Introduction

IkBα is a critical regulator of the transcription factor NFκB, which induces expression of a wide range of genes involved in immune and inflammatory responses, cell proliferation and apoptosis [1-5]. Deregulation of IkBα cellular levels and localization results in a variety of diseases, including chronic inflammatory disorders and many types of cancer and leukemia [6-15]. Even though IkBα has been originally discovered as a cytoplasmic inhibitor of NFκB, it is now clear that it has important nuclear functions as well.

NFκB proteins form homodimers or heterodimers consisting of p65 (Rel-A), p50, p52, c-Rel, and Rel-B [16-20]. In the classical model of NFκB activation, IkBα inhibits NFκB activity by masking the nuclear localization signals (NLS) of NFκB dimers and retaining them in an inactive state in the cytoplasm. Following cell stimulation by extracellular stimuli, including inflammatory cytokines, bacterial and viral products, apoptotic signals, and other forms of cellular stress, IkBα is phosphorylated at serine residues 32 and 36 through a cascade of inducible protein kinases that involve IKK, ubiquitinated, and selectively degraded by the 26S proteasome [21-26]. This results in unmasking of the NLS of the NFκB dimers, which then translocate to the nucleus and stimulate transcription of NFκB-dependent pro-inflammatory and anti-apoptotic genes.

Studies have shown that individual NFκB dimers bind various κB sites with differential affinity, which is affected by differences in the affinity of each dimer for the κB site, the ability to interact with associated transcription factors and inhibitors, chromatin environment, and by the post-translational modifications of NFκB proteins [27-32].

One of the first genes induced following NFκB activation is IkBα, since IkBα promoter also contains the NFκB binding region [33-35]. This newly synthesized IkBα can then enter the nucleus, remove NFκB from gene promoters, and transport NFκB proteins back to the cytoplasm [36-39]. This feedback regulation by post-induction repression represents a crucial regula-
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Regulatory mechanism terminating NFκB activation during persistent stimulation, and limiting the NFκB response. Loss of this negative feedback regulation as well as increased degradation of IκBα have been associated with increased NFκB activation in inflammatory diseases as well as in numerous types of cancer and leukemia [6-10].

**Regulation of IκBα nuclear transport and accumulation**

Sequences determining the nuclear localization of IκBα

IκBα is the most abundant and best-characterized member of the IκB protein family that currently consists of nine IκB proteins: IκBα, IκBβ, IκBε, Bcl-3, IκBz, IκBNS, IκBh, and the precursor proteins p100 and p105. All IκBα proteins are characterized by ankyrin repeat domain (ARD), enabling IκB proteins to form complexes with NFκB dimers and bind other proteins. The IκBα molecule consists of three main regions: N-terminal region where the inducible phosphorylation and ubiquitination occur, the ARD, and an acidic C-terminal sequence that is important for basal degradation of free IκBα [4, 5, 20, 40]. Even though IκBα does not contain the classical nuclear localization sequence (NLS; KK/RXK/R), and its small size (37 kD) would allow a simple diffusion through the nuclear pore complex (NPC), IκBα is transported to the nucleus by an active transport mediated by a nuclear import sequence localized within the ARD of IκBα [41-43].

The nuclear export of IκBα is facilitated by two nuclear export signals (NES) located at the amino terminus (N-NES) [44-47] and carboxyl terminus (C-NES) [37, 38]. The nuclear IκBα export is mediated by the NES receptor CRM1, also known as exportin 1, which belongs to the karyopherin β family and shares sequence homology in the Ran-GTP binding domain with members from this family [48, 49]. In unstimulated cells, IκBα continuously shuttles between the nucleus and the cytoplasm [38, 44]. However, in most cells, the nuclear export of IκBα is dominant over its import, resulting in the cytoplasmic localization of IκBα.

The nuclear translocation and accumulation of IκBα can be induced by three main mechanisms: by the post-induction repression in stimulated cells, by inhibition of the nuclear export of IκBα, and by the proteasome inhibition (Figure 1).

**Induction of nuclear IκBα accumulation by post-induction repression**

In continuously stimulated cells, the newly synthesized IκBα translocates to the nucleus, dissociates NFκB dimers from gene promoters and transports them back to the cytoplasm, thus terminating transcription [36-39]. This feedback regulation by post-induction repression represents a crucial mechanism terminating NFκB activation during persistent stimulation. Impaired post-induction repression may result in a persistent activation of NFκB and increased cell survival. Blocking the nuclear export of IκBα by CRM1 inhibitors increases the nuclear IκBα accumulation, suppresses NFκB activity and in-

![Figure 1. Schematic representation of the main mechanisms inducing the nuclear translocation and accumulation of IκBα. The nuclear translocation and accumulation of IκBα can be induced by the post-induction repression in stimulated cells [36-39], by blocking the nuclear export of IκBα by CRM1 inhibition [54-61], and by the inhibition of the 26S proteasome [69-71].](image-url)
duces apoptosis, representing an attractive therapeutic target.

