from apoptosis and in some cases stimulates their growth. Therefore, many current anti-tumor therapies seek to block NF- $\kappa$ B activity as a means for inhibiting tumor growth or sensitizing the tumor cells to more conventional therapies, such as chemotherapy.

Over-expression studies in tissue culture almost certainly do not accurately reflect physiological signaling events. Similarly, what controls the balance between the levels of the various heterodimeric complexes in vivo is not known. Studies in *Drosophila* have elegantly shown that very small differences in nuclear concentrations of these factors, in their affinities for target DNA sites, and in cooperation or competition between Rel proteins and other transcription factors can have profound physiological consequences in organisms. Lastly, in many situations, it is not known how or which of the many genes induced by Rel/NF- $\kappa$ B factors in a given response contribute to that response. The recent development of methods to analyze genome-wide changes in gene expression (e.g., cDNA microarrays), which has already begun to uncover additional Rel/NF- $\kappa$ B-responsive genes, may clarify which Rel/NF- $\kappa$ B target genes are activated in a given response.

As described above, the structures of several Rel/NF- $\kappa$ B dimers on DNA or bound to I $\kappa$ B are known.

Furthermore, there is little information about how any of the Rel/NF- $\kappa$ B complexes actually activate transcription: that is, what are the co-activators or basal factors with which they interact to activate transcription? Therefore, we cannot accurately simulate the dynamic nature of the complex as it releases from I $\kappa$ B, enters the nucleus, binds to DNA, and enhances gene expression.

The study of v-Rel unequivocally demonstrates that Rel/NF- $\kappa$ B transcription factors can be oncogenic, and one would like to know how the activating mutations in v-Rel have altered its structure as compared to c-Rel.

The involvement of Rel/NF- $\kappa$ B transcription factors in human inflammation and disease certainly establishes them as targets for therapeutics. Indeed, many common synthetic (e.g., aspirin), and traditional (e.g., green tea, curcumin) remedies target, at least in part, the Rel/NF- $\kappa$ B signaling pathway. It is likely that our knowledge of the molecular details of this pathway will enable us to develop more specific and potent inhibitors.