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Return to homeostasis: downregulation of NF-κB responses

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Activation of NF-κB transcription factors by receptors of the innate or adaptive immune system is essential for host defense. However, after danger is eliminated, NF-κB signaling needs to be tightly downregulated for the maintenance of tissue homeostasis. This review highlights key negative regulatory principles that affect the amount, localization or conformational properties of NF-κB-activating proteins to attenuate the NF-κB response. These mechanisms are needed to prevent inflammation, autoimmune disease and oncogenesis.

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Almost all danger-sensing receptors of the innate and adaptive immune systems activate NF-κB transcription factors to mediate effector function. Active NF-κB binds to consensus target sequences in various promoters and induces the expression of a plethora of genes encoding molecules that drive the proliferation, survival and differentiation of cells of the immune response, as well as factors such as proinflammatory cytokines that organize and execute immune and inflammatory responses. Thus, prompt activation of NF-κB is critical for host defense against various classes of pathogens. Still, NF-κB-driven immune responses should not last permanently. They need to be down-modulated and properly terminated, as aberrant NF-κB activity can directly lead to uncontrolled tissue damage and deleterious disease[1–3]. Many distinct negative regulatory mechanisms have evolved that operate at different molecular levels in the NF-κB signaling pathways to maintain tissue homeostasis. Although this review discusses the negative regulation of NF-κB in immunity, the NF-κB signaling cascade is ubiquitously active, and many of the principles discussed here are applicable to other cell types and organ systems.

The mammalian NF-κB family consists of the transactivation domain–containing RelA (p65), c-Rel and RelB proteins, and the transcriptionally inactive NF-κB1 subunit (p50 and its precursor, p105) and NF-κB2 subunit (p52 and its precursor, p100)[4]. These molecules form various homodimers and heterodimers, which in resting cells are typically retained in the cytoplasm by binding to inhibitory IκB proteins. Canonical and alternative activation of NF-κB are both based on signal-induced phosphorylation of IκB molecules by IκB kinases (IKKs)[4]. These events mark IκB proteins for degradative Lys48 (K48)-linked polyubiquitination, resulting in their proteolysis, which frees NF-κB dimers from cytoplasmic inhibition.

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Inhibition of canonical NF-κB activity by classical IκB proteins

The main pathway for NF-κB activation depends on the canonical IKK complex and impinges mainly on the activation of RelA- and c-Rel-containing dimers[4] (**Fig. 1**). This cascade is rapidly engaged by most receptors for proinflammatory cytokines, innate pattern-recognition receptors (including the Toll-like receptors (TLRs), Nod-like receptors, RIG-I-like helicases and C-type lectin receptors), antigen receptors on T lymphocytes and B lymphocytes, and others. All receptor systems use distinct proximal protein complexes to mediate signal-specific activation of IKK[5]. Once activated, IKK phosphorylates the classical IκB proteins IκBα, IκBβ and IκBε at specific serine residues, which leads to their proteasomal degradation. Although the degradation of IκB proteins is a decisive step in canonical NF-κB activation, the three IκB proteins are degraded with different kinetics and are subject to different transcriptional regulation by NF-κB[6,7]. IκBα is degraded most rapidly and IκBε is degraded most slowly in response to inflammatory stimuli[6,7]. Notably, the genes that encode IκBα and IκBε are directly regulated by NF-κB; thus, in contrast to IκBβ, IκBα and IκBε also function as regulators of a negative feedback loop[8–10].

