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The Regulatory Logic of the NF-κB Signaling System

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NF-κB refers to multiple dimers of Rel homology domain (RHD) containing polypeptides, which are controlled by a stimulus-responsive signaling system that mediates the physiological responses to inflammatory intercellular cytokines, pathogen exposure, and developmental signals. The NF-κB signaling system operates on transient or short timescales, relevant to inflammation and immune responses, and on longer-term timescales relevant to cell differentiation and organ formation. Here, we summarize our current understanding of the kinetic mechanisms that allow for NF-κB regulation at these different timescales. We distinguish between the regulation of NF-κB dimer formation and the regulation of NF-κB activity. Given the number of regulators and reactions involved, the NF-κB signaling system is capable of integrating a multitude of signals to tune NF-κB activity, signal dose responsiveness, and dynamic control. We discuss the prevailing mechanisms that mediate signaling cross talk.

How can the regulatory logic of a signaling system be understood? The regulatory logic refers to the properties of a system that are not evident by studying one molecular component in isolation, or even the interaction between two; hence, they are sometimes referred to as emergent system properties. Straightforward emergent properties of a signaling system are signal-dose responses (which may be linear or sinusoidal) and dynamic control of the response (which may be fast or slow ramping, transient or oscillatory). More complex emergent properties may pertain to the integration of different signals (synergistically or antagonistically), memory functions, or contingencies for prior stimulus exposures to signal transduction. Those properties are mediated by the molecular components arranged in a particular network topology. Quantitative measurements of the relevant biochemical reactions is a prerequisite for a molecular understanding of the regulatory logic, and tracking a multitude of reactions via a computational model is an effective and practical strategy.

Understanding the function of a network must begin with comprehensive accounting of the parts list, the list of molecular components. The NF-κB signaling system consists of two protein families, the NF-κBs (activators) and the IκBs (inhibitors) (Fig. 1). The NF-κB transcription factors are the result of combinatorial dimerization of five monomers that can

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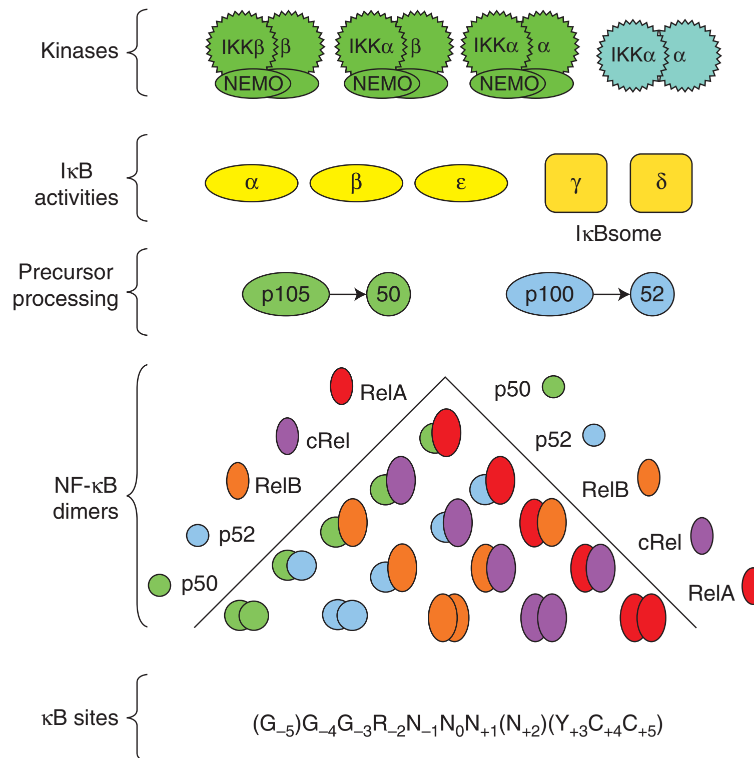

Figure 1. Molecular components of the IKK–IκB–NF-κB signaling system. The IκB kinases form canonical NEMO-containing (green) complexes and noncanonical IKKα complexes (blue), which control the degradation of IκB proteins as well as precursor processing. IκBα, IκBβ, IκBε, p105 (IκBγ), and p100 (IκBδ) are able to bind and sequester NF-κB dimers ("IκB activity"). The p50 and p52 NF-κB proteins are initially synthesized as the precursor proteins p105 and p100, respectively. The five NF-κB family members can potentially form 15 possible dimers, which may bind to a large family of κB sites in DNA.

