A firm hand on NFκB: structures of the IκBα–NFκB complex

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Two crystal structures of an IκB–NFκB complex have recently been determined. The structures show in detail how IκB controls the subcellular localization and activity of the eukaryotic transcription factor NFκB.

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The regulation of gene expression, which allows a living cell to express a defined set of genes according to its environmental conditions and stage of development, occurs primarily at the level of transcription. The precise temporal and spatial control of the transcription factor NFκB has become a paradigm for the complexity of transcriptional regulation in eukaryotes. IκB, the inhibitor and central regulator of NFκB, prevents nuclear import of NFκB, disrupts NFκB–DNA complexes in the nucleus and serves as an adapter for nuclear export of NFκB. Two X-ray crystallographic structures of the IκBα–NFκB complex have recently been determined by Huxford *et al.* at 2.3 Å [1] and by Jacobs and Harrison at 2.7 Å resolution [2]. The structures give the first detailed picture of the interactions between two representatives of the important NFκB and IκB families. They provide insight into how IκB fulfils its multiple functions.

The NFκB/Rel family of eukaryotic transcription factors

NFκB can be activated by various extracellular signals including cytokines, lipopolysaccharides (LPS), viral infection and oxidative stress. In response to these diverse signals, NFκB ultimately directs the transcription of a great variety of genes involved in immune and inflammatory responses (reviewed in [3,4]). Viruses such as human immunodeficiency virus 1 (HIV-1) or human T-cell leukaemia virus 1 (HTLV-1) use NFκB to control the transcription of their own genes.

NFκB is a heterodimer formed by two homologous subunits, p50 and p65. It is the prototype of the NFκB/Rel protein family, a large group of transcription factors comprising the mammalian proteins NFκB p50 and p52, their precursors p105 and p100, NFκB p65, c-Rel, RelB and the viral oncogene v-Rel. In insects, Rel proteins act as morphogens and direct a primitive immune defense. Roles for Rel proteins in vertebrate limb development have also been reported recently [5,6].

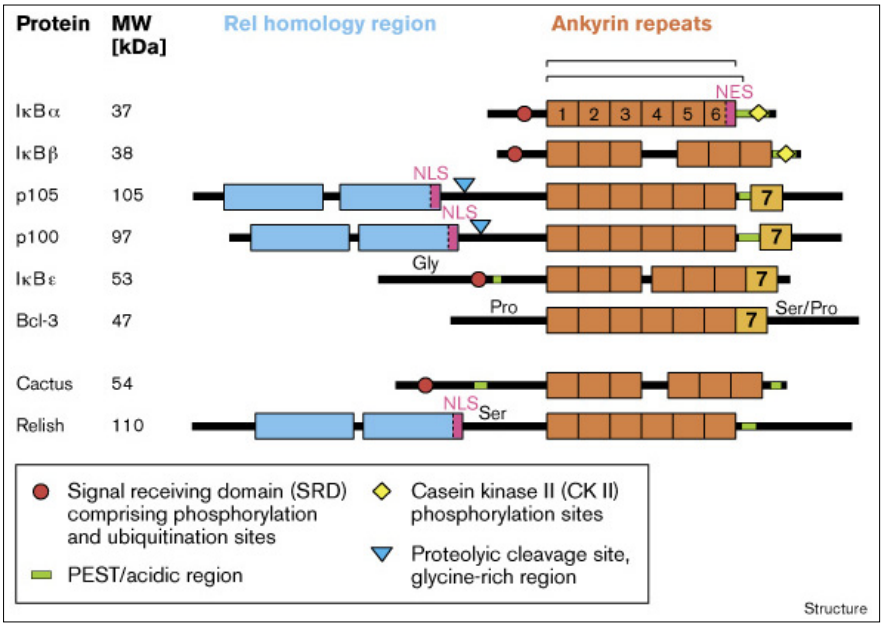
NFκB/Rel proteins can be divided into two classes: one contains a strong transactivation domain at the C terminus, which is responsible for stimulating transcription through interactions with other proteins, whereas the other lacks such a domain. All members of the NFκB/Rel family form stable dimers and modulate their DNA-binding specificity through homo- and heterodimerization. The resulting dimers are either activators or repressors of transcription depending on the presence or absence of a transactivation domain. The Rel homology region (RHR), a stretch of about 300 amino acid residues that contains a nuclear localization signal (NLS) at its C-terminal end, is required for dimerization and DNA binding. Crystal structures of Rel homo- and heterodimers in complex with DNA have shown that the RHR is composed of two immunoglobulin (Ig) like domains connected by a short flexible linker [7–11]. The C-terminal Ig-like domains form the dimer interface. Loop residues of both domains contact the DNA backbone, but only residues of the N-terminal Ig-like domains participate in base-specific recognition.

