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The Nuclear Factor – κ B

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Abbreviation:

ERK:extraregulated kinase

IKK: I κ B kinase

MAP: mitogen-activated protein

NIK: NF- κ B inducing kinase

PUFA: polyunsaturated fatty acids

RHR: Regions of high homology

ROS: reactive oxygen species

I κ B: inhibitor – κ B

IL:interleukin

NF- κ B: nuclear Factor – κ B

NLS: nuclear localization signals

PTK: tyrosine kinases

RNOS: reactive nitrogen oxide species

Table of contents

Abstract-----	2
Introduction-----	3
The structure of the p50/p65 dimer-----	4
The classical activation pathway of NF- κ B and some modification to the model-----	5
Nonclassical NF- κ B signaling pathways-----	6
The NIK pathway-----	6
The NF- κ B activation involved I κ B α but without I κ B α degradation-----	7
Novel I κ B α proteolytic pathway-----	8
Role and regulation of NF- κ B transcriptional activity -----	8
Reduction-oxidation (redox) regulation NF- κ B-----	9
References-----	10

Abstract

The Nuclear Factor – κ B (NF- κ B) family comprises a number of structurally related, interacting proteins that bind DNA as dimers. NF- κ B is a critical regulator in the development and maintenance of the immune system, also it is involve in stress responses and neoplastic progression. Members of this family are formed by dimeric combinations of subunits and are activated by a number of receptor-mediated signaling pathways. Once activated, they stimulate the transcription of specific sets of target genes mediating a plethora of diverse functions. This review mainly focuses on recent advance on the structure of NF- κ B family, the NF- κ B function *in vivo*, the activation pathway of NF- κ B and the regulation of NF- κ B activation.

1. Introduction

Firstly, the nuclear factor- κ B (NF- κ B) was identified as a factor that regulates the expression of the κ light chains in mouse B-lymphocytes, later, the NF- κ B was found also involved in diverse cellular functions such as immune response, cell growth, and development [1]. The NF- κ B family (Rel family) comprises five members, including: Rel (c-Rel), Rel A (p65), Rel B, NF- κ B1 (p105/p50), and NF- κ B2 (p110/p52). They are structurally related and can bind DNA as homo- or heterodimers [1-2]. The classical form of NF- κ B is the heterodimer of p50 and p65 subunits [14]. The NF- κ B family has been divided to two groups: NF- κ B1 and NF- κ B2 form one group, they are synthesized from precursor proteins of 105 and 110 kDa respectively; the others form another group [1-3]. All the Rel family members have amino-terminal Rel-homology regions (RHRs), The RHR is responsible for the dimerization, DNA binding and complex formation with inhibitor κ B proteins [1-4]. Figure 1 shows the sequence motifs of Rel/NF- κ B family proteins [4].

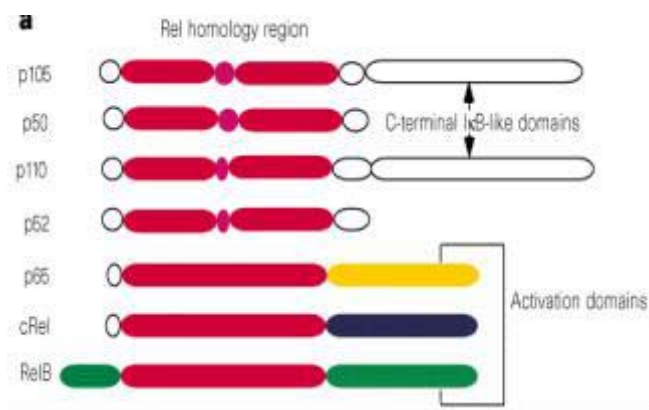


Figure 1 The Sequence motifs of Rel/NF- κ B family of proteins. p50 and p52 are proteolysis products of p105 and p110, respectively, in which the inhibitor κ B-like domains are removed. Regions of high homology (RHR) exist among the family members. p105 (p50) and p110(p52) also contain 32- and 18-amino-acid inserts, respectively, in the RHR. Non-homologous transactivation domains exist in p65, c-Rel, and RelB [4].

The NF- κ B family regulates the expression of genes encoding cytokines, adhesion molecules, and many other proteins [13]. Table 1 shows some of these genes [11].