**Induction of nuclear IkBα accumulation by CRM1 inhibition**

Leptomycin B (LMB) is a specific inhibitor of the nuclear protein export that interferes with the interaction between NES and CRM1 by covalently binding to a cysteine residue in the central domain of CRM1 [50-52]. It has been discovered in 1983 as a potent anti-fungal antibiotic produced by *Streptomyces* [53]. However, since then, LMB has been extensively used to study the nuclear-cytoplasmic shuttling of CRM1-binding proteins, including IkBα [54-57]. Studies from our laboratory have shown that in stimulated human leukocytes, LMB induces nuclear accumulation of IkBα by inhibiting IkBα nuclear export, resulting in the inhibition of NFkB activity, and increased leukocyte apoptosis [57-61]. Even though LMB possesses strong antitumor and anti-inflammatory properties [62, 63], its toxicity prevents it from being clinically useful [64, 65]. However, using high-content screening technologies and medicinal chemistry approaches based on modifying LMB, several recent studies identified novel selective nuclear export inhibitors (NEI) that maintain the high potency of LMB but are better tolerated [66-68]. These new NEIs have the potential to inhibit the constitutive activity of NFkB in cancer cells and chronic inflammatory disorders by increasing the nuclear levels of IkBα.

**Induction of IkBα nuclear accumulation by the proteasome inhibition**

We have shown that in addition to the post-induction repression and by blocking the nuclear export of IkBα by the CRM1 inhibition, the nuclear IkBα accumulation can be induced by the proteasome inhibition ([Figure 1]) [69-71]. Bortezomib (Velcade, PS-341) and other 26S proteasome inhibitors have been developed to inhibit the cytoplasmic degradation of IkBα, thus inhibiting the NFkB signaling in cancer cells [72-75]. However, studies from our laboratory have shown that bortezomib, MG132, MG115 and other proteasome inhibitors inhibit NFkB activity by an additional mechanism that consists of inducing the translocation of IkBα from the cytoplasm to the nucleus in prostate and ovarian cancer cells, HeLa cells, leukemia HL-60 cells, monocyctic cells and chronic T cell leukemia Hut-78 cells [69-71]. The proteasome inhibition-induced nuclear IkBα accumulation is dependent on *de novo* protein synthesis, since cycloheximide (CHX) completely blocks the proteasome-induced nuclear IkBα translocation [69]. This lack of IkBα nuclear translocation in response to the proteasome inhibition in CHX-treated cells could be explained by two mutually non-exclusive mechanisms. In the first model, treatment with CHX might prevent resynthesis of a protein that is otherwise necessary for the proteasome inhibition-induced nuclear translocation of IkBα, but has a short half-life; thus, treatment with CHX would significantly decrease its level. Alternatively, the proteasome inhibition-induced nuclear translocation of IkBα may require that the cellular (cytoplasmic) level of IkBα increases above certain threshold level. When cells are treated with CHX, *de novo* synthesis of IkBα is inhibited, and IkBα never reaches this threshold level, even after the degradation of IkBα is blocked by the proteasome inhibition. Similar mechanism has been suggested to account for the proteasome inhibition induced nuclear accumulation of glucocorticoid receptor and the varicella-zoster virus DNA binding protein ORF29p [76-78]. This model is also supported by previous studies that used cells transfected with constructs expressing IkBα and demonstrated that when IkBα is over-expressed, it localizes in the nucleus [79-81]. Since bortezomib has been approved by FDA for the treatment of multiple myeloma and is being tested in clinical trials as a combination therapy to treat other cancers as well [82-85], understanding the mechanism how it induces the nuclear translocation and accumulation of IkBα may lead to the development of more specific and effective therapies in the future.

**Induction of IkBα nuclear accumulation by UV light**

Interestingly, a recent study by Tsuchiya et al suggested that IkBα translocates into the nucleus and associates with the nuclear IKKβ also in response to UV radiation and other types of oxidative stress [86]. However, in contrast to the proteasome inhibition or to the increased nuclear IkBα accumulation induced by the CRM1 inhibition, this UV light-induced nuclear IkBα translocation does not result in the nuclear IkBα accumulation and inhibition of NFkB activity. On the contrary, the UV light-induced nuclear translocation of IkBα is followed by IkBα degra-
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Constitutive nuclear localization of IκBα in human neutrophils