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produce 15 possible dimers. IκB activities act as stoichiometric inhibitors of the DNA binding activities of these dimers, and all combinations are, in principle, possible. Two classes of IκB kinase (IKK) complexes (the canonical and noncanonical IKK complexes) control the half-life and therefore abundances of the IκB activities. The canonical IKK are defined as those complexes bound and regulated by NF-κB essential modulator (NEMO), and the noncanonical complexes require both IKKα and NF-κB inducing kinase (NIK) activities but are independent of NEMO (Scheidereit 2006). The canonical IKK complexes act on the classical IκB proteins IκBα, -β, -ε, and the IκBγ activity mediated by p105, whereas the noncanonical IKK complex acts on the IκBδ activity mediated by p100. Both of the latter nonclassical IκB activities (IκBγ and IκBδ) reside in a high-molecular weight complex (Savinova et al. 2009), which we term the IκBsome. In addition, both IKK activities can affect NF-κB dimer generation by controlling the processing of precursor proteins during or shortly after their translational synthesis. Canonical IKK may enhance processing of p105 to p50, thereby increasing the availability of p50-containing dimers, whereas noncanonical IKK controls processing of p100 to p52, thereby generating p52-containing dimers.

Considering the regulatory logic of the NF-κB signaling system, we distinguish regulatory mechanisms on two different timescales at which they operate: NF-κB dimer formation via NF-κB protein synthesis and dimerization, and the regulation of NF-κB dimer activity via

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IκB degradation and resynthesis. The former (NF-κB protein synthesis and dimerization) is largely associated with longer-term cell differentiation processes, whereas the latter (IκB degradation and resynthesis) controls reversible responses to often transient inflammatory stimuli. Hence, we first consider the regulatory logic of NF-κB dimer generation and then discuss the mechanisms that regulate dimer activity.

REGULATION OF NF-κB DIMER GENERATION

NF-κB refers to homo- and heterodimeric DNA binding complexes that consist of Rel homology domain (RHD) containing polypeptides. Of the 15 potential dimers (Fig. 1), three do bind DNA but lack transcriptional activity (p50:p50, p52:p52, and p50:p52), and three are not known to bind DNA (RelA:RelB, cRel:RelB, and RelB:RelB), leaving nine potential transcriptional activators (Hoffmann and Baltimore 2006). Dimer formation and DNA binding occur through the RHD. Upon dimerization, NF-κB dimers can interact with IκB proteins, as the ankyrin repeat domain (ARD) of IκB forms a large interaction surface around the dimeric interface (Hayden and Ghosh 2004; Hoffmann et al. 2006). IκB binding biases cellular localization of the NF-κB dimer to the cytoplasm, thereby inhibiting the DNA binding activity of the NF-κB dimers and allowing for stimulus-responsive activation by cytoplasmic IKK complexes.

Several biochemical reactions control the generation and therefore availability of NF-κB dimers. Synthesis of the RHD polypeptides is the first step, and is regulated at the level of transcription in a cell-type-specific manner. Furthermore, RHD polypeptide genes (except *rela*) are inducible by NF-κB activity (see nf-kb.org for a list of NF-κB target genes), potentially resulting in positive feedback as well as functional interdependence between them. In addition, the generation of p50 and p52, the two RHD polypeptides that lack transcriptional activation domains (TADs) but act as binding partners for cRel, RelB, and RelA, is controlled by proteolysis of p105 and p100. These proteolytic events may be responsive to canonical and noncanonical signals.

RHD polypeptides require dimerization to avoid degradation, presumably via unfolded protein degradation pathways. NF-κB dimer formation and stability may not only be determined by intrinsic association and dissociation rate constants between RHD polypeptides, but may also involve interactions with IκB proteins. One intriguing possibility is that association with the IκBs stabilizes the NF-κB dimers by slowing their dissociation into monomers, and possibly also the degradation of the intact dimers. If this hypothesis is found to be valid, IκB interactions with NF-κB dimers would not only inhibit NF-κB function, but would also contribute to generation of NF-κB dimers in the first place.

Equilibrium States

The generation of the cell-type-specific NF-κB dimer repertoire is a function of cell-type-specific homeostatic regulation that may be subject to signals conditioning the cell in a particular microenvironment. Homeostatic regulation may be tuned at several different mechanistic levels: (1) stimulus-responsive and cell-type-specific RHD polypeptide synthesis; (2) dimerization specificities governing the association and dissociation of each of the 15 monomer pairs; (3) IκB-NF-κB interactions contributing to dimer stability (Fig. 2A,B). As IκBs may have differential affinities for NF-κB dimers, the relative abundances of various IκB proteins may affect the repertoire of latent dimers available for activation.

