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Editor

Encyclopedia of Signaling Molecules

With 686 Figures and 86 Tables

 Springer

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ISBN 978-1-4419-0460-7 ISBN 978-1-4419-0461-4 (eBook)

DOI 10.1007/978-1-4419-0461-4

ISBN 978-1-4419-0462-1 (print and electronic bundle)

Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012942647

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IFN- β 2

► IL6

IFN- γ

► Interferon-Gamma

IkappaBzeta

► IkBz

IkBz

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Synonyms

IkappaBzeta; IkB-zeta; IL-1 inducible nuclear ankyrin-repeat protein (INAP); Inap; Mail; Molecule possessing ankyrin repeats induced by lipopolysaccharide (MAIL); Molecule possessing ankyrin-repeats induced by lipopolysaccharide; NF-kappa-B inhibitor zeta; NFKBIZ; Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta

Historical Background

IkB ζ , which is also known as molecule possessing ankyrin repeats induced by lipopolysaccharide (MAIL) or interleukin (IL)-1 inducible nuclear ankyrin-repeat protein (INAP), was discovered independently in three laboratories during 2000 and 2001 as a protein containing ankyrin repeats. The gene encodes a protein with an amino-terminal region nearly 450 amino acids in length of unknown structure that contains a nuclear localization signal (NLS) and

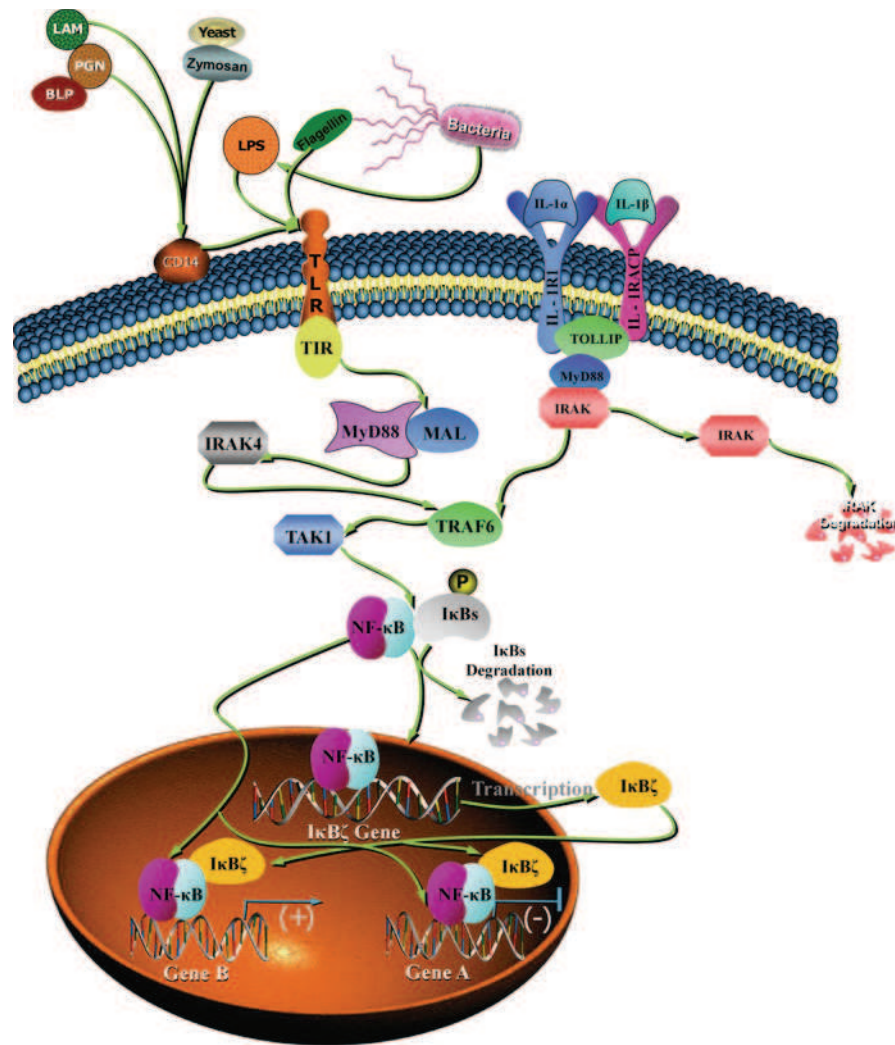
a transactivation domain (TAD) followed by the seven recognizable ankyrin repeats at its carboxyl terminal. Treatment of cells with lipopolysaccharides (LPS) or the cytokine interleukin (IL)-1 (Haruta et al. 2001; Kitamura et al. 2000; Yamazaki et al. 2001) induced the expression of IkB ζ , whereas tumor necrosis factor (TNF)- α (Totzke et al. 2006) treated cells did not show any IkB ζ expression. The induced IkB ζ is localized in the nucleus, where it interacts with the nuclear factor (NF)- κ B subunit and other nuclear proteins via their ankyrin repeat domain (ARD), leading to a positive or negative regulation of its transcriptional activity depending on genes (Muta 2006). Thus, the innate immune system utilizes NF- κ B as a major transcription factor and modulates its activity in a gene-specific manner via the regulatory factor IkB ζ , which is specifically induced upon stimulation of the innate immune system. This multistep regulation of the transcriptions would be fundamental in the selective expression of genes upon cell activation. In this chapter, we summarize recent findings in nuclear IkB ζ with an emphasis on its immunological aspects.

