Am J Cancer Res 2011;1(4):446-459
www.ajcr.us /ISSN:2156-6976/ajcr0000036

Review Article

Alternative nuclear functions for NF-κB family members

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Received February 2, 2011; accepted February 10, 2011; Epub February 16, 2011; Published April 1, 2011

Abstract: The NF-κB signalling pathway regulates many different biological processes from the cellular level to the whole organism. The majority of these functions are completely dependent on the activation of the cytoplasmic IKK kinase complex that leads to IκB degradation and results in the nuclear translocation of specific NF-κB dimers, which, in general, act as transcription factors. Although this is a well-established mechanism of action, several publications have now demonstrated that some members of this pathway display additional functions in the nucleus as regulators of NF-κB-dependent and independent gene expression. In this review, we compiled and put in context most of the data concerning specific nuclear roles for IKK and IκB proteins.

Keywords: NF-κB (nuclear factor -κB), IκB (inhibitor of NF-κB), IKK (IκB kinases), chromatin, gene transcription

Introduction

The NF-κB proteins and their negative regulators IκB

NF-κB was first identified in 1986 as a protein that binds to the specific decameric DNA sequence (gggACTTTCC) within the intronic enhancer of the immunoglobulin kappa light chain in mature B- and plasma cells [1]. At that time, it was demonstrated that NF-κB/DNA binding activity was induced by different stimuli such as pathogen derived products, cytokines or UV radiation, both in lymphocytes and in non-lymphoid cell lines, and this activation was independent of de-novo protein synthesis [1]. Nowadays, it is clearly established that NF-κB regulates multiple cellular activities such as proliferation, differentiation and survival, as well as general processes, including innate and adaptative immune response or organ development [2].

In mammals, there are five NF-κB proteins, RelA (p65), RelB, c-Rel, NF-κB1 and NF-κB2, being the two latter synthesized as precursor proteins of 105 kDa and 100 kDa respectively, that will be further processed to p50 and p52 proteins [3, 4]. NF-κB proteins are characterized by the presence of a conserved amino-terminal region called the Rel-homology domain (RHD) that includes the DNA-binding and dimerization domains, the nuclear localization signal (NLS) and the domain involved in IκB binding [3]. They associate as homo- or heterodimers being the most common the one including p65 and p50. Since only p65, RelB and c-Rel contain a transactivation domain (TAD) [3], dimers containing these subunits are the ones that activate transcription. In contrast, p50- and p52-only complexes are mainly associated with transcriptional repression through co-repressors recruitment [5, 6]. Other important players of the NF-κB pathway are the IκB family of proteins, which are characterized by the presence of ankyrin-repeats (**Figure 1**). IκB proteins are essential for regulating cytoplasmic localization of NF-κB dimers in non-stimulated conditions, by masking their NLS localized near the RHD [7]. This family of proteins includes IκBα, IκBβ, IκBε, BCL-3, IκBNS and IκBζ [8, 9]. In addition, precursor p100 and p105 proteins, which retain the ankyrin-repeat domains, are functionally equivalent to IκB and are considered as IκB-like pro-

teins (called IκBδ and IκBγ, respectively) [10-12]. Another IκB protein, named IκBη, has been recently identified [13].

The NF-κB signalling

Two different NF-κB signalling pathways have been identified: the canonical and the alternative pathways.

Canonical NF-κB pathway is primarily responsible for regulating immune response and can be triggered by multiple extracellular stimuli including bacterial and viral components [1, 14], as well as inflammatory cytokines such as interleukin-1β (IL-1β) or tumor necrosis factor alpha (TNFα) [15, 16]. Association of one of these components with its receptor leads to the recruitment of a multiprotein complex to the membrane (that is specific for the different receptors that activate the pathway) that results in the activation of the cytoplasmic IKK complex. This complex is formed by two kinase subunits with high sequence similarity, IKKα and IKKβ, and a regulatory subunit IKKγ/NEMO (NF-κB essential modulator). IKKα and IKKβ are characterized by the presence of an N-terminal kinase domain, a C-terminal helix-loop-helix (HLH) domain and a leucine zipper domain. NEMO, which is not related to IKKα or IKKβ, contains a C-terminal zinc finger-like domain, a leucine zipper and N-terminal and C-terminal coiled-coil domains (**Figure 1**). Despite they participate in the same cytoplasmic protein complex, IKKα and IKKβ have large non-overlapping functions and display different substrate specificities.