Newly synthesized IκBα enters the nucleus and associates with DNA-bound NF-κB dimers[11], which leads to export of the transcription factors to the cytoplasm via the nuclear-export sequence present in IκBα. Consistent with that, absence of IκBα impairs the termination of NF-κB activity[7], but the inhibitory function of IκBα is not absolute and can be counteracted by IκBβ[12,13], which does not contain a nuclear-export sequence[14]. When hypophosphorylated IκBβ binds to RelA and c-Rel in the nucleus, the NF-κB–IκBβ complexes are resistant to IκBα-mediated inhibition. IκBβ can thereby prolong the expression of specific genes, including those encoding proinflammatory cytokines such as tumor necrosis factor (TNF) or interleukin 1β[12,13]. In addition, the acetylation of RelA by lysine acetyltransferase CBP (p300) complexes can also prevent the association of IκBα with RelA and extend the duration of the presence of functional RelA complexes, at least on some promoters[15].

IκBε provides an additional negative-feedback loop, but IκBε expression is considerably delayed relative to IκBα expression[10]. Still, newly synthesized IκBε can also enter the nucleus to inhibit NF-κB

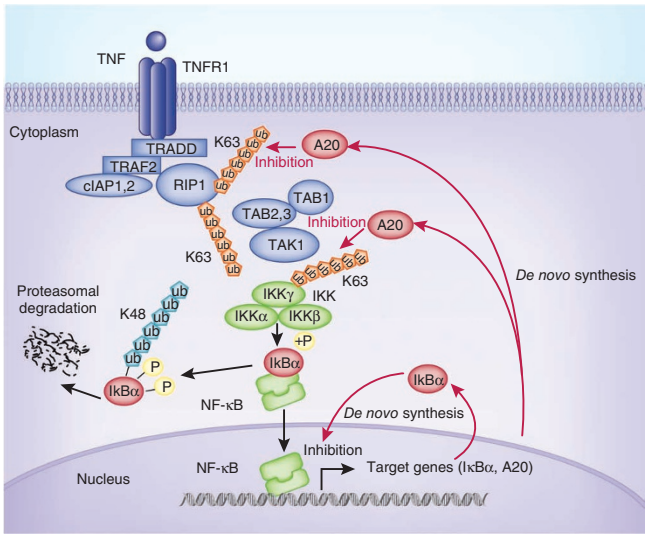

Figure 1 IκBα- and A20-dependent negative feedback loops in the canonical NF-κB pathway. Canonical activation of NF-κB by TNF is mediated via the recruitment of TRADD, TRAF2 and cIAP1 and cIAP2, together with RIP1, to the receptor. K63-linked polyubiquitination of RIP1 results in further recruitment of the IKK complex to the activated receptor and IKK activation. IKK phosphorylates IκBα, which triggers its K48-linked polyubiquitination and subsequent degradation by the proteasome. These events allow translocation of NF-κB into the nucleus and activation of gene transcription. Strongly induced NF-κB target genes include those that encode the negative regulators IκBα and A20. After protein synthesis, IκBα binds to nuclear NF-κB complexes and inhibits their function by shuttling NF-κB back into the cytosol. In addition, the ubiquitin-editing enzyme A20 deubiquitinates RIP1 and IKKγ, which leads to the disassembly of proximal NF-κB-activating complexes and shutting down of the inflammatory response.

function, acting to dampen potential IκBα-driven oscillations[10]. The signal-induced resynthesis of IκB proteins still represents one of the best-understood and well-accepted feedback loops for the down-modulation of canonical NF-κB signaling (**Fig. 1**).