Induction of IkB ζ and Its Functions

Many cellular responses are mediated by orchestrated gene expression. When cells are exposed to diverse inflammatory stimuli, such as microbial components, a large number of genes are induced to elicit inflammatory responses. These genes include cytokine/chemokine, antimicrobial peptides, and cell adhesion molecules, many of which are known to be induced through activation of transcription factor NF- κ B (Akira and Takeda 2004; Hayden and Ghosh 2008; Hoffmann and Baltimore 2006). In resting cells, typical cytoplasmic IkB proteins (IkB- α , - β and - ϵ) mask the NLS of NF- κ B, thereby preventing its translocation into the nucleus. The activation of cells with appropriate stimuli, particularly toll-like receptor (TLR) ligands or various host immune mediators such as proinflammatory cytokines and IL-1 superfamily proteins, induces activation of IkB kinase complex, which leads to the degradation of the cytoplasmic IkBs by the ubiquitin-proteosomal pathway. The NF- κ B liberated from the IkBs is then translocated to the nucleus, where it binds to the promoter/enhancer region of the target genes, resulting in the regulation

I κ B ζ , Fig. 1 Roles of I κ B ζ in inflammatory response.

Activation of the TIR-containing receptors by TLR ligands elicits phosphorylation and ubiquitination-induced degradation of the cytosolic I κ B proteins, which allows nuclear translocation of NF- κ B. In the nucleus, NF- κ B activates transcription of a subset of genes A, which includes I κ B ζ . The expression of I κ B ζ also requires a specific mRNA stabilization signal that comes from the TIR-containing receptor as well as activation of NF- κ B. The expressed I κ B ζ associates with NF- κ B, and the complex engages transcription of another subset of genes B. Simultaneously, I κ B ζ inhibits transcription of the subset of genes A.



of transcription via recruitment of several co-activators and co-repressors. This transcriptional activation leads to the expression of primary/early response gene A, depicted in Fig. 1, which includes three atypical members, I κ B ζ , Bcl-3, and I κ BNS. The induced I κ B ζ associates with NF- κ B, and this complex then activates another subset of inflammatory gene B (Fig. 1). Simultaneously, I κ B ζ inhibits the transcriptional regulation of gene A.