One of the best-characterized stimuli activating the classical NFκB pathway is TNFα. Association of TNFα with NFκB receptor 1 (TNFR1) triggers the sequential recruitment of different protein adaptors including TRADD, RIP and TRAF2 to the membrane. Then, polyubiquitinated TRAF2 mediates the recruitment of the IKK complex to the TNFR1 signalling complex through the ubiquitin-binding domain of NEMO [17]. The scaffold proteins TAB2 and TAB3 subsequently bind to Lys63-polyubiquitylated substrates, such as RIP1, resulting in TAK1 and then IKKβ activation. Once activated, IKKβ phosphorylates IκB (IκBα, IκBβ and/or IκBε) thus inducing its β-TRCP -dependent ubiquitination and degradation by the proteasome [18]. The timing of degradation and resynthesis of the different IκB proteins is responsible for regulating the fine tune kinetics

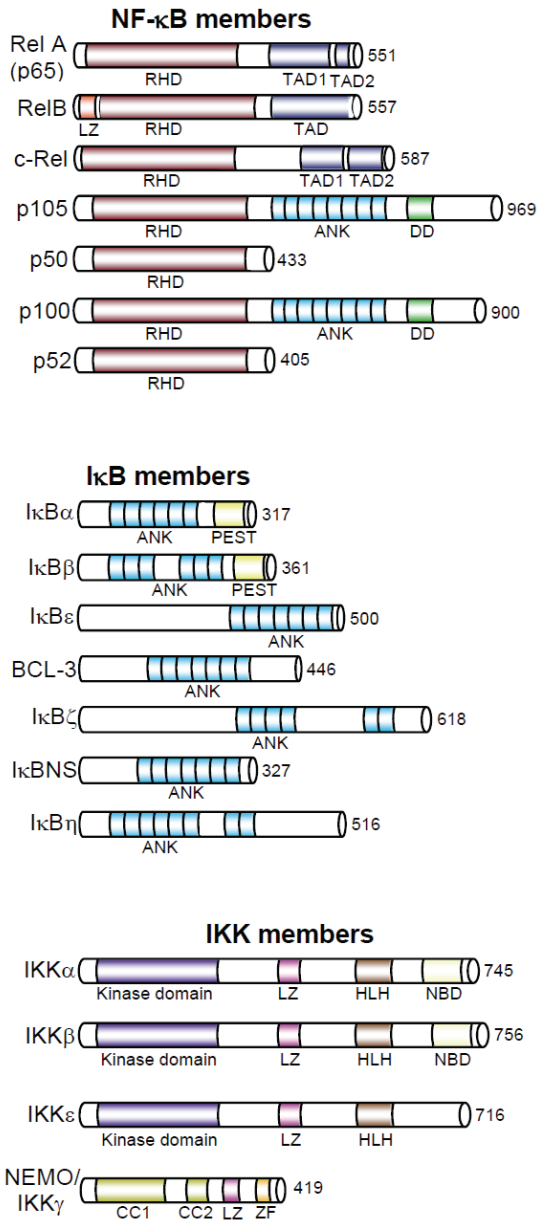

Figure 1. Members of the NF-κB, IκB and IKK families. The domains that typify each protein family are indicated. Domains and their abbreviations: ANK: ankyrin-repeat motifs; CC: coiled-coil; DD: region with homology to death domain; HLH: helix-loop-helix; LZ: RelB-transactivation-domain containing a putative leucine-zipper-like motif; NBD: NEMO-binding domain; PEST: domain rich in proline (P), glutamate (E), serine (S) and threonine (T); TAD: transcriptional activation domain; ZF: zinc-finger domain.

of nuclear entrance and export of particular NF-κB complexes, which in turn is responsible for

specific patterns of gene transcription [19].

Alternative NF-κB pathway is activated by developmental signals through specific receptors, including lymphotoxin β receptor (LTβR) [20, 21], BAFFR or CD40 [22, 23]. This pathway does not require the kinase activity of IKKβ but absolutely depends on the NF-κB-inducing kinase (NIK) and IKKα [20, 24]. Signaling through alternative NF-κB results in phosphorylation-dependent ubiquitination of p100, leading to its partial proteolytic degradation into p52 [20, 25] that translocates to the nucleus associated with RelB to activate specific transcription. More recently, it has been demonstrated that p100 can also inhibit the DNA-binding activity of p65-p50 and RelB-p50 dimers, thus activation of the alternative pathway also results in the activation of classical NF-κB pathway downstream of NIK and IKKα [10].

In addition to IKKα and IKKβ, a third IKK homologue with kinase activity, IKKε, has been identified as a regulator of the interferon antiviral response [26-28].