TABLE 1. THE ARRAY OF PROTEINS ENCODED BY GENES DIRECTLY CONTROLLED BY NF-KB	
Protein type	Protein
Cytokines/growth factors	IL-1 α , IL-1 β , IL-2, IL-3, IL-6, IL-8, IL-12, TNF- α , Lymphotoxin- α ,
	Interferon- β , Granulocyte Colony Stimulating Factor, Macrophage Colony
	Stimulating Factor Granulocyte-Macrophage Colony Stimulating Factor
Cytokine receptors	IL-2 receptor α chain

Stress proteins	Serum amyloid A protein, Complement factors B, C3 and C4 α_1 glycoprotein
Adhesion molecules	Intracellular adhesion molecule-1, Vascular cell adhesion molecule-1, Mucosal addressin cell adhesion molecule-1, E-selectin
Immunoregulatory molecules	Immunoglobulin κ light chain, MHC class I and II, T-cell receptor α and β , β_2 -Microglobulin, Invariant chain, Transporter associated with antigen processing, Proteasome subunit, Inducible nitric oxide synthase, Inhibitory κ B, p53

2. The structure of the p50/p65 dimer and the NF- κ B/ DNA interaction.

The overall structure of the p50/p65 heterodimer has been determined by Chen *et al.* [4]. It is consistent with that of other Rel family proteins. Each subunit consists of two immunoglobulin like domains connected by a 10-amino-acid flexible linker. Dimers form through a β -sheet sandwich of the carboxy-terminal dimerization domains. Unlike most DNA-binding proteins, which use α -helices for base-pair recognition, Rel family dimers use loops from the edges of the N- and C-terminal domains to mediate DNA contacts (Figure 2).

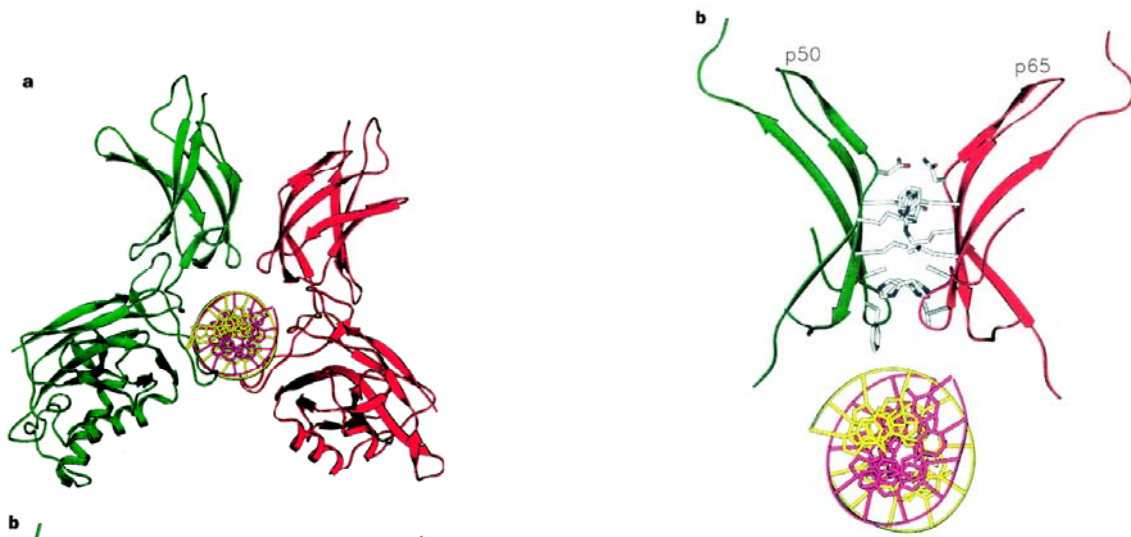


Figure 2. The structure of the heterodimer bound on the Ig κ B DNA. **a**, Ribbon drawing of the entire complex viewed down the DNA helical axis. **b**, The hydrophobic core of the dimer interface between p50 and p65 consists of an array of nonpolar hydrocarbons, aromatic rings and uncharged polar residues pointing from the β -sheets in towards the interface [4].

Secondary structures of the subunits are equivalent, apart from a 32-amino-acid insert in the

N-terminal domain of p50 that adds a second β -helix [4]. The RHRs of Rel family is responsible for the dimerization, DNA binding and complex formation with inhibitor κ B (I κ B) [2]. NF- κ B specifically recognizes κ B DNA elements with a consensus sequence of 5'-GGGRNYYYCC-3' (R is an unspecified purine; Y is an unspecified pyrimidine; and N is any nucleotide) [4]. Chen *et al.* report the crystal structure at 2.9 Å resolution of the p50/p65 heterodimer bound to the κ B DNA of the intronic enhancer of the immunoglobulin light-chain gene. They revealed a 5-base-pair 5' subsite for interaction with p50, and a 4-base-pair 3' subsite for p65. This structure indicates why the p50/p65 heterodimer interface is stronger than that of either homodimer. A comparison of this structure with those of other Rel dimers reveals that both subunits adopt variable conformations in a DNA-sequence-dependent manner. Their results explain the different behaviour of the p50/p65 heterodimer with heterologous promoters [4]. An interesting and related result is provided by Chen *et al.* They found that the dual function of NF- κ B, as an inhibitor or activator of apoptosis, depends on the relative levels of RelA and c-Rel subunits. The ratio between c-Rel and RelA (p65) subunits will determine whether the activation of NF- κ B will trigger apoptotic or survival signal. They speculate that the different subunits has different affinity with certain promoters [7,9,13].