Deubiquitinases upstream of IKK

Before discussion of negative regulatory mechanisms that operate upstream of the canonical IKK complex ensues, the general principles of IKK activation need a brief introduction. As indicated, distinct immunoreceptors use specific complexes composed of selected adaptor molecules, ubiquitin ligases and protein kinases for context-specific control of IKK[4,5]. Ligation of the TNF receptor TNFRI, for example, results in recruitment of the adaptor TRADD to the receptor, which assembles a signalosome with the E3 ubiquitin ligases cIAP1, cIAP2 and TRAF2 and the protein kinase RIP1 (**Fig. 1**). RIP1 is then polyubiquitinated through non-degradative linkage via Lys63 (K63), presumably by cIAP proteins[16]. These K63-linked polyubiquitin chains on RIP1 associate with the ubiquitin-binding domain of IKKγ[17,18], which results in recruitment of the IKK complex to the receptor in proximity to upstream kinases such as TAK1 or MEKK3. The subsequent activation of IKK is potentially promoted by conformational changes in IKKγ after the binding of polyubiquitin[19]. Innate pattern-recognition receptors, including TLRs, use intermediates such as MyD88, TIRAP, TRIF, TRAM, TRAF6, IRAK1, IRAK4 and TAK1 (ref. 20), whereas signaling through antigen receptors requires CARD11, Bcl-10 and MALT1, together with TRAF2, TRAF6 and TAK-1 for NF-κB activation[21]. Notably, stimulus-induced K63-linked polyubiquitination of scaffold proteins is a prerequisite for canonical NF-κB signaling in all cases[4], but K63-linked polyubiquination is a reversible event.

Indeed, several deubiquitinase (DUB) enzymes are critically involved in the negative regulation of canonical NF-κB signaling[22]. The best studied DUB that down-modulates NF-κB is A20 (ref. 23). Remarkably, A20 has, in addition to its DUB region, a C2-C2 zinc-finger E3 ubiquitin ligase domain that allows dual ubiquitin-editing function[24]. As the expression of A20 is also directly induced by NF-κB activity, A20 constitutes an additional negative feedback loop[25] (**Fig. 1**). Once activated, the DUB domain of A20 removes the K63-linked polyubiquitin chains from RIP1, which leads to destabilization of the IKK-activating complex. In addition, the E3 ligase domain promotes subsequently degradative K48-linked polyubiquitination of RIP1, which results in its proteasomal degradation for robust termination of signal transmission. As the removal of K63-linked polyubiquitin from RIP1 is a prerequisite for the K48-linked polyubiquitination of the same molecule, proper timing is secured. Notably, the DUB activity of A20 is not selective for RIP1 but targets also other IKK activators, including TRAF6 (ref. 26), IKKγ[27], RIP2 (ref. 28) and MALT1 (ref. 29). Thus, A20 down-modulates not only the TNFRI pathway but also canonical NF-κB cascades that originate from pattern-recognition receptors or antigen receptors[25]. The activity of A20 itself is modulated by Tax1-binding protein 1, which facilitates the interaction of A20 with RIP1 or TRAF6 (ref. 30). Further, A20 acts together with the HECT ubiquitin ligase Itch to promote K48-linked RIP1 ubiquitination[31]. Interestingly, Itch can also acts together with the E3 ubiquitin ligase Nedd4 to mediate the degradative ubiquitination of Bcl-10 to down-modulate antigen-induced NF-κB activity[32]. Additional regulators of A20 function are the adaptors ABIN-1 and ABIN-2, which contribute to termination of the NF-κB response, presumably by recruiting A20 to ubiquitinated IKKγ[33]. Consistent with an essential role for A20 in the resolution of inflammatory responses *in vivo*, A20-deficient mice die as a result of unrestrained inflammation[23].

The A20 family member Cezanne (cellular zinc finger anti-NF-κB) is another DUB enzyme that attenuates NF-κB signaling[34]. Its expression is also induced by NF-κB activation, for example, after TNFR ligation, in which it suppresses the buildup of polyubiquitinated RIP1 proteins. NF-κB-induced expression of Cezanne provides a further negative feedback loop in the TNFR pathway. Still, the potential functions of Cezanne in other NF-κB-activating cascades and its role in the resolution of inflammation *in vivo* remain to be discovered.