In most resting unstimulated cells, IκBα is localized in the cytoplasm and by binding to NFκB dimers, it inhibits their nuclear translocation [1-3, 87]. In contrast, in human neutrophils (polymorphonuclear leukocytes), majority (more than 60%) of the total cellular IκBα is localized in the nucleus (Figure 2) [88]. Interestingly, neutrophils are cells that have one of the shortest life spans in the body. They circulate in the blood and in the absence of infection, they undergo apoptosis within 24 hours after the release from bone marrow [89, 90]. Even though the NFκB subunits p50 and p65 are present in the nucleus of resting neutrophils as well [88, 91], the nuclear IκBα prevents NFκB activation by binding to nuclear p65 NFκB [57]. In response to neutrophil stimulation with lipopolysaccharide (LPS) or pro-inflammatory cytokines, IκBα is phosphorylated by the enzymes of the IKK complex and degraded by the proteasome both in the cytoplasm and in the nucleus [57, 92]. However, compared to macrophages and other inflammatory cells, the extent of NFκB activation in human neutrophils is considerably lower [93, 94], and this is associated with a decreased production of NFκB-dependent pro-inflammatory cytokines [95, 96]. Interestingly, this high nuclear accumulation of IκBα in resting cells is unique to human neutrophils, since in mouse neutrophils, IκBα is localized mainly in the cytoplasm (unpublished data).

The mechanisms responsible for the high nuclear accumulation of IκBα in resting human neutrophils are not understood. There are two possible scenarios. In the first model, the high nuclear IκBα accumulation is the result of an increased nuclear import that is dominant over the nuclear export in human neutrophils. In the second model, the high nuclear IκBα level could be caused by the intranuclear binding of IκBα. This hypothesis is supported by our results indicating that the nuclear IκBα associates with the components of the nuclear matrix in human neutrophils [97]. Our study showed that a further increase of the nuclear accumulation of IκBα in the neutrophils increases caspase-3 activity and accelerates neutrophil apoptosis [57]. Thus, it seems likely that the high nuclear IκBα accumulation in human neutrophils represents one of the underlying mechanisms responsible for the high rate of spontaneous apoptosis in these cells (Figure 3). Since neutrophil apoptosis plays a critical role in the inflammatory response that characterizes sepsis, acute lung injury (ALI), and other inflammatory
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Figure 4. Schematic representation of the regulation of IL-8 transcription by S536 p65 and nuclear IκBα in LPS-stimulated human macrophages. In LPS-stimulated human macrophages, the IL-8 promoter is occupied predominantly by S536 phosphorylated p65 homodimers, which do not bind to IκBα. Consequently, the IL-8 expression is not inhibited by the LMB-induced nuclear IκBα [60]. In contrast, the gene promoters of IL-1β and IL-6 are occupied by p65/p50 heterodimers, and their transcription is repressed by the LMB-induced nuclear IκBα [60].

First, IκBα forms a ternary complex with NFκB-DNA, and subsequently, the negatively charged PEST domain of IκBα would displace DNA and dissociate NFκB from the promoter [111]. In vivo, several additional mechanisms are likely to be involved in the termination of NFκB activity. These mechanisms include termination of NFκB activation by p65 phosphorylation/dephosphorylation and acetylation, which regulate affinity for IκBα, nucleosome remodeling and the nuclear degradation of p65 NFκB by the associated proteasome [114-121]. Studies from our laboratory have demonstrated that the recruitment of IκBα to NFκB-dependent promoters is genes specific [60, 61, 70]. In LPS-stimulated human macrophages, the newly synthesized nuclear IκBα induced by post-induction repression is recruited to TNFα, IL-1β, and IL-6 gene promoters, resulting in the transcriptional suppression of these genes [60]. In contrast, the nuclear IκBα is not recruited to IL-8 promoter and the IL-8 expression is not inhibited by the LMB-induced nuclear IκBα [60]. In vivo, the IL-8 promoter is occupied predominantly by p65 NFκB homodimers phosphorylated on serine 536 [60]. Interestingly, this modification was shown to inhibit p65 binding to IκBα in vitro [119]. These studies indicate that the genes occupied by S536 phosphorylated p65 homodimers are not inhibited by the nuclear IκBα (Figure 4). IKKα, IKKβ and IKKc can phosphorylate p65 on serine 536 [122-127]. However, it is not clear at present whether this phos-
phorylation occurs before p65 binds to DNA or after, as a part of the preinitiation complex assembly. In this context, both IKKα and IKKβ were shown to be recruited to the promoters of NFκB-dependent as well as NFκB-independent genes [128-132], and could phosphorylate the promoter-bound p65, resulting in a prolonged transcription and decreased binding to the nuclear IκBα. Furthermore, the strength of the in vivo nuclear IκBα-p65 NFκB interaction might be influenced by the DNA sequence of κB response elements in the regulatory regions of NFκB-dependent genes. This would be consistent with studies demonstrating that a single nucleotide can influence the recruitment of specific NFκB dimers and the required cofactors for efficient gene transcription [133, 134].