The predominantly expressed cytoplasmic I κ B proteins, I κ B- α , - β , and - ϵ , act exclusively as NF- κ B inhibitors, whereas the nuclear I κ B proteins, Bcl-3 and I κ B ζ , can both act as either a positive or negative regulator of NF- κ B target genes. Bcl-3 can act as a positive regulator of NF- κ B either by removing

transcriptionally inactive p50 and p52 dimers from the I κ B sites, thus allowing transcriptionally active heterodimers to take their places or by forming a ternary complex with DNA-binding p50 and p52 homodimers, thereby facilitating gene expression (Yamazaki et al. 2001; Totzke et al. 2006; Dechend et al. 1999; Fujita et al. 1993; Yamamoto et al. 2004). Unlike other classical I κ B proteins, I κ B ζ is strongly expressed in response to treatment with different proinflammatory stimuli. I κ B ζ inhibits the transcriptional activity of NF- κ B by associating with the p50/p65 heterodimer. More importantly and in contrast to other classical I κ B proteins, I κ B ζ can also induce the expression of genes such as IL-6 and IL-12p40 by binding with the p50 homodimer.

I κ B ζ is encoded by a primary responsive gene, *Nfkbiz*, and its induction depends on NF- κ B activation, suggesting that I κ B ζ regulated genes are induced via two-step machinery (Motoyama et al. 2005; Yamazaki and Takeshige 2008). Very little I κ B ζ is detected in unstimulated cells, and it is induced by various microbial substances that stimulate TLRs and IL-1 β receptors, but not by TNF- α . Subsequent studies have shown that TNF- α induced transcription of *Nfkbiz* gene, but did not stabilize I κ B ζ mRNA, indicating that the stimulus-specific expression of I κ B ζ is determined post-transcriptionally. All TLRs possess ectodomains that recognize ligands from microbial substances and endodomains that facilitate the downstream signaling, which has been shown to be associated with the ectodomain through a transmembrane segment. With the exception of TLR3, which mediates downstream signaling through TRIF/TRAM (which is ▶ Myd88 independent), the other TLRs share common signaling pathways initiated by an adaptor protein, Myd88 (Myd88 dependent), which is necessary for the I κ B ζ induction. In macrophage cells, LPS stimulation leads to three variants (L, S, and D) that are generated by alternative splicing of I κ B ζ . The longer form, I κ B ζ (L, 1–728), is predominantly expressed upon LPS stimulation, while the shorter form I κ B ζ (S) has been observed at mRNA and protein levels in minor species. Although I κ B ζ (D) mRNA has been detected in macrophages, its corresponding protein level has not been found (Yamazaki et al. 2005). Overall, I κ B ζ (L and S) are functionally active when expressed in the cells.