CYLD (cylindromatosis) is a third DUB with established negative regulatory roles in the NF-κB system[35–37]. It can deubiquitinate a wide range of activators, including TRAF2, TRAF6, TAK1 and IKKγ[36,37]. Consistent with that, TNF induces more rapid degradation of IκBα in CYLD-deficient cells[38], but CYLD also attenuates NF-κB signaling after stimulation via TLR2 or TLR4 or ligation of antigen receptors[37]. Although CYLD expression, in contrast to the expression of A20 and Cezanne, is not directly induced by NF-κB, its activity is still controlled in a stimulus-dependent manner by phosphorylation or subcellular localization[38,39]. Thus, in addition to the IκB proteins, these DUB enzymes are general feedback regulators of canonical NF-κB activity that down-modulate a broad range of inflammatory pathways.

Dissociation of signaling complexes

A signal-specific layer for the negative regulation of selected NF-κB responses is provided by dominant-negative adaptors (**Fig. 2**). These mechanisms are best characterized in the regulation of MyD88- and TRIF-dependent TLR pathways[20]. The binding of ligand to the lipopolysaccharide (LPS) receptor TLR4 results in the recruitment of

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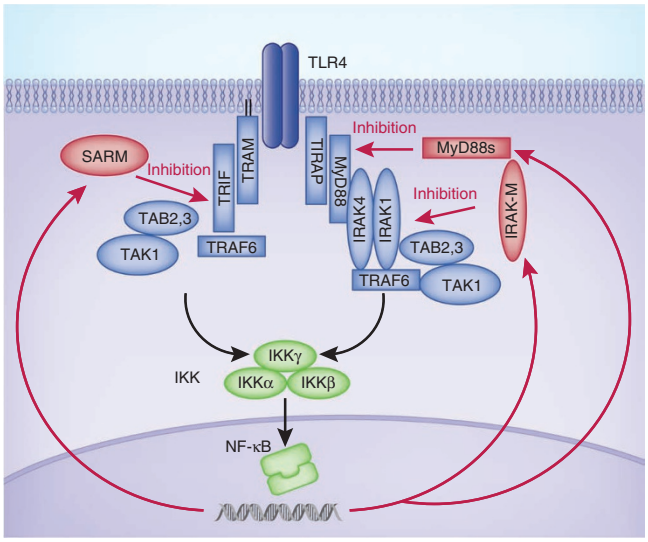

Figure 2 Dominant-negative adaptors. TLR4 signaling activates the canonical IKK complex via MyD88-dependent and TRIF-dependent mechanisms. MyD88 assembles complexes that contain IRAK kinases together with TRAF6, TAB2, TAB3 and TAK1. TRIF can directly recruit TRAF6 and recruit TAB2, TAB3 and TAK1. The active NF-κB pathway subsequently induces expression of the alternative MyD88 splice product MyD88s, the kinase-inactive IRAK family member IRAK-M and the negative regulatory adaptor molecule SARM. These dominant-negative factors presumably affect the stability of the IKK-activating complexes.

MyD88 and TRIF to the receptor via TIRAP and TRAM, respectively[20]. MyD88 activates canonical IKK signaling through IRAKs together with TRAF6 and, presumably TAB2, TAB3 and TAK1, whereas TRIF engages TRAF6 or other TRAF proteins directly. Intriguingly, stimulation of cells with LPS induces *de novo* generation of the short MyD88 isoform MyD88s via alternative splicing[40]. In contrast to full-length MyD88, MyD88s lacks the intermediate domain between the death domain and Toll–interleukin 1 receptor (TIR) domain that is required for the interaction of MyD88 with IRAK4. Thereby, MyD88s can act as a dominant-negative signaling factor that interferes with the MyD88-IRAK4 interaction to shut down LPS-induced NF-κB activation[40]. Interestingly, the MyD88s-mediated inhibition of TLR signaling is selective for the NF-κB pathway and does not affect activity of the transcription factor AP-1 (ref. 41).