IKKα protein and chromatin remodelling

Although IKKα is a constitutive component of the cytoplasmic IKK complex, this kinase is specifically accumulated in the nucleus of mouse embryonic fibroblasts upon TNFα stimulation [29, 30] (**Figure 2**). Nuclear IKKα associates with the chromatin at specific NF-κB-target gene promoters, such as IκBα and IL-6, to induce phosphorylation of serine 10 of histone H3 thus facilitating gene expression [29, 30]. Similarly, lipopolysaccharide (LPS) stimulation induces nuclear translocation of NIK that results in the activation of IKKα, histone H3 phosphorylation and enhanced IκBα and rantes gene transcription [31]. This mechanism also regulates NF-κB-independent transcription, as it has been demonstrated for EGF-mediated activation of c-fos in fibroblasts [32], and estrogen-dependent transcription of cyclin D1 and c-myc in breast cancer cells [33]. In the other hand, IKKα phosphorylates CREB-binding protein (CBP) upon TNFα stimulation, favoring its association with p65 at expenses of p53. As a result, NF-κB-dependent transcription is enhanced and p53-mediated gene expression is suppressed, leading to increased cell proliferation and tumor growth [34]. Moreover, nuclear IKKα regulates expression of maspin, a metastasis suppressor, in prostate cancer cells presumably by facilitating recruitment of DNA methyltransferase activity to its promoter [35]. In a mouse model of prostate cancer it has been shown that tumors and metastasis arising after castration contain infiltrating B cells that produce lymphotoxin β (LTβ) and are responsible for activating nuclear translocation of IKKα associated with STAT3 in tumor cells [36].

However, other chromatin-related functions for IKKα are independent of its kinase activity. This is the case of chromatin-bound IKKα in keratinocytes that prevents the recruitment of the Suv39h1, which is responsible for Lys9 trimethylation of histone H3, to the 14-3-3σ promoter leading to gene activation. Thus, in the absence of functional IKKα, 14-3-3σ is not expressed and cells are induced to proliferate resulting in the loss of skin homeostasis and eventually increased cell transformation [37]. These results are in agreement with the requirement for IKKα in skin development [37-39] and with the fact that the skin defects observed in the IKKα knockout mice can be rescued by reintroduction of a kinase-inactive IKKα under the control of Keratin 14 promoter [40]. Moreover, IKKα negatively regulates HDAC3 recruitment to specific NF-κB-dependent gene promoters, such as icam-1 and mcp-1 but not IκBα, correlating with enhanced p65-dependent transcription [41].

IKKα and nuclear transcriptional repressors/ activators

In addition to histone H3, nuclear IKKα phosphorylates different substrates including silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) at residue Ser2410 inducing its dissociation from the chromatin, and being a prerequisite for activation of NF-κB-dependent genes, such as ciap-2 and IL-8 in response to laminin attachment [42]. Further studies demonstrated that this IKKα-induced derepression occurs in two distinct phases. In basal conditions, SMRT and HDAC3 are associated with chromatin-bound p50 homodimers promoting basal repression. Upon stimulation (initial phase), IKKα-mediated phosphorylation of SMRT initiates transcriptional derepression by releasing SMRT and HDAC3 from the chromatin. Then, SMRT is degraded by the proteasome facilitating the binding of transcriptionally active p65-p50 dimer to specific gene promot-