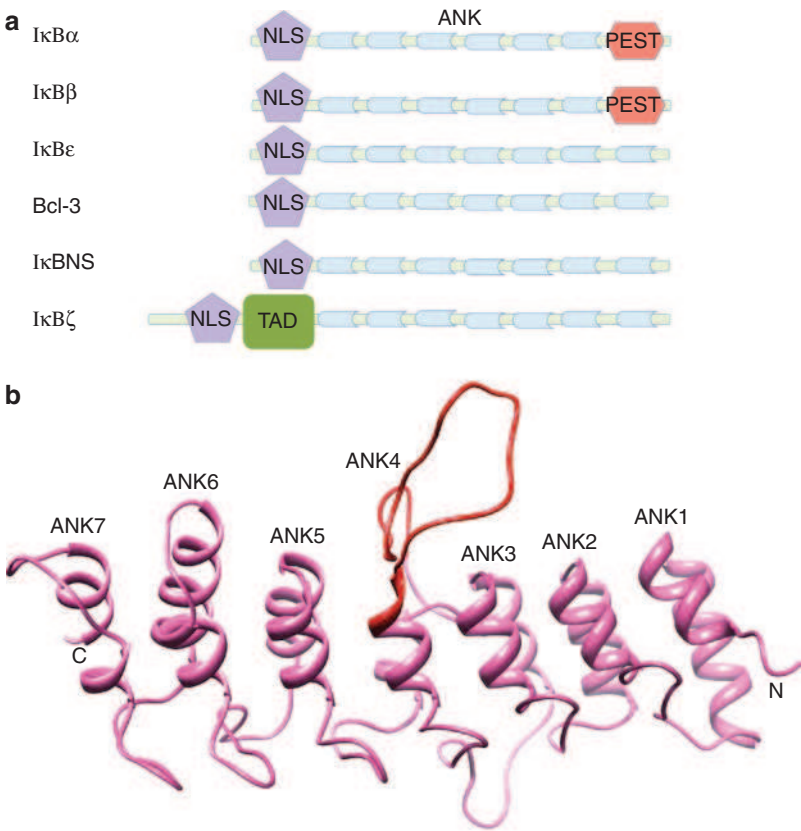
I κ B ζ (1–728 amino acids) can be divided into N-terminal (1–450) and C-terminal (451–728) portions. The N-terminal region contains NLS and TAD, whereas the C-terminal region contains the ANK repeat domain, which plays an important role in interaction with NF- κ B subunits, thereby regulating its functions (Fig. 2a). Truncated mutation studies of I κ B ζ (especially its N-terminal region) have shown -K₁₆₃-R₁₆₄-X₁₂-K₁₇₇-R₁₇₈- to be indispensable for NLS, and the mutation of this portion has revealed that I κ B ζ localized in the cytosol and effectively inhibited NF- κ B, which is similar to the cytosolic I κ B proteins. I κ B ζ was initially characterized as a negative regulator of NF- κ B, but subsequent studies demonstrated that it could also act as a positive regulator of NF- κ B. Analysis using GAL4-fusion protein of I κ B ζ revealed that its N-terminal region (329–403) exhibits transcriptional activity after association with

NF- κ B (p50/p50) subunit (Yamazaki et al. 2005). To date, numerous functions of I κ B ζ have been reported, when bound to other nuclear proteins. (1) Overexpression of I κ B ζ augmented IL-6 production in response to LPS by interacting with p50 homodimer, whereas TNF- α production is inhibited through interaction with p50/p65 heterodimer, indicating specific target gene activity (Motoyama et al. 2005). (2) I κ B ζ physically and functionally interacts with STAT3, which is a member of the NF- κ B signaling pathway, thereby inhibiting the transcriptional activation of STAT3 (Wu et al. 2009). (3) Human I κ B ζ expressed in response to TNF- α binds to the DNA-binding region of p50/p65 heterodimer, consequently leading to the inhibition of the TNF- α response (Totzke et al. 2006). (4) I κ B ζ mediates pre-initiation complex assembly and histone H3K4 methylation, leading to the activation of secondary response genes, thereby suggesting a role of I κ B ζ in the nucleosome remodeling (Kayama et al. 2008). (5) I κ B ζ is expressed in IL-17-producing helper T (T_H17) cells that play an important role in resistance to experimental autoimmune encephalomyelitis (EAE) (Okamoto et al. 2010).

Physiological Roles of I κ B ζ

Studies of I κ B ζ -deficient mice have demonstrated that I κ B ζ plays a role as a positive and negative regulator of NF- κ B-mediated transcription (Yamamoto et al. 2004). Microarray studies have shown that I κ B ζ is an indispensable component of the LPS-induced transcription of genes represented by IL-6 and of the genes listed in Table 1. It should also be noted that IL-6 plays an important role in many inflammatory diseases including sepsis, heart attacks, and stroke, as well as in many human cancers including hepatocarcinoma, multiple carcinoma, and ovarian cancer. However, the transcription complex on IL-6 appears to differ depending on the stimuli. The role of I κ B ζ as a negative regulator was not evident in the isolated cells, probably because of redundant negative regulators of NF- κ B. The inhibitory roles of I κ B ζ in NF- κ B-mediated transcription are critical in fine-tuning to balance inflammatory reactions to maintain homeostasis in vivo. I κ B ζ knockout animals have atopic-like dermatitis and eye inflammation that supports a role of I κ B ζ in innate host defense. However, the inflammation caused by I κ B ζ deficiency is not so