IRAK-M is another protein that inhibits TLR signaling in a dominant-negative way[42]. However, IRAK-M is not a product of alternative splicing but is an inactive IRAK homolog whose expression is induced after TLR stimulation[43]. IRAK-M interferes with the formation of IRAK-TRAF6 complexes, and IRAK-M-deficient cells have more cytokine production[42]. Moreover, mice that lack IRAK-M develop enhanced inflammatory responses to bacterial infection *in vivo*. Furthermore, endotoxin tolerance, a protective mechanism against septic shock, is much lower in IRAK-M-deficient mice[42], and the induction of IRAK-M is associated with LPS tolerance in models of human endotoxemia[44].

A third example of a dominant-negative adaptor in the TLR pathway is the TIR domain–containing protein SARM[45]. The expression of SARM is also much higher after LPS stimulation. However, SARM does not affect MyD88 signaling but does selectively inhibit the TRIF pathways through direct interaction with TRIF. Although it is unknown exactly how SARM inhibits TRIF function, it is possible that the interaction between SARM and TRIF interferes with the stability of the TRIF effector complex.

Although the functions of dominant-negative adaptors in non-TLR pathways are less well understood, it is very likely that such molecules also contribute to the down-modulation of IKK–NF-κB activity in other settings. Nevertheless, related mechanisms are responsible for the signal-induced dissociation of IKK-activating complexes to attenuate signaling through antigen receptors. IKKβ is essential for the positive regulation of NF-κB signaling after its initial T cell antigen receptor–mediated activation. Subsequently, IKKβ phosphorylates its upstream molecule Bcl-10 directly[46] to mediate the dissociation of Bcl-10 from CARD11 and subsequent disassembly of the NF-κB-activating CARD11–Bcl-10–MALT1 signalosome[46]. Additional data have indicated that IKKβ-mediated phosphorylation of Bcl-10 marks Bcl-10 for degradative ubiquitination to turn off the signal[47]. Another component that first promotes and then terminates antigen-induced activation of NF-κB is casein kinase 1α (CK1α)[48]. CK1α engages the active CARD11–Bcl-10–MALT1 complex after stimulation of the antigen receptor by directly interacting with CARD11 to initially contribute to activation of the NF-κB response. Then, CK1α phosphorylates CARD11 to dampen signaling. How CK1α down-modulates CARD11 signaling is unclear, but it has been suggested that CK1α-mediated phosphorylation could contribute to CARD11 degradation[48].

Termination of canonical NF-κB responses in the nucleus

Although IκB-mediated reshuttling of nuclear NF-κB is important for the termination of NF-κB responses at the level of DNA, additional negative regulatory mechanism operate in the nucleus (**Fig. 3**). These include IκB-independent displacement of active NF-κB from DNA and proteolytic degradation of active NF-κB dimers. After the initial observation that mice that lack the SUMO E3 ligase PIAS1 are more sensitive to LPS-induced shock[49], it was demonstrated that PIAS1 can directly interfere with the binding of RelA dimers to DNA, particularly at early time points of NF-κB activation[50]. Similarly, PIAS4-deficient mice are also hypersensitive to septic shock and also have enhanced DNA-binding activity of RelA[51], which indicates that both PIAS proteins might displace