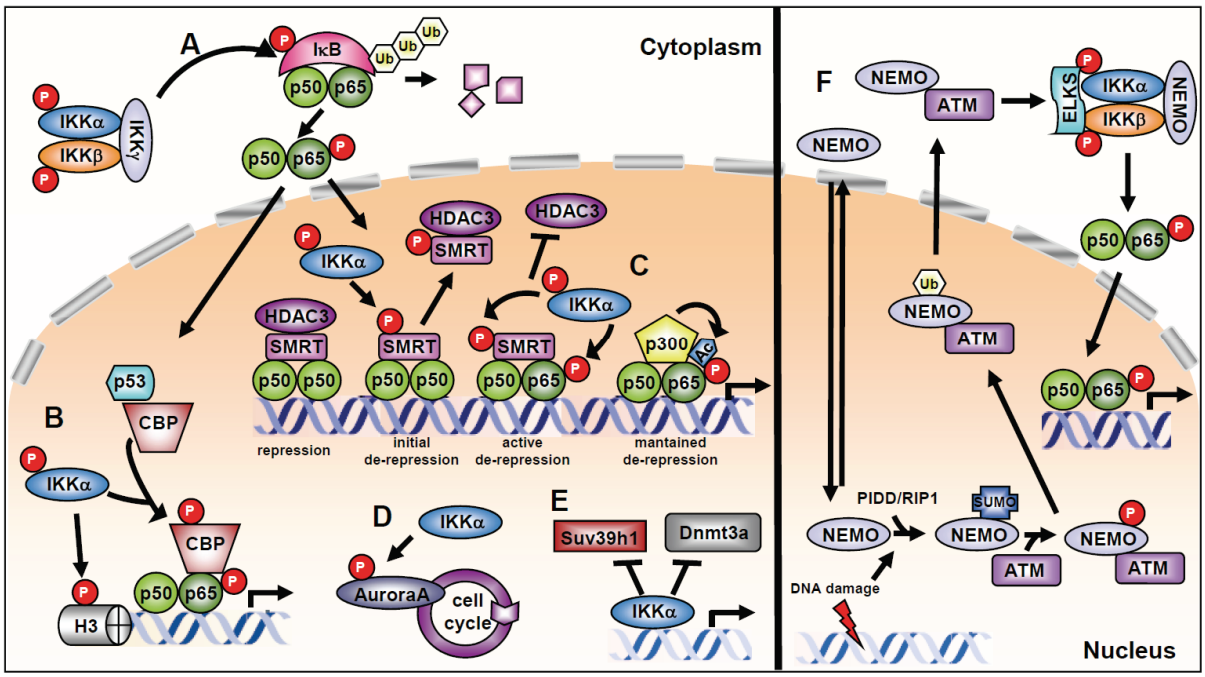

Figure 2. Alternative nuclear functions of IKKα and NEMO. **A.** The most common IKK function consists in phosphorylating IκB proteins leading to its proteasome-dependent degradation, however they also phosphorylate NF-κB proteins. **B.** Nuclear IKKα phosphorylates Serine 10 on histone H3 facilitating the accessibility of the transcriptional machinery to the chromatin. Moreover, IKKα phosphorylates the histone acetylase CBP promoting its preferential binding to p65 at expenses of p53. The functional consequence of these events is specific transcriptional activation. **C.** IKKα phosphorylates the nuclear co-repressor SMRT-bound to transcriptionally repressive p50 homodimers resulting in the displacement of SMRT and HDAC3. Then, transcriptionally active p65-p50 dimers are recruited to specific gene promoters. IKKα phosphorylates p65 and SMRT preventing HDAC3 recruitment and favouring p300 recruitment that acetylates p65, which is required for full NF-κB gene expression. **D.** Nuclear IKKα phosphorylates Aurora A thus promoting cell cycle progression. **E.** In human keratinocytes, IKKα physically prevents the binding of epigenetic silencers Suv39h1 and Dnmt3a to the 14-3-3σ promoter, resulting in enhanced transcriptional activity of this gene. **F.** Upon DNA damage, cytoplasmic NEMO enters into the nucleus to sense the genotoxic stress. Then, NEMO is sequentially sumoylated, phosphorylated and ubiquitinated, and becomes exported back to the cytoplasm where it promotes IKK complex activation and NF-κB-dependent gene expression of diverse anti-apoptotic genes. P, phosphate; Ub, ubiquitin.

ers. In a second phase, chromatin-associated IKKα phosphorylates p65 (Ser536) thus favouring p300 recruitment that acetylates p65 at Lys310, which is required for full NF-κB gene expression [43]. In colorectal cancer cells, IKKα is aberrantly activated and recruited to the promoter of diverse Notch-dependent genes, such as hes1 and herp2. Once in the chromatin, IKKα phosphorylates SMRT leading to its release and resulting in Notch-dependent gene expression [44]. Inhibition of IKK activity restores SMRT chromatin binding, inhibits Notch-dependent gene expression, and prevents tumor growth in nude mice. Moreover, IKKα also phosphorylates the nuclear corepressor N-CoR creating a functional 14-3-3 binding domain that facilitates its nuclear export in colorectal cancer cells [45].

Moreover, Lawrence et al. demonstrated that IKKα phosphorylates p65-NF-κB at Ser536 thus triggering its proteasomal degradation to terminate NF-κB-dependent activation of pro-inflammatory gene promoters [46]. Nuclear IKKα also phosphorylates the steroid receptor coactivator 3 (SRC-3) upon TNFα stimulation thus activating NF-κB signalling [47]. Other IKKα substrates includes β-catenin [48-50] or FOXO3a [51], but it has not been demonstrated whether they are phosphorylated in the nucleus.

IKKα and cell cycle

One of the consequences of alternative IKKα functions is enhanced cell proliferation. As mentioned before, IKKα activates cyclinD1 and c-myc transcription in breast cancer cells [33]. Moreover, IKKα is important for estrogen-induced cell cycle progression through regulation of PCAF-mediated acetylation of E2F1, which increases its DNA-binding activity and protein stability [52]. In addition, IKKα plays a role during M phase by inducing phosphorylation and activation of Aurora A, one of the mitotic kinases that regulate cell cycle progression [53]. In keratinocytes, IKKα permits 14-3-3σ expression, which is a crucial regulator of the G2/M checkpoint in response to DNA damage. Importantly, squamous cell carcinoma cells contain mutations in IKKα that prevent its association with histone H3 and fails to induce 14-3-3σ expression, indicating the relevance of this mechanism on human skin homeostasis [37].

Nuclear functions for IKKβ

In 2000, Makris et al. showed that IKKβ, together with IKKα and NEMO localizes in the nucleus of normal human skin. However this finding has not been further characterized [54]. Later on, few reports have addressed a putative nuclear role for IKKβ, and data indicating the possibility that IKKβ is recruited to the promoter of specific NF-κB target genes in a TNFα-dependent manner is controversial [29, 30]. However, it has been shown that in NIH-3T3 cells, IKKβ, together with IKKα, binds to the promoter of Notch target genes hes1 and herp2 in response to TNFα, correlating with transcriptional activation [55]. More recently, it has been demonstrated that nuclear IKKβ acts as an adaptor protein together with β–TrCP and heterogeneous ribonucleoprotein U (hnRNP-U) to promote degradation of nuclear IκBα upon UV irradiation. The functional consequence of this novel role of nuclear IKKβ is the suppression of NF-κB-dependent anti-apoptotic genes, inducing cell death [56, 57]. Other reports suggest that IKKβ associates with different nuclear proteins in a context-dependent manner. For example in HeLa cells, IKKβ regulates genome integrity by direct binding to Aurora A thus inducing its degradation [58]. Furthermore, IKKβ promotes breast cancer by phosphorylating FOXO3a triggering its cytoplasmic export and proteasomal degradation [51].