IκBz, Fig. 2 IκB family members. (a) All IκB proteins harbor ankyrin repeats at their carboxy terminal region. The NH₂ terminal regulatory region of IκB-α, -β, and -ε contains specific sequences that are phosphorylated and ubiquitinated. The NH₂ terminal region of IκBζ contains the nuclear localization and transactivation domains that are necessary for the transcriptional process. (b) The homology models of the IκBζ ARD domain are shown in *bright pink*. The 28 amino acid residues occurring within the ANK4 are shown in *red*



IκBz, Table 1 Genes that require IκBζ for LPS-mediated induction

Category	Subset of genes
Cytokines	IL-6, IL-12 p40 subunit, IL-18, granulocyte macrophage stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), growth-differentiation factor (GDF) 15, Epstein-Barr-virus-induced gene (EBI) 3
Chemokines	CXC chemokine ligand (CXCL)5, CXCL13, chemokine ligand (CCL)7, CCL17
Enzymes	Histidine decarboxylase, caspase11, inositol polyphosphate-5-phosphatase B, deltex 2B, glutathione reductase, guanylate nucleotide-binding protein (GDP) 1
Receptors	Formyl peptide receptor 1, macrophage receptor with collagenous structure (MARCO)
Biological active peptides	Endothelin 1, ghrelin
Transcription factors	Basic leucine zipper transcription factor (BATF), CCAAT/enhancer-binding protein (C/EBP)-δ
Antimicrobial substances	Lipocalin 2/neutrophil gelatinase-associated lipocalin (NGAL)
Others	Tax-1 binding protein, extracellular proteinase inhibitor, solute carrier family 11 member 2 (S1c11a2), Src-like adaptor protein (SLAP), immunoglobulin heavy chain, immunoglobulin light chain, membrane spanning 4-domains (MS4A1), thrombospondin 1, immediate early response 3 (IER3/IEX1), disabled-2

clear. Accordingly, it will be important to test whether the skin and eye inflammation in IkB ζ knockout mice is due to the lack of host defense molecules such as lipocalin, or lack of essential cytokines such as IL-6. However, the other function of IkB ζ , suppression of NF- κ B activity, cannot be excluded. Atopic dermatitis and ocular inflammation may also occur due to the over expression of cytokines that IkB ζ might inhibit via the C-terminal ankyrin repeat.

The factors that distinguish genes that are activated or inhibited by IkB ζ appear to be present in the promoter of each gene. Biochemical studies have indicated that in addition to NF- κ B binding sites, IkB ζ is also required for transcriptional activation. Since the transcriptional activation activity of IkB ζ on the GAL4-reporter system is much weaker than that of the NF- κ B p65 subunit, other transcription factors are necessary for efficient IkB ζ -mediated transcription (Yamazaki et al. 2008). IkB ζ acts as a negative regulator of the promoter harboring canonical NF- κ B-binding sequences alone. Moreover, all the nuclear IkB proteins are homologous to each other; therefore, these nuclear proteins may act as competitors for IkB ζ or vice versa. In fact, IkBNS has been reported to inhibit LPS-mediated IL-6 production (Kuwata et al. 2006).