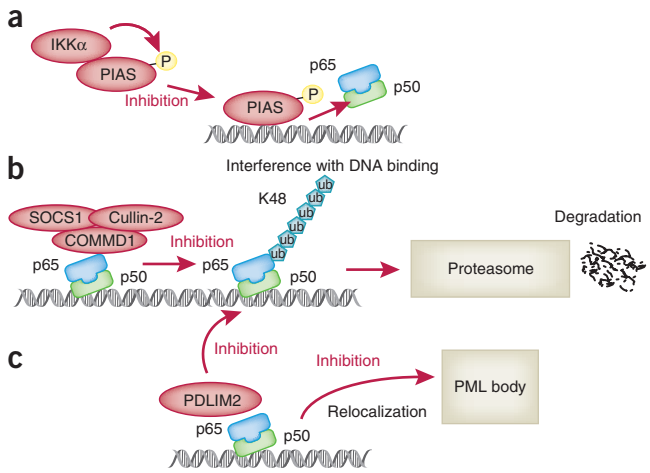

Figure 3 Interference with NF-κB function in the nucleus. (**a**) After being phosphorylated by IKKα, PIAS1 can interfere with the binding of RelA–NF-κB complexes to DNA. (**b**,**c**) The COMMD1–SOCS1–Cullin-2 E3 ubiquitin ligase complex (**b**) and the E3 ligase PDLIM2 (**c**) terminate RelA–NF-κB responses in the nucleus by inducing K48-linked polyubiquitination of RelA, which results in proteasomal degradation of RelA. In addition, PDLIM2 (**c**) promotes the relocalization of RelA to transcriptionally silent promyelocytic leukemia nuclear bodies (PML body).

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RelA from target sequences. Still, PIAS1 and PIAS4 regulate the expression of distinct NF-κB target genes differently, as PIAS1 has substantial effects on TNF-induced expression of IκBα or the precursor to interleukin 1β, whereas PIAS4 has a substantial effect on expression of the chemokine CXCL1 (refs. 50,51). The exact regulation of the interference of PIAS proteins with the DNA binding of NF-κB is unknown. However, a fraction of IKKα is constitutively associated with PIAS1 and can directly phosphorylate PIAS1 after inflammatory activation[52]. This modification induces the dissociation of PIAS1 from the kinase and its movement to the promoters of NF-κB target genes to mediate transcriptional termination.

IKKα has additional negative regulatory roles in the termination of nuclear canonical NF-κB responses, as it accelerates the turnover of RelA and c-Rel[53,54]. While activated in the cytoplasm by proinflammatory signals, IKKα phosphorylates RelA specifically at Ser36 and thereby enhances its later proteasomal degradation in the nucleus. These effects explain at least in part the enhanced inflammatory responses of IKKα-deficient macrophages.

At least two E3 ubiquitin ligases control the degradation of nuclear RelA[55]. One of these is PDLIM2, which is essential for the prevention of uncontrolled inflammation *in vivo*[56]. PDLIM2 has a PDZ (postsynaptic density 65–discs large–zonula occludens 1) domain in addition to its E3 ligase activity that mediates degradative RelA ubiquitination. The former has chaperone function and promotes the transport of RelA to promyelocytic leukemia nuclear bodies[50]. Thus, PDLIM2 not only targets nuclear RelA for proteasomal degradation but also relocalizes DNA-bound RelA to areas of transcriptional silencing. The other known E3 ligase that terminates the RelA responses in the nucleus is the EC2S complex, which contains SOCS1, Cullin-2 and COMMD1 (refs. 57,58). Functionally, COMMD1 bridges RelA to SOCS1 and Cullin-2 after stimulation with proinflammatory molecules. The EC2S complex then mediates ubiquitin-dependent degradation of RelA, which seems to be particularly important for the termination of NF-κB responses at later stages of cell stimulation[57]. It is still unknown whether PDLIM2 and the EC2S complex act together in the termination of RelA NF-κB responses under some conditions or whether the two ligases operate in signal-specific ways. However, as PDLIM2-deficient dendritic cells are completely defective in LPS-induced polyubiquitination of RelA, PDLIM2 function cannot be compensated for, at least under these conditions.

A study has demonstrated important functions for lysine methylation of RelA mediated by the lysine methyltransferase Set9 for the initiation of proteasome-mediated degradation of promoter-associated RelA[59]. Consistent with that, depletion of Set9 by small interfering RNA enhances TNF-mediated expression of NF-κB target genes. Still, the mechanisms by which methylation of RelA dictates NF-κB turnover in the nucleus are unclear. One possibility is that lysine methylation recruits an as-yet-unidentified E3 ligase to RelA[59].