Nuclear IKKγ/NEMO

NEMO is an essential component of the cytoplasmic IKK complex. However, this protein displays a well-characterized role as a sensor of genotoxic stress in the nucleus (**Figure 2**). Specifically, DNA damage induces nuclear translocation of NEMO, where it is first sumoylated [59]. Then, Ataxia Telangiectasia Mutated (ATM) protein phosphorylates NEMO to promote its ubiquitin-dependent nuclear export. Concomitantly, ATM is also exported in a NEMO-dependent manner to the cytoplasm, where it associates to ELKS, which functions as an IKK complex activator. The functional consequence of these events is IκB degradation and nuclear translocation of NF-κB, which results in the transcriptional activation of anti-apoptotic genes [60]. Further studies have identified protein inhibitor of activated STATy (PIASy) as the enzyme responsible for NEMO sumoylation [61] and calcium mobilization as an essential signal for NEMO nuclear export [62]. Moreover, it has been shown that poly(ADP-ribose)-polymerase-1 (PARP-1), responsible for sensing DNA strand breaks, is the DNA proximal regulator of NEMO, PIASy, and ATM assembly, which depends on poly(ADP-ribose) (PAR) synthesis [63]. Recently, several groups have contributed to dissect activation of IKK complex from the nucleus and have shown that ELKS ubiquitination is dependent on ATM and NEMO, and promotes the assembly of TAK1/TAB2/3 and NEMO/IKK complexes thus activating NF-κB signalling [64]. In addition, ATM activates TRAF6 leading to Ubc13 polyubiquitination and generation of an ATM/TRAF6/cIAP1-complex that promotes TAK1 activation and NEMO monoubiquitination [65]. In contrast, Verma et al proposed that nuclear NEMO could repress NF-κB by competing with p65 and IKKα for their interaction with CBP [66].

IκB proteins, more than NF-κB inhibitors

The most important role of IκB proteins is the inhibition of NF-κB dimers by means of their cytoplasmic retention, a function that is partially redundant among the different IκBs [67]. However, different IκB homologues have specific degradation and resynthesis rates and thus, they regulate NF-κB activation with precise kinetics following a particular stimulation [19]. In addition to this complexity, several reports have demonstrated the existence of nuclear func-