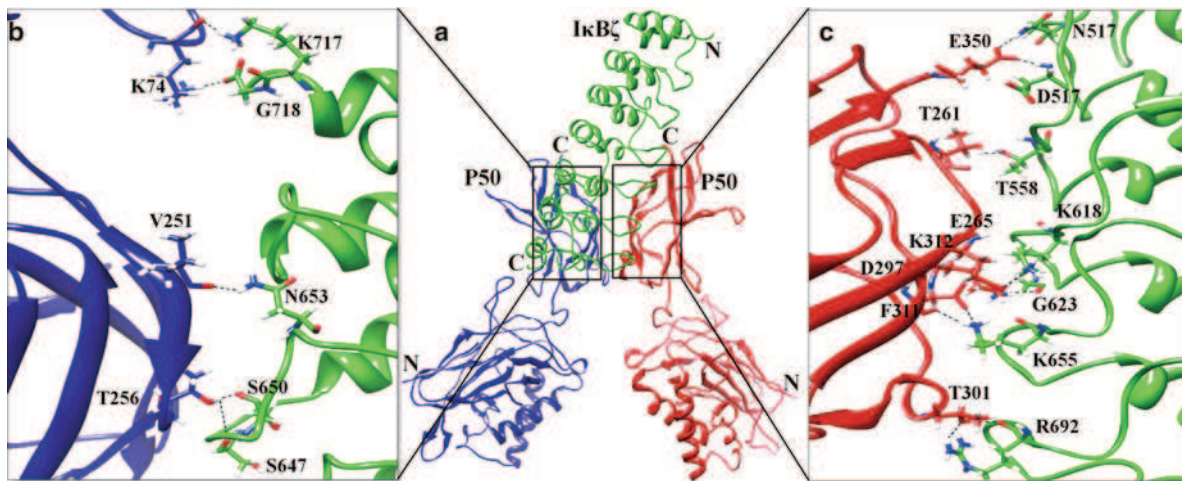
Structure-Based Activation and Inhibition Mechanism of Nuclear IkB ζ

The primary sequence of human and mouse IkB ζ share about 70% homology with the N-terminal region (1–450) and 97% sequence identity with the C-terminal ARD. Although there is sequence variation at the N-terminal region, the NLS and TAD are conserved. There has been speculation about the differences in the N-terminal region of human and mouse IkB ζ , which differ in their ability to bind with NF- κ B proteins. Mouse IkB ζ binds only to p50/p50 homodimer, whereas human IkB ζ binds to both the p65 and p50 subunits. Sequence analysis of the N-terminal region has not revealed any significant homology to proteins in the NCBI database. In addition, secondary structure prediction showed that the N-terminal has no ANK repeat followed by the C-terminal ARD. This type of architecture has also been reported in proteins such as the yeast ribosomal binding protein yar-1 (Lycan et al. 1996). No crystal

structure is yet available for IkB ζ ARD, but recent modeling studies have shown the IkB ζ three-dimensional structure, which was built based upon the Bcl-3 crystal structure (Michel et al. 2001) (Fig. 2b). Each ANK repeat of the IkB ζ models depicted two antiparallel α -helices, followed by a loop of variable length at a right angle. Each repeat began and ended with short β -hairpin turns that protruded away from the α -helix. This non-globular fold was stabilized through intra- and inter-repeat hydrophobic interactions. The represented structural motifs stack upon one another in a linear fashion to form a curved architecture. These motifs are known to facilitate protein–protein interaction, but have no known enzymatic activity (Mosavi et al. 2004). The presence of ankyrin repeats and their role in inflammatory signaling immediately suggested structural and functional homology with the ARD-containing classical IkB proteins. Despite the similarities, IkB ζ differs structurally from the classical IkB inhibitors in its unique amino-terminal region, the number of ankyrin repeats, the presence of a unique 28 amino acids insertion within the helices of the α 1 and α 2 of ANK4 (similar to one that has been observed in IkBNS), and the complete absence of a PEST-like region within the IkB ζ carboxy-terminus (Fig. 2b).