Negative control of alternative NF-κB signaling

The alternative NF-κB pathway is centered on the unstable kinase NIK[5] (**Fig. 4**) and is particularly required for the development and survival of mature B cells[60,61]. It is physiologically activated by a restricted set of members of the TNFR superfamily and mainly controls the activation of RelB-p52 heterodimers. RelB preferentially forms dimers with the NF-κB2 precursor p100, which contains ankyrin repeats similar to those of the classical IκB proteins; these allow IκB function to retain RelB-p100 complexes in the cytoplasm. Receptor stimulation causes rapid NIK stabilization, which leads to subsequent IKKα activation. Active IKKα in turn phosphorylates p100, which results in the ubiquitination and partial degradation of p100 and release of RelB-p52 dimers into the nucleus. Interestingly, under certain conditions, p100 can also function as an IκB that controls RelA-p50 activation in response to NIK, which provides crosstalk between the alternative and canonical NF-κB cascades[62].

How upstream signals mediate NIK stabilization is not precisely understood. However, NIK stabilization correlates directly with the stimulus-induced degradation of TRAF3 (ref. 63), which bridges NIK via TRAF2 to cIAP1 and cIAP2. These cIAP proteins serve as potent K48-specific E3 ligases for NIK that mediate degradative ubiquitination to constitutively repress alternative NF-κB signaling under unstimulated conditions[64,65]. Failure to repress steady-state signals results in severe disease, as both *Traf3*$^{-/-}$ and *Traf2*$^{-/-}$ mice develop constitutive activation of alternative NF-κB signaling, with NIK stabilization, perinatal inflammation and early mortality[66–68]. The mechanisms that down-modulate alternative NF-κB signaling after initial ligand-induced activation are also beginning to be elucidated. An IKKα-dependent feedback loop that attenuates signal-induced NIK stabilization and presumably controls the magnitude and kinetics of the pathway is involved in this[69]. After being activated by NIK, IKKα directly phosphorylates NIK at the carboxyl terminus, which

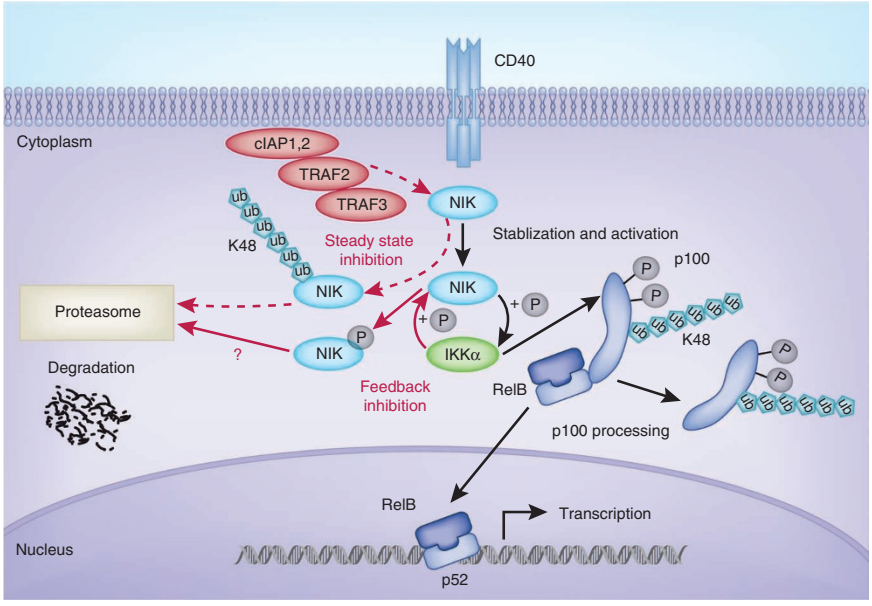

Figure 4 Negative regulation of alternative NF-κB signaling. Engagement of alternative NF-κB signaling by TNF superfamily receptors, such as CD40, induces NIK stabilization and subsequent IKKα-dependent phosphorylation of p100, which results in p100 processing and translocation of RelB-p52 dimers to the nucleus. Key negative regulatory mechanisms depend on NIK proteolysis. Under unstimulated conditions, the stability of NIK protein is negatively regulated by TRAF3, TRAF2 and cIAP1 and cIAP2, which control K48-linked polyubiquitination of NIK, resulting in proteasomal degradation. After receptor ligation and signal initiation, IKKα phosphorylates not only p100 but also NIK in a feedback loop to promote subsequent NIK destabilization through still-uncharacterized pathways that also involve the proteasome.