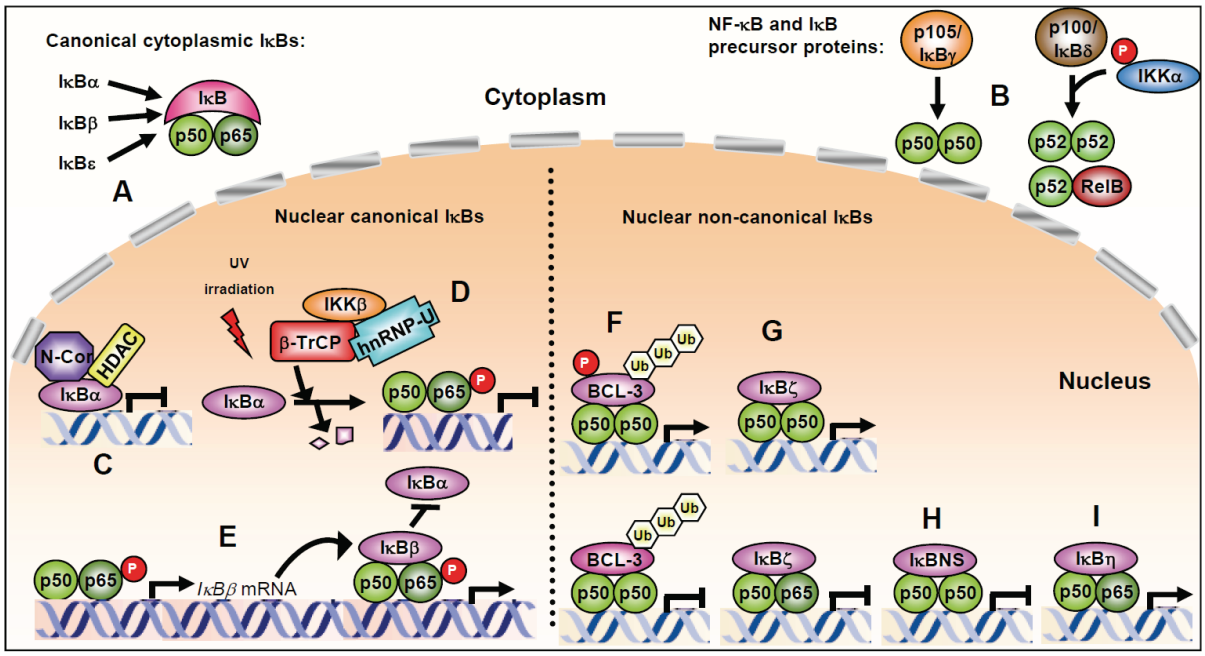

Figure 3. Nuclear functions of IκB proteins. **A.** The classical function of canonical IκB proteins, IκBα, IκBβ and IκBε involves their binding to cytoplasmic NF-κB dimers, preventing their nuclear translocation. **B.** Under certain conditions, precursor p100 and p105 proteins become processed to p52 and p50 proteins, which participate in NF-κB signaling. In contrast, unprocessed p100 and p105 are functionally comparable to IκB proteins, and have being called IκBδ and IκBγ, respectively. **C.** Nuclear IκBα, together with other transcriptional repressive elements, such as HDACs or N-CoR, is recruited to the promoter of Notch-target genes, correlating with their transcriptional silencing. **D.** Upon UV radiation, IκBα enters into the nucleus where it is degraded by the β–TrCP:IKKβ:hnRNP-U complex. **E.** After degradation of cytoplasmic IκBβ, newly synthesized unphosphorylated IκBβ enters into the nucleus and stably binds to NF-κB dimers. This prevents association of NF-κB with IκBα and enhances NF-κB dependent gene expression. **F.** BCL-3 is phosphorylated and ubiquitinated to facilitate transcriptional activation of a subset of NF-κB dependent genes. In contrasts, ubiquitinated and non-phosphorylated BCL-3, stabilizes inhibitory p50 homodimers thus repressing gene transcription. **G.** IκBζ facilitates or prevents gene transcription depending on its association with p50:p50 homodimers or p50:p65 heterodimers. **H.** IκBNS stabilizes repressive p50 homodimers. **I.** IκBη positively regulates NF-κB transcriptional activation of a subset of proinflammatory cytokines.

tions for specific members of this family of proteins (**Figure 3**).

Nuclear canonical IκBs

IκBα

In 1997, Arenzana-Seisdedos et al. demonstrated for the first time that IκBα subcellular distribution is not restricted to the cytoplasm. Instead, IκBα was shown to be a dinamic protein that constitutively shuttles from the cytoplasm to the nucleus to actively induce NF-κB export during post-activation repression phase [68]. Other groups have confirmed the existence of nuclear IκBα under specific conditions [69]. Importantly, nuclear translocation of IκBα does not require a conventional nuclear localization sequence (NLS) [70], although it is exported back to the cytoplasm by CRM1 via a conventional nuclear export sequence (NES) [71, 72]. Further studies demonstrated that nuclear IκBα not only regulates NF-κB binding to the DNA but also associates with other nuclear proteins such as HDACs and the nuclear corepressors N-CoR and SMRT [55, 73, 74]. Specifically, in non-induced cells IκBα together with HDACs is recruited to the promoter of Notch target genes correlating with transcriptional repression, whereas in response to NF-κB activation, IκBα is released from the chromatin correlating with Notch-dependent transcriptional activation [55]. Recently, it has also been demonstrated that not only TNFα but also UV

radiation promotes IκBα nuclear import that is important for its IKKβ-mediated degradation [57].

IκBβ

Several reports indicate that IκBβ is also present in the nuclear compartment [75-77]. Further investigations have demonstrated that following degradation of the basal IκBβ upon LPS or IL-1 treatment, the newly synthesized unphosphorylated IκBβ accumulates in the nucleus and stably binds NF-κB dimers to prevent their association with IκBα. The IκBβ/NF-κB trimer is efficiently retained in the nucleus, since IκBβ lacks a functional NES [71], and binds DNA leading to enhanced transcriptional activation of specific genes [78]. Recently, the functional relevance of this mechanism has been demonstrated *in vivo* by demonstrating that IκBβ functions during inflammation response. In this sense, mice lacking IκBβ showed a dramatic reduction on the expression of inflammatory cytokines, in particular TNFα, following intraperitoneal LPS injection. Analysis of the TNFα promoter demonstrated that LPS induced a persistent recruitment of IκBβ together with p65 and c-Rel, suggesting that optimal TNFα expression requires the binding of this ternary complex. Thus targeting IκBβ expression could be a therapeutical strategy to treat chronic inflammatory diseases such as rheumatoid arthritis [79]. A second report that describes the phenotype of the IκBβ-deficient mice has also demonstrated that these animals are protected from LPS-induced lethality although the authors identify IL-1β, instead of TNFα, as the crucial inflammatory cytokine targeted by IκBβ [80].

