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NF-κB, a redox regulated transcription factor

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Abbreviation:

GPx	Glutathione peroxidase
IKK	IκB kinase
MEKK	mitogen-activated protein kinase/extracellular signal regulated kinase kinase kinase
NF-κB	Nuclear factor kappa B
NIK	NF-κB-inducing kinase
ROS	Reactive oxygen species
TNF	Tumor necrosis factor
TRAFs	TNF receptor-associated factor
Trx	Thioredoxin

Table of Contents **page**

Abstract

NF-κB is an important transcription factor. Before activated, the p50 and p65 dimer binds to I-κB. After the degradation of I-κB, NF--κB entry into nucleus. It regulates many genes' expression. In turn, it is also regulated by many factors including the redox condition in the cell. Oxidative stress can up regulate NF--κB, while the DNA binding ability requires the NF--κB subunit to keep the reduce states. This mini review describes the molecular characteristics, the mechanism of its activation, how the activated dimers bind to DNA, the possible mechanism of redox regulation and the method to detect the NF-κB level in cell extract.

Introduction

NF-κB was first described by Sen and Baltimore as a nuclear transcription factor in B-cells that bound to the B-site of the immunoglobulin κ enhancer [1]. Now we know that NF-κB is not only found in B-cells, but also can be found in almost every cell type. Its DNA binding sites, κB sites, have been identified in the promoter region of many mammalian genes. So NF-κB actually acts as a promoter instead of enhancer. The number of genes that are regulated by NF-κB is large. Typically, cytokines (IL-2, IL-6, IL-8, β-interferon, TNFα, and β), growth factors (GM-CSF, G-CSF, M-CSF), cell adhesion molecules, immunoreceptors (T-cell receptor, MHC class I and II, IL-2 receptors) or acute phase protein are the targets of NF-κB regulation [2].

NF-κB is a heterodimer of subunits known as p50 and p65 (RelA), both of which exhibit homology to the product of the *rel* oncogene [3]. Rel family members can form both hetero- and homodimers with distinct specificities in various combinations. The NF-κB complex is activated by phosphorylation, ubiquitination, and consecutive proteasomal degradation of the IκB component [4]. Activation of NF-κB also controlled by redox status of the cell. For example, activating NF-κB tends to trigger the formation of reactive oxygen species (ROS); NF-κB activation can be triggered by H_2O_2 or organic hydroperoxides; NF-κB activation is inhibited by a broad range of chemically unrelated antioxidants [5]. This review will focus on the molecular characteristics of NF-κB and the regulation pathways of its activation.

Molecular Characteristics

Mammalian Rel/NF-κB family polypeptides include p65 (RelA), p50, p52, RelB, and the proto-oncoprotein c-Rel. These subunits associate in various combinations to form homodimers and heterodimers with distinct but overlapping functions. Among the most abundant and best understood of these dimers are the p50/p65 heterodimer and the homodimers of p50 and p65 [6]. The Rel homology region of p50 (and so do other subunits) consists of two distinct domains: N-terminal domain is the larger one which contains residues that contact DNA base pairs; the C-terminal domain have two functions, one is forming the dimer interface, the other is a DNA phosphate interaction [7]. NF-κB is not active in healthy tissue because there is a class of specific inhibitory proteins, called IκBs, that prevent nuclear translocation and DNA binding of the transcription factor [8]. Figure 1 shows the schematic form of the complex.

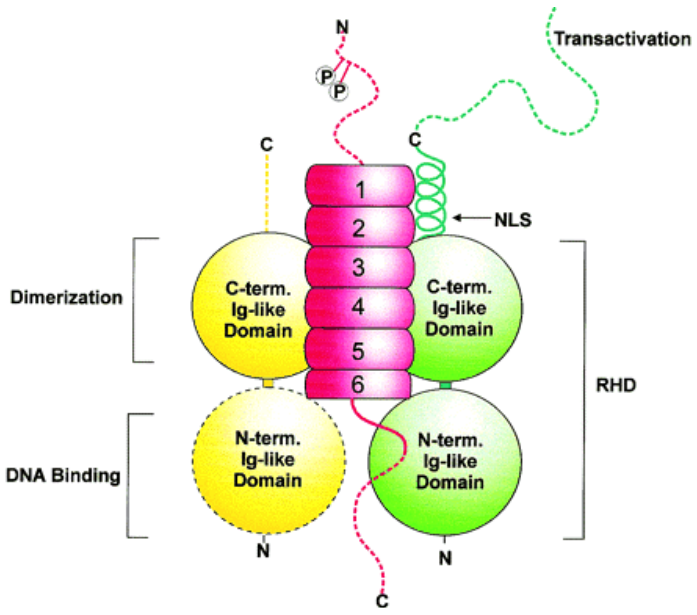

Figure 1. Schematic Presentation of the IB/p65/p50 Structure Combining Features. (Adapted from [8].) Shown in middle is IkB. The numbers refer to the ankyrin repeats. Shown on the left is p50 and on the right p65 with their two Ig-like domains.
NLS, nuclear locating signal. RHD, rel homology domain; P, phosphate groups on serines 32 and 36; C and N, C and N termini of the three proteins.