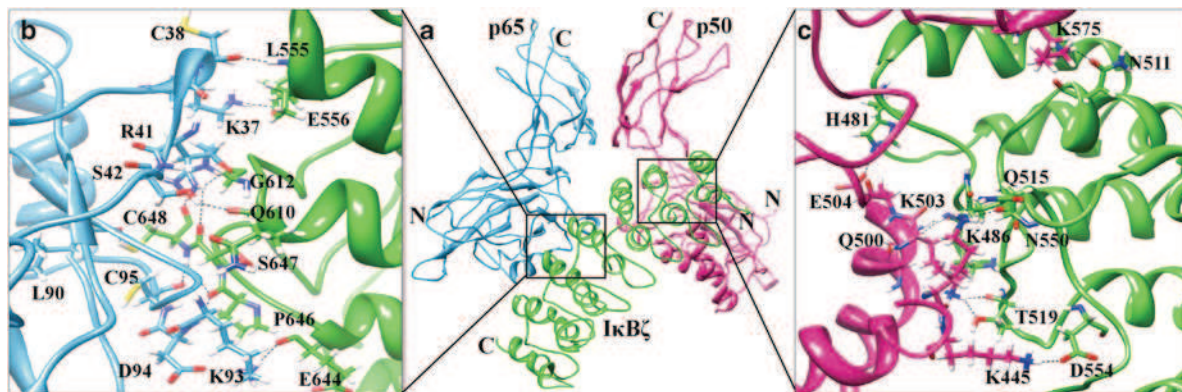
Recent studies have indicated that human TNF- α -induced IkB ζ is associated with both the p50/p65 subunits of NF- κ B in the nucleus, and inhibits the transcriptional activity of anti-apoptotic protein (Totzke et al. 2006). The structural basis of this inhibitory and activation mechanism was explained by docking studies (Manavalan et al. 2011), which have shown that IkB ζ ARD associates with the DNA-binding domain of p50/p65 subunits of NF- κ B and contains important residues that interact with the bases as well as sugar phosphate backbone present in the p50 and p65 subunit (Fig. 3). Hence, there will be no more p50/p65 subunits available to the promoter region, which ultimately results in inhibition of the transcription mediated by p50/p65 subunit. Generally, p65 subunits contain the TAD at the C-terminal end, which is important for its transcriptional activity (O'Shea and Perkins 2008). IkB ζ inhibits p65 transactivation activity through its binding with the N-terminal DNA-binding domain.

Furthermore, IkB ζ -p50/p50 complex revealed that ANK3-7 interact with the dimerization domain of the p50 subunit (Fig. 4). The binding orientation of IkB ζ



IkBz, Fig. 3 Docking studies predict that the cofactor IkB ζ ARD binds at the side of the p50/p65 heterodimer interface. (a) The p50/p65 heterodimers, represented as a ribbon diagram, are shown in magenta and cyan, respectively. Docked IkB ζ is green in the ribbon diagram. (b) p65-IkB ζ binding interface.

Side chains of the amino acid contributing to the hydrogen bonding formation (marked as *black*) are represented by a stick model with the residue name and numbers shown next to them. (c) The p50-IkB ζ binding interface is shown in a similar fashion as in (b)



IkBz, Fig. 4 IkB ζ ARD-p50 homodimer interface. (a) The p50/p50 dimers are blue and red in the ribbon diagram. Docked IkB ζ is green in the ribbon diagram. (b) The p50 (chain A)-IkB ζ binding interface. Side chains of the amino acid contributing to

the hydrogen bonding formation (marked as *black*) are represented by a stick model with the residue name and numbers shown next to them. (c) The p50 (chain B)-IkB ζ binding interface is shown in a similar fashion as in (b)

with this homodimer is similar to that of the classical IkB α -p50/p65 heterodimer. Although the binding orientation is the same, there might be some differences in the regulation of NF- κ B-dependent gene expression by IkB α and IkB ζ . Activation of p65-containing NF- κ B heterodimer by LPS or IL-1 leads to the expression of NF- κ B-dependent genes, including IkB proteins IkB α and IkB ζ . Following translocation, IkB α enters the nucleus, where it targets NF- κ B p50/p65 dimers and removes them from DNA through the acidic PEST

motif of IkB α and the basic DNA containing surfaces of the NF- κ B p65 subunit that likely disrupt protein/DNA binding. In contrast, IkB ζ enters into the nucleus and targets the p50/p50 homodimer, which is already bound to the promoter region, thereby blocking the transcription due to the unavailability of the TAD. Overexpression experiments have suggested that IkB ζ exhibited transactivation potential (Motoyama et al. 2005); hence IkB ζ mediates transcriptional activity by binding with DNA-bound p50/p50 homodimer,