3. The classical activation pathway of NF- κ B and some modification to the model

The Rel/NF- κ B family of transcription factors is sequestered in the cytoplasm of most mammalian cells by inhibitor proteins belonging to the I κ B family [1-3]. The I κ B family includes I κ B α , I κ B β , I κ B γ , I κ B ϵ , Bcl-3, and the precursors of NF- κ B1 (p105) and NF- κ B2 (p110). Members of the I κ B family of proteins are characterized by the presence of six or more ankyrin repeats [13]. It has been known after phosphorylation on serine 32 and serine 36 of I κ B α , or serine 19 and serine 23 of I κ B β , or serine 157 and serine 161 of I κ B ϵ by I κ B kinase complex (IKK), the I κ B is degraded by

the ubiquitin/26S proteasome pathway, allowing NF- κ B to translocate to the nucleus and regulate many genes' expression [11-13]. The IKK is composed of three subunits: two, IKK α and IKK β , are bona fide kinases, while the third, IKK γ (NEMO), has no catalytic activity but plays a critical regulatory role [1,4]. Conventional view holds that a central point of NF- κ B regulation is the translocation of activated NF- κ B from the cytoplasm to the nucleus. Cytoplasmic retention of the inactive I κ B/NF- κ B complex is thought to be due to the masking of the dual nuclear localization signals (NLS) located on NF- κ B by I κ B. Upon receiving a variety of signals, I κ B undergoes phosphorylation, is then ubiquitinated at nearby lysine residues and finally degraded by the proteasome [3,7,12]. New discoveries made it necessary to modify this simplistic model. Study of the crystal structure of I κ B complexed to NF- κ B p50 revealed that only one NLS is masked by I κ B, while the other is still free. The presence of a free NLS gives credence to recent observations that I κ B/NF- κ B complexes are dynamic in nature and can shuttle between the cytoplasm and nucleus [3].

4. Nonclassical NF- κ B signaling pathways

4.1. The NIK pathway

The classical signaling pathway leading to the activation of NF- κ B is mediated by IKK β and results in the phosphorylation of I κ B. This pathway lies downstream of all known proinflammatory stimuli including TNF, IL-1, LPS, and double-stranded RNA. In contrast, the nonclassical NF- κ B activation pathway involves IKK α and leads to the phosphorylation of p110 – the precursor of NF- κ B2 p52, which can be stored in cells (see Figure 3), and its subsequent processing to p52. IKK α is itself activated by the upstream kinase NIK [3].

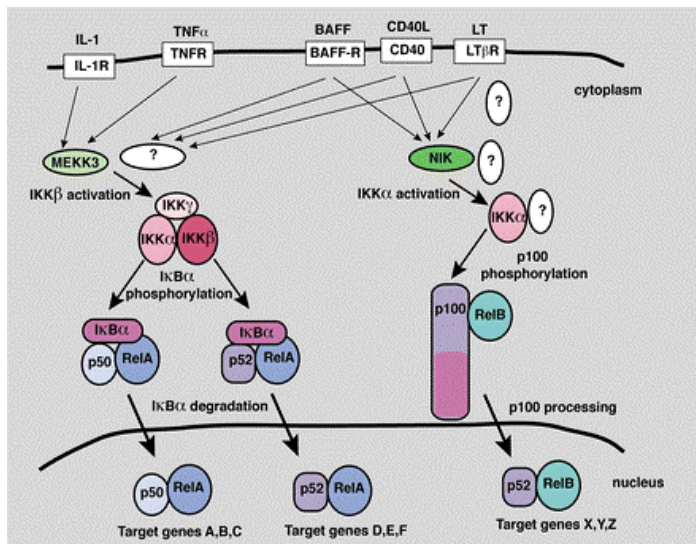


Figure 3. Canonical (Left) and noncanonical (Right) Pathways for the activation of NF- κ B, in the canonical pathway, the MEKK3 activate the IKK β . Subsequently, the NF- κ B is activated. In the noncanonical pathways the kinase NIK activated IKK α , then the NF- κ B is activated [3].