How the IκB family controls NFκB activity

Considering the importance and variety of genes regulated, NFκB itself has to be tightly controlled. This control is achieved mainly through interactions with proteins of the IκB family, members of which include IκBα, IκBβ, the C-terminal regions of NFκB p105 and p100, and the recently discovered IκBε and Bcl-3 (Figure 1). IκB proteins contain six or seven 'ankyrin repeats' — sequence stretches of 33 amino acid residues in length that were first found in the erythrocyte protein ankyrin (reviewed in [12]). Structural analyses of different ankyrin-repeat-containing proteins have shown the role of this repeat as a modular building block forming a specific protein–protein interaction surface.

IκB and NFκB proteins form tight complexes. One IκB molecule binds to one NFκB dimer and masks its NLS sequences, thereby sequestering it in the cytosol. Joint efforts of several groups have unravelled the mechanism by which this inhibition can be released (Figure 2). Extracellular signals lead to the activation of the 900 kDa IκB kinase (IKK) complex, which phosphorylates two serine residues in the N-terminal signal receiving domain (SRD) of IκB [13]. This in turn results in the ubiquitination of adjacent lysine residues followed by the degradation of IκB in the proteasome and its dissociation from NFκB. The newly exposed NLS sequences of NFκB can interact with cytosolic import factors, leading to the transport of NFκB into the nucleus where it can bind to DNA sites in its target promoters and regulate transcription.

Figure 1



Schematic representation of IκB proteins. The mammalian family members IκBα, IκBβ, p105, p100, IκBε and Bcl-3 and the insect homologues Cactus and Relish are depicted. Nuclear localization signals (NLS) and nuclear export signals (NES) are depicted in pink. A seventh ankyrin repeat found in some family members is depicted in light orange. Rel homology regions in p105, p100 and Relish are shown in blue and regions that are rich in certain types of amino acids are labelled. The upper and lower brackets above IκBα indicate the ordered IκBα residues in the models of Jacobs and Harrison and Huxford *et al.*, respectively.

How is NFκB-mediated gene expression shut off? It has been established that NFκB also activates the transcription of the gene encoding IκBα. Newly synthesized IκBα can enter the nucleus where it disrupts the DNA binding of NFκB and forms a tight complex with NFκB. This complex is then exported into the cytosol. The export of the complex is mediated by the presence of a leucine-rich sequence motif within the sixth ankyrin repeat of IκBα: this motif has been identified as a nuclear export signal (NES) [14], while the principles governing nuclear transport are only just beginning to emerge (reviewed in [15]).

IκBα is the most widely studied IκB family member and regulates the activity of the prototypic NFκB p50–p65 heterodimer. In IκBα, six ankyrin repeats are preceded by the N-terminal SRD. The C-terminal part of the protein following the ankyrin repeats contains an acidic 'PEST' region that is rich in proline, glutamate, serine and threonine residues. This region becomes phosphorylated at serine and threonine residues by casein kinase II (CK II) and is associated with basal protein degradation.

Structure determination of IκBα–NFκB complexes

Determining the crystal structure of the IκBα–NFκB complex was no trivial task, the main hurdle being that of obtaining crystals. Prior to crystallization, complex formation had to be studied carefully. The correct combination of protein constructs was chosen guided by proteolytic digestion and protein–protein interaction assays, such as gel electrophoresis under native conditions, surface plasmon resonance and size-exclusion chromatography [1,2,16].