In addition, the regulation of NFκB-dependent transcription by the nuclear IκBα depends on the subunit composition of NFκB complexes. Our studies indicate that in the chronic T cell leukemia Hut-78 cells, the expression of NFκB-dependent anti-apoptotic genes cIAP1 and cIAP2 is inhibited by the bortezomib-induced nuclear IκBα, while expression of Bcl-2 is not suppressed [70]. Analysis of the in vivo binding of NFκB proteins to cIAP and Bcl-2 promoters by chromatin immunoprecipitation showed that NFκB p65 and p50 subunits are recruited to cIAP1 and cIAP2 promoters, whereas the Bcl-2 promoter is occupied only by NFκB p50. Thus, these data suggest that cIAP1 and cIAP2 promoters associate with NFκB p65/50 heterodimers and this binding and transcription are inhibited by the bortezomib-induced nuclear IκBα. In contrast, Bcl-2 promoter is occupied predominantly by NFκB p50/50 homodimers and its transcription is not inhibited by IκBα.

NFκB-independent function of the nuclear IκBα

The nuclear IκBα not only regulates NFκB binding to NFκB-responsive promoters and NFκB-dependent transcription, but it also physically interacts with different repression elements including nuclear co-repressors, and histone acetyltransferases and deacetylases (HDACs), resulting in transcriptional repression [132, 135]. In resting cells, IκBα together with HDACs are recruited to the promoters of Notch target genes correlating with transcriptional repression, whereas in response to NFκB activation, IκBα is released from the chromatin, resulting in Notch-dependent transcriptional activation [136, 137]. In addition, IκBα negatively regulates HIV-1 expression by directly binding to the HIV-encoded Tat protein, resulting in the nuclear export and cytoplasmic sequestration of the HIV transactivator [138]. According to this study, IκBα acts as a potent repressor of HIV-1 transcription by inhibiting both NFκB and Tat transacting factors, which are major players in the transcriptional activation and elongation of HIV-1 transcripts [138].

Conclusion

The studies carried out within the last decade clearly demonstrated that in addition to the cytoplasmic retention of NFκB dimers in unstimulated cells, IκBα has important functions in the nucleus as well. Nuclear IκBα is involved in the regulation of numerous pro-inflammatory and anti-apoptotic NFκB-dependent genes as well as NFκB-independent genes through its interactions with HDACs and other transcriptional co-regulators. The nuclear translocation and accumulation of IκBα can be induced by the post-induction repression in stimulated cells, by blocking the nuclear export of IκBα by CRM1 inhibitors, and by the proteasome inhibition. A better understanding of the mechanisms regulating the nuclear shuttling of IκBα in stimulated cells, IκBα nuclear translocation and accumulation in response to the proteasome inhibition and the nuclear IκBα accumulation in resting human neutrophils could lead to the development of new therapies aimed at the inhibition of NFκB activity by increased nuclear localization of IκBα. In addition, an important future goal will be to analyze the in vivo NFκB post-translational modifications, and DNA and NFκB subunit preferences of the nuclear IκBα, since they might hold a key to more specific anti-inflammatory and anti-cancer therapies.

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References

[1] Baeuerle PA, Baltimore D. IkB: a specific inhibitor of the NFκB transcription factor. Science...
Regulation and function of nuclear IκBα


[32] Bacherlere F, Rodriguez MS, Dargebont C, Rosset D, Thomas D, Virelizier JL, Arenzana-Seisdedos F. Nuclear export signal of IκBα interferes with the Rev-dependent posttranscrip-
Regulation and function of nuclear IkBα


[47] Huang TT, Miyamoto S. Postrepression activation of NFκB requires the amino-terminal nuclear export signal specific to IkBα. Mol Cell Biol 2001; 21: 4737-47.


Regulation and function of nuclear IκBα


[80] Cressman DE, Taub R. IκBα can localize in the nucleus but shows no direct transactivation potential. Oncogene 1993; 8: 2567-73.


Regulation and function of nuclear IκBα


[125] O’Mahony AM, Montano M, Van Beneden K,

[126] Buss H, Dörrie A, Schmitz ML, Hoffmann E, Resch K, Kracht M. Constitutive and IL-1-inducible phosphorylation of p65 NFkB at serine 536 is mediated by multiple protein kinases including IkB kinase (IKK)-α, IKKβ, IKKe, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated IL-8 transcription. J Biol Chem 2004; 279: 55633-43.


[133] Natoli G. Tuning up inflammation: how DNA sequence and chromatin organization control the induction of inflammatory genes by NFkB. FEBS Lett 2006; 580: 2843-2849.