The key step of NF-κB activation has been characterized as an inducible phosphorylation of ser32

and ser36 near the N-terminus of IκBα or ser19 and ser23 of IκBβ [5]. Figure 2 shows the IκB degradation procedure.

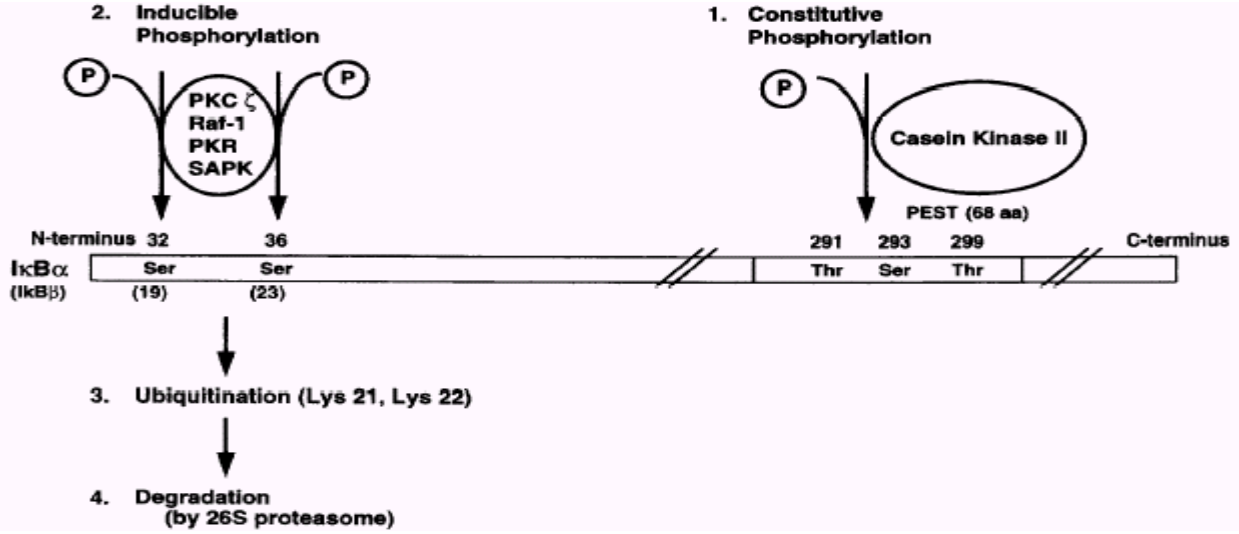

Figure 2. Sequence of IκB processing. Step I is constitutive phosphorylation of the PEST domain by casein kinase II. Step 2 is the serine residues phosphorylation. Step III is multiubiquitination at lysines 21 and 22. Step IV is degradation by 26S proteasome. (Adapted from [5].)

As shown in Fig. 2, casein kinase II carries out constitutive phosphorylation of the C-terminal 68 amino acids, a Pro, Glu, Ser, Thr-rich domain of IκB (PEST domain) [5]. Phosphorylation of serine residues 32 and 36 near the N-terminal targets IκBα to ubiquitination at lysine residues in the n-terminal domain and, in consequence, to proteasomal degradation [5].

Redox regulation of NF-κB activation

TNFα is a strong activator of NF-κB. The possible mechanism is TNFα can induce MnSOD [5]. Mitochondrial H_2O_2 formation induced by TNFα may be released into the cytosol where it could induce or facilitate NF-κB activation [5].