In the end both groups used a similar combination of constructs: the C-terminal dimerization domain of p50 including the NLS was combined with the entire p65 RHR. Huxford *et al.* used a p65 protein construct ending just after the NLS, whereas Jacobs and Harrison used a p65 protein extending 14 residues further. In both cases, the IκBα construct (M_r = 25 kDa) contains all six ankyrin repeats but lacks the flexible N-terminal SRD. In the structure determined by Huxford *et al.*, 22 additional residues of the C-terminal PEST region are present, the first 11 of which are ordered in the structure but do not adopt secondary structure. The crystal structures of the complex were solved by a combination of molecular replacement and multiple isomorphous replacement methods.

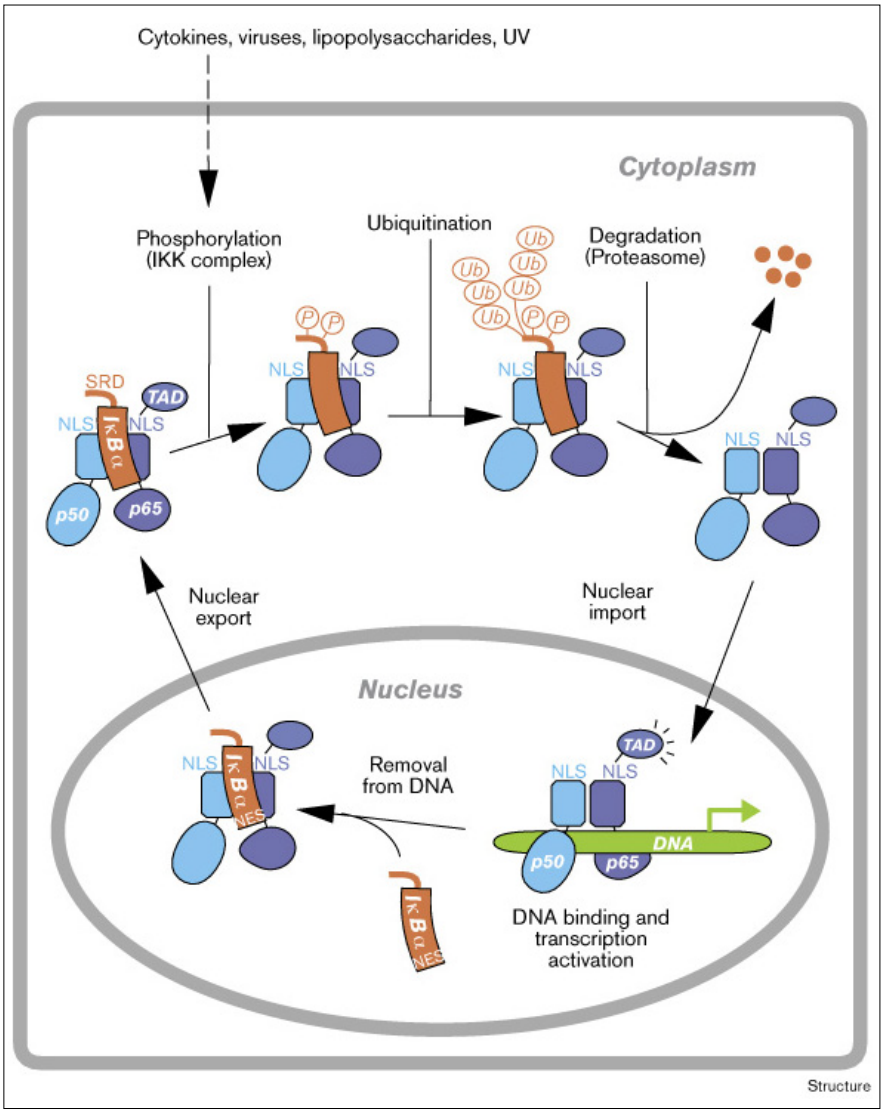
A conserved mode of IκB binding

The structures show that IκBα binds to the dimerization domains of the NFκB p50–p65 dimer and to the NLS of p65 (Figures 3 and 4). The polypeptide chains of IκBα and NFκB run in an antiparallel fashion putting the N-terminal SRD of IκBα close to the p65 transactivation domain. This topology is consistent with a model proposed for the autoinhibition of p105 and p100. It was suggested that the IκB-like region at the C terminus of the p105 and p100 precursors folds back onto their RHR to bring about autoinhibition. It is likely that the glycine-rich region separating the two parts of the precursors simply loops out.