IκBε

IκBε actively shuttles between the cytoplasm and the nucleus with a kinetics that is delayed compared with IκBα. Similar to IκBα, IκBε contains a NES-like sequence and it has been proposed that it might also facilitate cytoplasmic export of NF-κB dimers [81]. However, additional chromatin-related functions for IκBε have not yet been reported.

Nuclear non-canonical IκB proteins

In addition to the IκB proteins that are essential for regulating NF-κB localization, other IκB-like proteins are also found in the nucleus of the cells and can be induced in response to specific stimuli (**Figure 3**). This family of proteins include: BCL-3, IκBζ, IκBNS and IκBη.

BCL-3

BCL-3 was initially considered as an oncogene involved in B-cell leukaemias, however further studies revealed that BCL-3 codifies for a cellular protein with IκB-related functions. In addition, it is structurally related to IκBα and contains seven ankyrin repeats. The main role of nuclear BCL-3 is to counteract the inhibitory effects of p50:p50 homodimers [14, 82-84] and to facilitate transcriptional activation by p52:p52 homodimers [85]. Further studies suggested that BCL-3 acts as an adaptor of these homodimers with histone acetylases such as Tip60 [86]. Nuclear BCL-3 is regulated by proteasomal degradation upon GSK-3-dependent phosphorylation [87-89]. In addition, Yang et al. demonstrated that BCL-3 interacts cooperatively with the peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1 alpha (PGC-1α) to coactivate nuclear receptors estrogen-related receptor alpha (ERRα) and PPARα [90]. Recently, it has been reported that expression of BCL-3 inhibits granulopoiesis in an NF-κB p50-dependent manner and limits acute inflammatory damage in a murine lung injury model [91, 92].

IκBζ

IκBζ was first identified in a screening for novel genes up-regulated following LPS stimulation in macrophages [93]. In this report, it was shown that IκBζ is localized in the nuclear compartment and negatively regulates NF-κB activity thus preventing excessive inflammatory responses. However, *in vivo* studies using the IκBζ $^{-/-}$ mice demonstrated that IκBζ is indispensable for specific gene expression such is the case of Il-6 or Il12β and Csf2 genes [94] in association with p50 homodimers [94, 95]. Further ChIP experiments in bone marrow derived macrophages revealed that IκBζ facilitates the recruitment of C/EBPβ and different remodelling factors to the chromatin [96]. IκBζ can also induce IFN-γ production in myeloid KG-1 cells [97] likely involving STAT4 recruitment and specific acetylation of Lys9 of histone H3 [98]. Recently, a crucial role for IκBζ in regulating IL-17-producing helper T cells (T_H17) development, a specific T-cell subset characterized by its patho-

logical role in autoimmune diseases, has been identified. This function is displayed in the nucleus in cooperation with orphan nuclear receptors (RORα and RORγ) and involves physical association of IκBζ to the il-17 promoter [99].

IκBNS

This IκB member was first identified in thymic nuclear lysates as a protein capable of interacting with NF-κB dimers following TCR stimulation, and its expression was associated with apoptosis of immature thymocytes [100]. Further studies demonstrated that IκBNS$^{-/-}$ mice showed a reduced TCR-dependent proliferation of T-cells, associated with a reduction in IL-2 levels [101]. However in macrophages treated with LPS, IκBNS is recruited to the il-6 promoter together with p50 correlating with its transcriptional suppression [102]. In agreement with these data, IκBNS$^{-/-}$ mice are highly susceptible to LPS-induced endotoxin shock and intestinal inflammation [103].

IκBη

Recently, a novel protein structurally related with IκBα has been identified and named IκBη. The IκBη protein is ubiquitously and constitutively expressed in different cell types displaying a nuclear distribution. IκBη does not regulate NF-κB nuclear translocation but it regulates transcription of a subset of proinflammatory cytokines during innate immune response through DNA binding [13, 104].

Specific nuclear functions for NF-κB members in the skin

Skin is the largest organ of the body and constitutes its principal protective barrier from chemical, microbial and physical insults. Mammalian epidermis is composed of several layers: basal, spinous, granular and cornified. Basal keratinocytes proliferate, differentiate and fully maturate undergoing enucleation to generate the cornified layer. Because skin needs to respond to constant environmental stimuli, the mechanisms that regulate the balance between cellular proliferation and cell loss due to desquamation are of critical importance. Several studies indicate that NF-κB is essential in the maintenance of skin homeostasis [105-107], and *in vivo* studies using IKKα-deficient mice demonstrated that nuclear IKK plays a pivotal role in this process [37-39, 108]. Since IKKα is dispensable for either IKK complex activation and IκBα degradation these results indicate an alternative role for IKKα in the skin. In this sense, we already mentioned that the skin phenotype of IKKα knockout mice is rescued by re-introduction of an epidermal-specific IKKα inactive transgene [40]. Moreover, these defects were also attenuated by exposure of mutant skin to wild type dermis suggesting that IKKα promotes the expression of a putative soluble factor which is the responsible for proper keratinocyte differentiation [108]. Other studies have demonstrated that IKKα predominantly localizes in the nucleus of normal human and mouse skin [39, 54] and it has been shown that reduced levels of IKKα, mutations that generate truncated IKKα or aberrant cytoplasmic IKKα localization are significantly associated with poorly differentiated SCC and skin papillomas [109, 110]. In agreement with these findings, overexpression of IKKα transgene in a model of chemically-induced skin carcinogenesis antagonizes the tumoral progression and metastasis, indicating that IKKα plays a role as a tumor suppressor in the skin [109, 111]. Although the mechanisms underlying the specific role of IKKα in the skin are not fully identified, IKKα is known to associate with histone H3 in keratinocytes, thus protecting the 14-3-3σ locus from Lys9 trymethylation by Suv39h1 and facilitating 14-3-3σ expression, which is crucial for the maintenance of the genomic stability [37]. In addition, IKKα interacts with Smad2/3 after a TGF-β stimulation to facilitate transcriptional activation of cell-cycle regulators, such as Mad1, Mad2 and Ovol1, promoting cell growth arrest and keratinocyte differentiation [112].