Antioxidants can inhibit the activation of NF-κB. Those antioxidants include thiols (N-acetyl-L-cysteine, L-cysteine, 2-mercaptoethanol, aurothioglucose), α-lipoic acid (which is intracellularly reduced to generate a dithiol), other sulfur-containing scavengers (pyrrolidine dithiocarbamate, diethyldithiocarbamate, disufiram), phenolic antioxidants (butylated hydroxyanisol (BHA), nordihydroguaretic acid (NDGA), α-tocopherol) and chelators (which prevent transition metal-mediated free radical chain propagation, such as orthophenanthroline or deferrioxamine) [5]. Glutathione peroxidases (GPx) and Thioredoxin (Trx) are two important substances in this regulation process. Research showed that selenium supplementation increased GPx activity and decreased H_2O_2-induced NF-κB activation [9]. Overexpressing GPx in human breast cell inhibited the activation of NF-κB induced by TNFα or H_2O_2, and both κB -dependent gene transactivation and NF-κB DNA binding were reduced [10]. The role of Trx is contradicting. One research showed that overexpressing Trx inhibited NF-κB [11] while another showed that only the reduced form of Trx activated NF-κB [12]. It seems that Trx plays a different role in NF-κB activation. This idea is support by Japanese research [13]. It showed Trx treatment inhibited NF-κB -dependent transcription at the level of downstream of TRAFs and upstream of NIK: Trx inhibited TRAF2-, TRAF5- and TRAF6-induced NF-κB activation but did not inhibit NIK-, IKKα and MEKK-induced activation.

The actual role in the activation is at the DNA binding step. Most transcription factors, including NF-κB, favor DNA binding under reduced condition [15]. NF-κ

B has a critical cysteine residue in its DNA binding region that must be in the reduced state for DNA binding to occur [15].

DNA binding

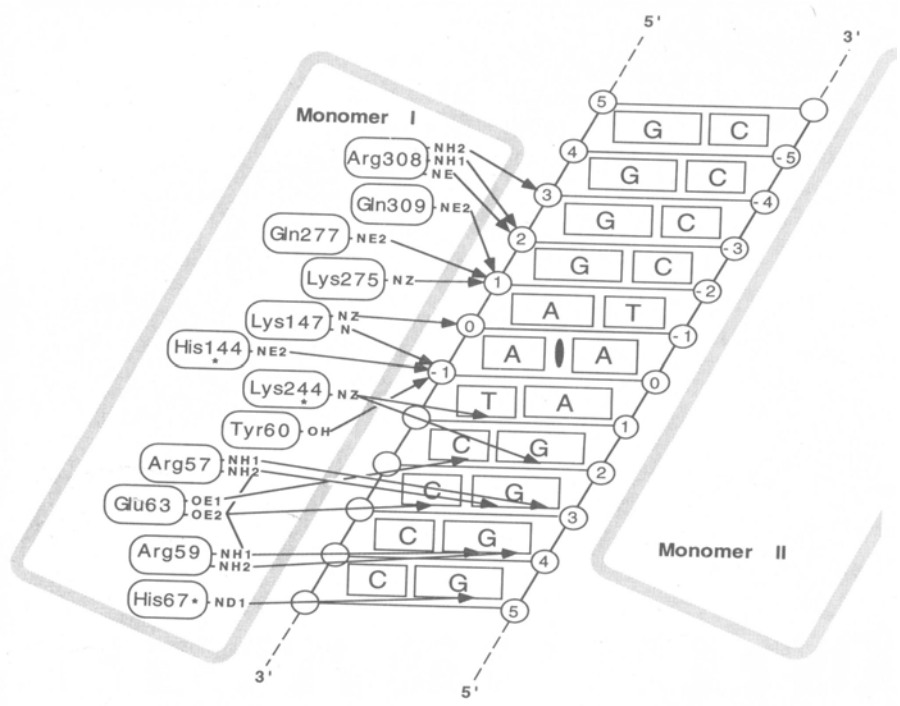

Figure 3. The schematic diagram of the polar interaction between p50 and DNA. (Adapted from [7].)

Upon activated, NF-κB translocates to nucleus and binds to DNA promoter region of target genes. The DNA motifs that NF-κB can recognize is 5'-GGGPuNNPyPy CC-3' [5]. Figure 3 shows the schematic diagram of the polar interaction between p50 and the central 11 base pairs of the DNA. The phosophodiester bonds of the sugar-phosphate backbone of the native duplex preferentially adopt a distinct conformation in the 5' and 3' regions of the κB site [14]. The mutant of this site is incapable of adopting the native DNA's conformation, suggesting that κB-DNA sequence also plays a role in NF-κB -DNA complex formation. The combined flexibility of the NF-κB dimers and their target

DNA allows NF-κB to adopt multiple conformations in a promoter specific manner [14]. Fraction of DNA bound (FB) can be calculated as below [14]:

$$FB = \frac{\begin{array}{c}([\text{NF-}\kappa\text{B}]/K_{\text{monomer(p65)}} + [\text{NF-}\kappa\text{B}]/K_{\text{monomer(p50)}}) \\ +([\text{NF-}\kappa\text{B}]^2/aK_{\text{monomer(p65)}}\,K_{\text{monomer(p50)}})\end{array}}{\begin{array}{c}1 + (2[\text{NF-}\kappa\text{B}]/K_{\text{monomer(p65)}} + 2[\text{NF-}\kappa\text{B}]/K_{\text{monomer(p50)}}) \\ +([\text{NF-}\kappa\text{B}]^2/aK_{\text{monomer(p65)}}\,K_{\text{monomer(p50)}})\end{array}}$$

$K_{\text{monomer(p65)}}$ is the affinity of the p65 monomer for its DNA half-site, $K_{\text{monomer(p50)}}$ is the affinity of the p50 monomer for its DNA half-site, and α is a cooperativity factor for the binding of the second monomer. The p50/p65 heterodimer binds DNA tighter than either homodimer (Figure 4) [14].