As seen in previous structures, each ankyrin repeat consists of a β loop and two antiparallel α helices perpendicular to the loop. The six ankyrin repeats of IκBα form a stack that curves slightly towards the NFκB dimerization domains.

Figure 2

Schematic diagram of NFκB regulation. IκB determines the subcellular localization of NFκB by preventing nuclear import and serving as an adapter for nuclear export. NFκB subunits p50 and p65 are shown in cyan and purple, respectively, IκBα is shown in orange and DNA is in green. See text for details.

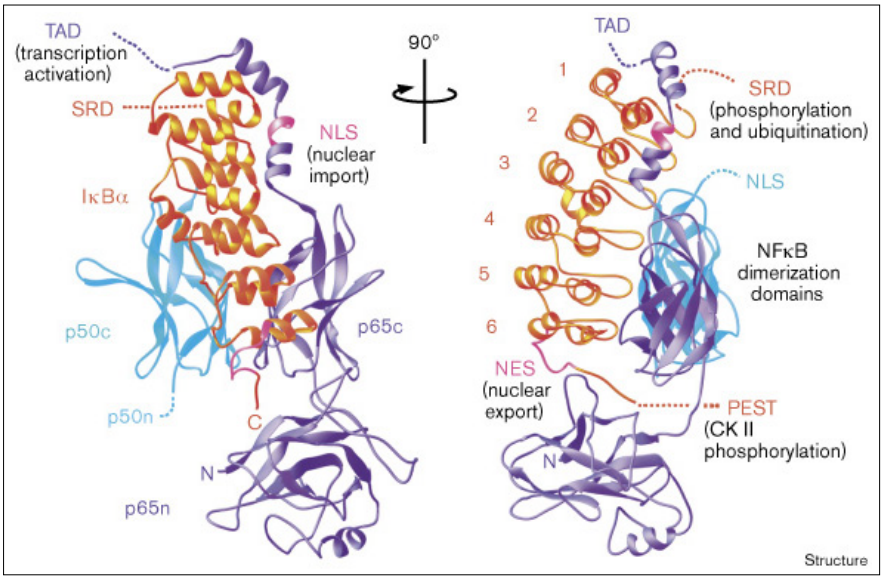


This arrangement prompted Jacobs and Harrison to use the picture of a 'cupped hand', where the loops of the six repeats represent the fingers and the inner and outer helices form the concave and convex surface of the palm, respectively. In each repeat, the C-terminal end of the inner helix packs between the inner and outer helices of the next repeat. The curvature of the stack results from the packing interactions of large hydrophobic sidechains between the outer helices of the repeats. Ankyrin repeats 3 and 4 are separated by six residues; this insert is even larger in IκBβ, IκBε and the *Drosophila* homologue Cactus (Figure 1). The NES at the C-terminal end of the sixth ankyrin repeat of IκBα is exposed in the complexes.

The IκBα–NFκB interface is not continuous but instead consists of three mostly polar patches: ankyrin repeats 1 and 2 interact with the p65 NLS and residues C-terminal to it; the tips of the 'fingers' in repeats 4 to 6 contact strands a, a′ and g of the dimerization domain of p50 along the edge of the dimer interface; and repeats 5 and 6 contact loop c′d of the dimerization domain of p65 via the C-terminal ends of their inner helices. Additional polar interactions are observed between the last ankyrin repeat and the p65 N-terminal domain.

The conservation of most of the contact-forming residues in homologues of the NFκB/Rel and IκB families, suggests that the observed register of ankyrin repeats and the interactions with the described regions of NFκB are essentially identical throughout both families. As seen in many protein–protein complex structures, functional diversity among various homologues might arise from subtle differences in

Figure 3



Two perpendicular views of a ribbon diagram of the IκBα–NFκB complex structure. Depicted is the model of Jacobs and Harrison. NFκB subunits p50 and p65 are shown in cyan and purple, respectively, IκBα is in orange and the nuclear localization signal (NLS) and nuclear export signal (NES) are drawn in pink. The ankyrin repeats of IκBα are numbered. (The figure was drawn with the program RIBBONS [20].)

molecular contacts. However, cell type specific changes in the abundance of the two partners might also lead to the formation of preferred IκB–NFκB combinations.