Other IKK proteins, IKKβ and NEMO have also been found in the nucleus of normal skin [54]. However, mice with skin-specific deletion of IKKβ show a markedly thickened epidermis but do not display any alteration in keratinocyte differentiation or proliferation [113]. Moreover, in the combined IKKβ and TNFRI deficient mice the skin defects are rescued suggesting that inflammation is responsible for this phenotype [113]. In contrast, conventional NEMO-deficient mice showed a significant gender disparity, with mutant males displaying a severe hepatocyte apoptosis and females developing skin lesions, which recapitulated the symptoms of the human genetic disorder called incontinentia pig-

menti, characterized by keratinocyte hyperproliferation, skin inflammation and apoptosis [54, 114, 115]. A similar inflammatory phenotype was observed in the epidermis-specific NEMO mutant mice, however this animals do not show any sign of hyperproliferation or keratynocyte differentiation defects [116]. Similar to IKKβ, TNFRI deletion rescued the phenotype caused by NEMO deficiency suggesting that TNFα-mediated inflammation is involved in the skin defect [116]. Together this data indicate that the role of IKKβ and NEMO in the skin is associated with the classical NF-κB pathway.

Another NF-κB member that is required for skin homeostasis is IκBα. Mice deficient for IκBα, either conventional or skin specific knockouts, display inflammatory skin disorders associated with increased keratinocyte proliferation and T-cell infiltration, thus resembling a psoriasis-like phenotype [117-119]. Selective deletion of IκBα in the keratinocytes resulted in increased proliferation and reduced differentiation without epidermal inflammation. Simultaneous deletion of IκBα in keratynocytes and T-cells mimicked the inflammatory phenotype observed in conventional IκBα mutants, which is rescued by additional deletion of p65-NF-κB. These results demonstrate the importance of NF-κB in keratinocyte/T-cell crosstalk associated with skin inflammation [119] but indicate that the differentiation-associated phenotype and the inflammatory phenotype are driven by different mechanisms.

Conclusions and future directions

Altogether, the data summarized in this review indicates that IKK and IκB proteins are more than cytoplasmic regulators of the canonical or non-canonical NF-κB pathways. Alternative functions for IKK and IκB members are involved in multiple biological and pathological processes including skin differentiation, inflammation and cancer. However, the existing literature regarding these functions is still incomplete or even controversial, being difficult to separate the contribution of specific proteins to the nuclear or the cytoplasmic functions. Our current view is that some IKK and IκB functions are NFκB-dependent whereas others are in the crossroad of NF-κB with other signalling pathways and are used to orchestrate specific pathway crosstalks. One example of the former is the IKKβ function in the developing liver as indicated by the massive apoptosis observed in the conventional knockouts [120-122] that is identical to the phenotype of the p65-deficient mice [123] and can be rescued by the additional elimination of TNFR1 [124, 125]. In contrast, conventional NEMO deficient mice show, apart from the liver apoptosis phenotype [120-122], an additional skin phenotype [54, 115] that is NF-κB-independent. Moreover, a similar skin phenotype is found in the IKKα knockout [40]. As a conclusion, generation and characterization of specific mice models will be required to fully understand the relative contribution of IκB and IKK proteins to specific pathways in the different tissues. For that reason, engineering knock-in mice with mutant IKK/IκB proteins with the ability to function only in the nucleus or cytoplasm will be required to understand these alternative functions. Accurate identification of the specificity of these functions for the different IKK and IκB proteins is an exciting field of research and will be extremely useful for future identification of new and more specific therapeutical targets.

Acknowledgements

LE is an investigator at the Carlos III program. MM is an investigator of the Sara Borrell program (CD09/00421). This work was supported by Instituto de Salud Carlos III Grant PI10/01128 and Fundación Mutua Madrileña to LE and RTICCS/FEDER (RD06/0020/0098) to AB.

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