4.2. The NF- κ B activation involved I κ B α but without I κ B α degradation.

Recently, Natarajan *et al.* showed that oxidative stress, generated by exposure to hypoxia followed by reoxygenation (H/R), failed to activate IKK in human microvascular endothelial cells (HMEC-1) [5]. They provided an alternative mechanism for NF- κ B activation during H/R stress without I κ B α degradation. This mechanism involves activation of protein tyrosine kinases (PTK) that phosphorylate I κ B α . Involvement of PTK was reinforced by the demonstration that the PTK inhibitor, herbimycin A, prevented H/R-mediated NF- κ B activation. Tyrosine phosphorylation alters the association between I κ B and NF- κ B with sufficient intensity to allow transient NF- κ B translocation to the cell nuclei, immunofluorescence imaging of NF- κ B protein reveals it to be shuttled between the nucleus and cytoplasm [5]. Overall, their studies suggest that tyrosine phosphorylation of I κ B represents a proteolysis-independent mechanism of NF- κ B activation [5].

4.3. Novel I κ B α proteolytic pathway

Rel/NF- κ B (a p50-c-Rel dimer) is constitutively nuclear in murine B cells, such as WEHI231 cells. In these cells, p50, c-Rel, and I κ B α are synthesized at high levels but only I κ B α is rapidly degraded [11]. In the canonical pathways for the activation of NF- κ B, prior phosphorylation at serine at 32

and 36 is an essential requirement for most signal-inducible I κ B α degradation pathways [1-4]. Miyamoto *et al.* demonstrate that all I κ B α is found complexed with c-Rel protein in the cytoplasm. Additionally, rapid I κ B α proteolysis is insensitive to proteasome inhibitors and S32/36 phosphorylation is not an absolute required, but the rapid I κ B α proteolysis can be inhibited by calcium chelators and some inhibitors of calpain, which is a calcium-dependent cysteine protease [11]. The conditions that prevent degradation of I κ B α also inhibit nuclear p50-c-Rel activity. So a novel calpain depend I κ B α proteolytic pathway was established [11].

5. Role and regulation of NF- κ B transcriptional activity

It is becoming evident that NF- κ B activation also involves posttranslational modification of NF- κ B/Rel proteins, particularly p65. Indeed, p65 had previously shown to be phosphorylated following cellular stimulation [3]. It has been found that while induction of IL-6 by TNF depends on an NF- κ B response element within the IL-6 promoter, it also requires concurrent activation of ERK and p38 MAP kinases. Pharmacological inhibition of these kinases leads to an attenuation of TNF-mediated IL-6 induction despite I κ B degradation, NF- κ B nuclear localization, and unaltered DNA binding capacity. This observation raises the possibility that the phosphorylation of NF- κ B by MAP kinases is required for transcriptional activation. While neither p50 or p65 are direct substrates for MAP kinases, p65 can be phosphorylated by one nuclear kinase located downstream of ERK and p38 at Ser 276 [1,3], an event required for transcriptional activity. It thus represents, together with the phosphorylation of particular nucleosome components (e.g., histone H3), an essential step leading to selective transcriptional activation of specific NF- κ B-dependent genes. It is likely different protein kinases modulating the transcriptional activity of NF- κ B by regulated phosphorylation of p65 and other Rel proteins, as well as of chromatin components [3].

6. Reduction-oxidation (redox) regulation of NF- κ B

Reduction-oxidation (redox) regulation has been implicated in the activation of the transcription factor NF- κ B [8-9]. However, the significance and mechanism of the redox regulation remain elusive, mainly due to the technical limitations caused by rapid proton transfer in redox reactions and by the presence of many redox molecules within cells [2]. Nishi *et al.* established some versatile methods and demonstrated that the redox state of NF- κ B is spatially regulated by its subcellular localization. While the p65 subunit and most cysteine residues of the p50 subunit are reduced similarly in the cytoplasm and in the nucleus, Cys-62 of p50 is highly oxidized in the cytoplasm and strongly reduced in the nucleus. The reduced form of Cys-62 is essential for the DNA binding activity of NF- κ B. Several lines of evidence suggest that the redox factor Ref-1 is involved in Cys-62 reduction in the nucleus. They propose that the Ref-1-dependent reduction of p50 in the nucleus is a necessary step for NF- κ B activation [2].

7. Summary

The NF- κ B is a critical transcription factor for many important genes and is involved in many important physiological and pathological processes. Members of this family are formed by dimeric combinations of subunits and are activated by a number of receptor-mediated signaling pathways. The activation of NF- κ B is extremely complex and under strict regulations. Once activated, they stimulate the transcription of specific sets of target genes mediating a plethora of diverse functions.

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