The NLS structure and blocking of nuclear import

Compared to its structure in the DNA-bound state, the overall structure of NFκB is affected by IκB binding in two ways (Figures 3 and 4). Firstly, the p65 NLS becomes ordered through interactions with ankyrin repeats 1 and 2. Secondly, the p65 N-terminal domain moves dramatically.

Jacobs and Harrison observe a helical conformation of the NLS induced by interactions with ankyrin repeats 1 and 2. This conformation is stabilized by a subsequent helix that packs against the top of ankyrin repeat 1. The residues forming this second, stabilizing helix are missing in the Huxford *et al.* structure and although the position of the p65 NLS is similar, its exact conformation remains elusive. In contrast, the p50 NLS is poorly ordered in both structures. It remains to be seen if N-terminal residues of the SRD of IκBα fold back to mask the p50 NLS, confirming the results of Latimer *et al.* [17], or if steric hindrance by ankyrin repeats 1 and 2 and the p65 NLS is sufficient to block the interactions of the p50 NLS with import factors.

The conformational difference between the p65 and p50 NLS in the complex is in accordance with the observed stoichiometry of one IκB molecule bound to one NFκB dimer. As Jacobs and Harrison point out, the α helix containing the p65 NLS would clash with a second IκB molecule bound at the opposite side of the dimer. Thus, ordering of the p65 NLS guarantees the asymmetry of the complex.

Whereas the p65 NLS sequence (Lys-Arg-Lys-Arg) adopts a helical conformation in the IκBα–NFκB complex, a short peptide comprising a simian virus 40 NLS (Lys-Lys-Lys-Arg-Lys-Val) soaked into crystals of the cytosolic import factor karyopherin α, was found to bind in an extended conformation [18]. Both NLS sequences belong to the group of monopartite NLS sequences [19] and apparently can adopt different conformations depending on the molecular context.

Domain movement and disruption of DNA binding

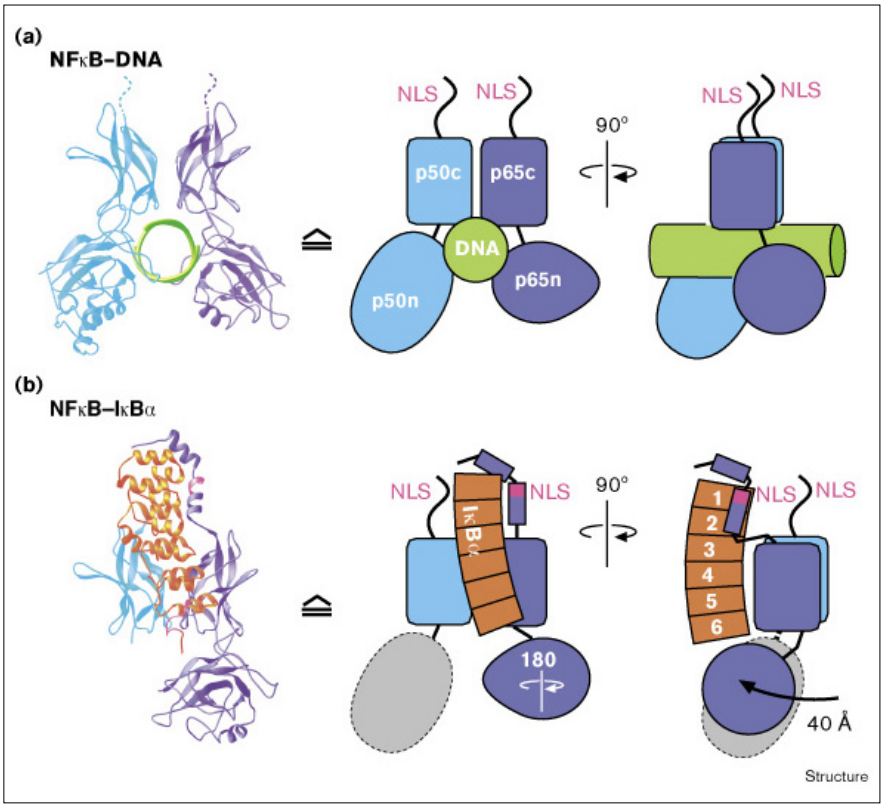
The N-terminal domain of p65 undergoes a dramatic rigid-body movement upon IκBα binding. Compared with its DNA-bound state, an almost 180° rotation and a translation of about 40 Å puts the domain in a position where it loosely associates with the bottom of ankyrin repeat 6 and the first residues of the PEST region. Similar movements might occur when NFκB dissociates from a DNA site.