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leads to NIK destabilization. Consistent with that, a NIK mutant that lacks the IKKα-targeted serine residues is more stable than wild-type NIK and results in more noncanonical NF-κB activity. These negative regulatory events apparently do not involve TRAF-cIAP complexes. Moreover, IKKα does not trigger detectable NIK ubiquitination, although this IKKα-mediated feedback degradation of NIK depends on the proteasome[69]. Thus, identification of the cellular cofactors that mediate IKKα-dependent destabliziation of NIK will be an important task for the future.

Conclusions and perspectives

The past decade has seen a tremendous increase in the understanding of how distinct pathways activate NF-κB in a context- and cell type–specific manner. However, the mechanisms that counteract these responses are much less well defined. Although this review has highlighted several principles of NF-κB down-modulation, further mechanisms for NF-κB inhibition exist. Numerous post-translational modifications of NF-κB subunits, such as reversible phosphorylation or acetylation of RelA, have been reported that positively or negatively regulate NF-κB function by altering its association with transcriptional cofactors or with DNA[70]. Another emerging concept of NF-κB control is its regulation through microRNAs[71,72] that target the 3′ untranslated region of mRNA and thereby decrease mRNA abundance and protein expression. Various microRNAs have been identified that are induced by NF-κB-dependent transcription. One of these is miR-146, which targets the expression of IRAK-1 and TRAF6 and thereby down-modulates the MyD88-dependent NF-κB response[73]. Another example is miR-155, which downregulates the expression of TAB2 and MyD88 in cells of the innate immune response[74,75]. Functional data that define the role of microRNA in NF-κB regulation and inflammation *in vivo* are still missing. However, it is very likely that such studies will identify additional pathophysiologically relevant layers of feedback for negative control of NF-κB.

Thus, much remains to be learned about the downregulation of NF-κB pathways. However, it is already very clear that failing to counteract NF-κB responses can have severely deleterious consequences for human health. Genetic defects that drive human inflammation or cancer have been detected at almost every molecular level. Somatic inactivating mutations of the gene encoding IκBα have been found in Hodgkin's disease, a lymphoid malignancy associated with constitutive NF-κB activity[76]. Disrupting mutations of the gene encoding IκBα have also been observed in glioblasoma, a cancer of the central nervous system[77]. Additional studies of human lymphomas have identified the DUB enzyme A20 as a key tumor suppressor in the B cell lineage that is commonly inactivated by loss-of-function mutations[78,79] or proteolytic cleavage by the MALT1 oncoprotein[80]. Genetic polymorphisms of the gene encoding A20 are also associated with the autoimmune disorder systemic lupus erythematosus[81], as well as rheumatoid arthritis[82] and psoriasis[83], and deficiencies in the DUB enzyme CYLD promote the oncogenic process in the familial tumor-predisposition syndrome cylindromatosis[35]. IRAK-M contributes to early onset persistent asthma[84] and, finally, mutations in the genes encoding TRAF3, NIK and other factors, or proteolytic processing of NIK by the IAP2-MALT1 oncoprotein that prevent down-modulation of alternative NF-κB signaling, drive the pathogenesis of multiple myeloma and MALT lymphoma, which are both malignancies of the B cell lineage[85–87]. These clinical results highlight the importance of attenuating NF-κB. Hopefully, further insights into the negative regulatory mechanisms of NF-κB responses will contribute to the development of new diagnostics and therapeutics for human inflammatory and malignant diseases.

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COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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