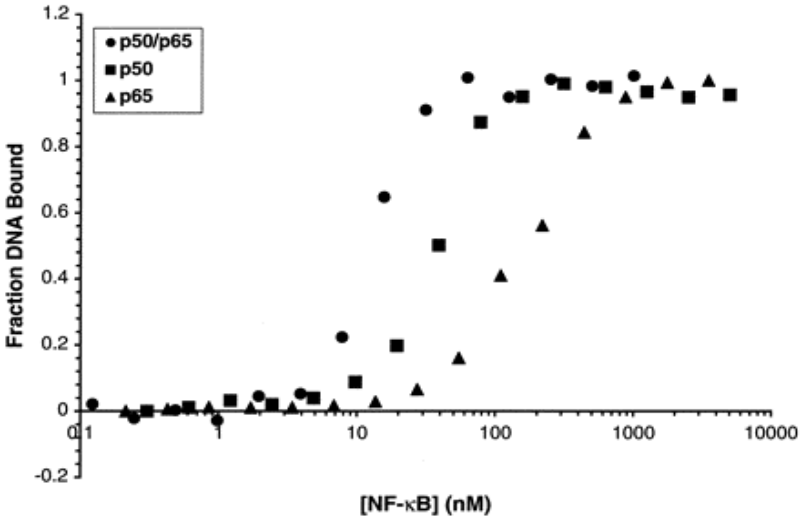

Figure 4. Semi-logarithmic plot of concentration (nM) of p50/p65, p50, and p65 versus fraction DNA bound from fluorescence anisotropy data for representative data sets. (adapted from [14])

Detection of NF-κB

Since NF-κB is basically a protein, the simplest way to detect it is Western blot. But more important, it is a transcription factor, so the gel shift assay is better.

Figure 5 is the typical results of this kind of assay. The oligonucleotide used for the assay was 5'-TCTGAGGGACTTTCCTGATC-3', which contains the heterodimer target site Ig-κB (underlined).

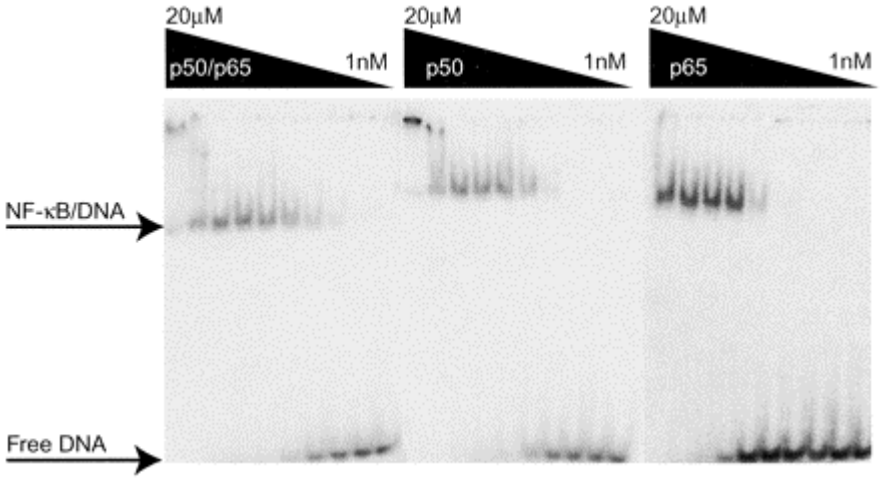

Figure 5. Sample electrophoretic mobility shift assays of p50/p65 heterodimer, p50 homodimer, and p65 homodimers (left to right). DNA concentration was held constant in each lane and titrated with decreasing NF-κB concentrations. Arrows indicate the location of the NF-κB dimer-DNA complex and free duplex DNA. (Adapted from [14].)

Summary

NF-κB is an important transcription factor that regulates many genes expression. Its activation is regulated by redox condition in some cell. After IκB degradation, the NF-κB dimers enter into nucleus and bind to κB site on promoter region. Thus turn on the target genes. Gel shift assay is the best method to detect the NF-κB level.

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