How does IκB disrupt the NFκB–DNA complex? The conformational changes of NFκB p65 observed in the two structures are comparable, but the two groups put different emphasis on their interpretation. Huxford *et al.* suggest that the observed interactions of IκBα with the N-terminal domain of p65 reflect an allosteric mechanism, whereby IκB binding disrupts NFκB DNA binding through the observed strong domain movements. They also emphasize the role of the electrostatic interactions of negatively charged residues in the sixth ankyrin repeat and the acidic PEST region with positively charged residues of the p65 N-terminal domain.

In contrast, Jacobs and Harrison stress the "paucity of interactions" between the N-terminal and C-terminal

Figure 4

Comparison of the NFκB–DNA and NFκB–IκBα complexes. Ribbon diagrams of **(a)** the NFκB p50–p65 heterodimer–DNA complex structure and **(b)** the NFκB–IκBα complex structure are shown on the left. On the right, two schematic views of the complexes are shown related by a 90° rotation around the vertical axis. When bound to IκBα, the p65 NLS adopts a helical conformation. Compared with its position in the DNA-bound state, the N-terminal domain of p65 undergoes an almost 180° rotation and a translation of approximately 40 Å. The same colour code is used as in Figures 2 and 3. The N-terminal domain of NFκB p50 lacking in the NFκB–IκB complex structures is depicted in grey.



domains of NFκB, which allows the connecting linker to adopt its favourite conformation, resulting in the observed position of the p65 N-terminal domain for both copies in the asymmetric unit. The N-terminal domains show high thermal mobility in both structures [1,2] and in the view of Jacobs and Harrison crystal-packing forces rather than specific interactions with IκBα determine their exact positions. Because the C-terminal part of IκBα approaches the DNA-binding region of NFκB and would clash with bound DNA from the end of the sixth ankyrin repeat onwards, Jacobs and Harrison favour a model where DNA binding is prevented through steric hindrance. As it is "...the beautiful answer that asks the more beautiful question..." only further experiments will be able to clarify unambigously by which mechanism IκB disrupts the NFκB–DNA complex.

A related question is how the nuclear IκB homologue Bcl-3 functions. This homologue, like IκBε and the C-terminal regions of p105 and p100, contains a seventh ankyrin repeat which would interfere with bound DNA. However, Bcl-3 is mostly located in the nucleus and can form stable ternary complexes with NFκB p52 or p50 homodimers bound to DNA. For the formation of these complexes the conformation of the C-terminal region of Bcl-3 must be different.

Concluding remarks

The two X-ray structures of the IκB–NFκB complex provide a wealth of information for the interpretation of genetic and biochemical data and represent an important step towards the understanding of NFκB–IκB regulation. Two further noteworthy aspects of the complex should be mentioned. Firstly, NFκB is an important drug target due to its role in inflammatory disorders, such as asthma and rheumatoid arthritis. The clear definition of the IκB–NFκB interface, as a result of the X-ray structures, may allow the design of small molecules that can alter IκB binding and could ultimately lead to novel anti-inflammatory drugs. Secondly, the structures emphasize the two faces of IκB in nuclear transport: on one side IκB prevents nuclear import by masking an NFκB NLS, while on the other it provides an NES sequence for nuclear export. In this sense we can consider IκB as an NFκB-specific component of the nuclear transport machinery.

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