thereby providing a transactivation domain to the NF- κ B complex. Such IkB ζ mediated transcription is important for the production of IL-6, antimicrobial peptides, lipocalin, hDB-2, and the genes listed in Table 1. Finally, it should be noted that, when compared with other IkB proteins, IkB ζ possesses numerous functions that occur via binding with different nuclear proteins. Recent studies of MD (molecular dynamics) simulation of IkB have revealed that IkB ζ possesses more thermodynamically flexible residues than other IkB members. These findings demonstrate that structural flexibility is the major factor that enables IkB ζ to interact with different sets of nuclear proteins (Manavalan et al. 2010).

Summary

Studies of IkB ζ have provided evidence for multistep regulation of inflammatory responses in TLR signaling. Upon cell activation by appropriate stimuli, primary responses are induced by rapid activation of the major transcription factor (NF- κ B), which is activated through posttranslational modifications such as phosphorylation without *de novo* protein synthesis. During this period, transcriptional regulators such as IkB ζ are induced via stimuli-specific mechanisms. Secondary response genes are activated and primary responses are gradually diminished via the combinations of major transcription factors and inducible regulators. Since the genes that are activated via secondary responses also include other transcription factors, stimulus-specific transcriptional activation would proceed in a multistep fashion with time after the stimulation. In vitro studies have shown that these nuclear IkB proteins interact with the p50 or p52 subunits of NF- κ B. Only p50/p52 double knockout mice, but not single knockout mice, exhibit severely defective immune disorders such as osteopetrosis. However, some immunological phenotypes occur in mice lacking only one nuclear IkB protein. This condition may be compensated by utilizing other IkB proteins. Further studies are required to clarify and discover new and detailed physiological aspects of the nuclear IkB proteins in the future by using mice devoid of two or all three nuclear IkB proteins. In conclusion, it can be seen that nuclear IkB ζ not only contributes to NF- κ B mediated transcription, but also plays an important role

in innate immune responses by modulating the expression of proinflammatory cytokines.

Acknowledgments This work was supported by the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (2012016803).

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IkB-zeta

► [IkBz](#)

IL-1 Inducible Nuclear Ankyrin-Repeat Protein (INAP)

► [IkBz](#)

IL6

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Synonyms

B cell stimulatory factor-2 (BSF-2); IFN-β2

Historical Background

IL-6 was cloned as B cell stimulatory factor-2 (BSF-2) in 1986 (Hirano et al. 1986). This molecule has various biological activities; a strong stimulatory effect on growth of murine plasmacytoma and human myeloma, its functions as a hepatocyte stimulating factor, and the induction of acute phase reaction. IL-6 knockout (KO) mice revealed the inhibition of the antiviral antibody response after immunization with a vesicular stomatitis virus, compared with wild-type (WT) mice. Thus, IL-6 is a pleiotropic cytokine that is involved in the physiology of virtually every organ system.

Introduction

CD4⁺ T cells (Th) are essential regulators of immune responses and inflammatory diseases. They can be divided into different subsets such as Th1, Th2, and regulatory T (Treg) cells, whose development is specified by the transcription factors T-bet, GATA3, and fork head box p3 (Foxp3), respectively (Fig. 1). The development of Th1 cells, which activate macrophages and are highly effective in clearing intracellular pathogens, is coupled to the sequential actions of interferon-γ (IFN-γ) and interleukin-12 (IL-12). Th2 cells, which differentiation is driven by IL-4, are important for the production of immunoglobulin E and the clearance of extracellular organisms. In addition to these effector subsets, CD4⁺ T cells can differentiate into distinct regulatory subsets (Treg), which express the fork head/winged helix transcription factor Foxp3. Transforming growth factor-β1 (TGF-β) promotes