Different cell types have indeed been reported to contain different repertoires of NF-κB dimers. Murine embryonic fibroblasts (MEFs), just like many of the transformed cell lines commonly used (HeLa, HEK293), contain primarily latent RelA:p50 heterodimer, as well as RelA:RelA and p50:p50 homodimers. cRel and RelB expression can be detected in these cells, but appears functionally negligible. In contrast, B-cells are abundant with readily activatable cRel and p50 containing dimers

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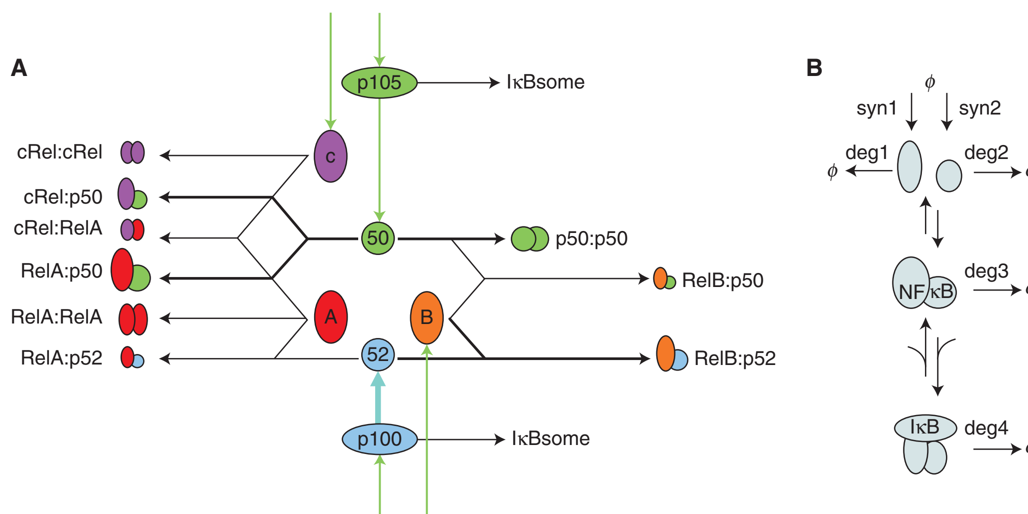

Figure 2. Mechanisms determining NF-κB dimer generation. (*A*) Synthesis of RHD polypeptides and their dimerization affinities control the generation of NF-κB dimers, whose relative abundances in MEFs are indicated by their relative size. Green arrows indicate regulation by canonical IKK and the blue arrow regulation by noncanonical IKK. The amount of processing of the p105 and p100 proteins alters the amount of p50 and p52 available for dimer interaction, and abundances of certain subunits is influenced by the amount of others. (*B*) IκB-NF-κB interactions may play a role in dimer generation. A theoretical model of NF-κB dimer metabolism indicates that dimerizaton may reduce monomer degradation (if deg3<deg2 and/or deg1), and further, that IκB interactions with the dimer may not only block dimer dissociation into monomers, but also dimer degradation (if deg4<deg3).

(Sen 2006). Dendritic cells (DCs) depend on both cRel and RelB for maturation (Ouaaz et al. 2002; Gerondakis et al. 2006), although the regulation of these cRel and RelB-containing dimer activities remain to be characterized.

Although cell-type-specific expression of RHD polypeptides and NF-κB dimers has been established, the molecular regulation that underlies the shifts in the NF-κB dimer repertoire during cell differentiation remains to be delineated. An early study examined the expression of NF-κB monomers and dimers in transformed cell lines that represent B-cell subtypes along the B-cell differentiation pathway (Liou et al. 1994). This work tracked differential NF-κB dimer availability, but what molecular mechanisms mediate these changes in the homeostatic NF-κB dimer repertoire has remained unclear. First insights about these molecular mechanisms come from the detailed analysis of NF-κB dimer misregulation in knockout cells deficient in one or more NF-κB or IκB genes (Basak et al. 2008). The mechanisms by which compensation or interdependence of NF-κB dimer regulation is mediated can thereby be characterized and the insights may be applicable to normal differentiation processes.

There are several levels in which the generation of different NF-κB dimers is interdependent, allowing for cross talk and/or robustness through compensation. Cross talk may be mediated by the fact that synthesis of the four RHD polypeptides RelB, cRel, p50, and p52 is dependent on RelA-containing dimers at the level of gene expression (Fig. 2A). Hence, phenotypes resulting from RelA deficiency may (in part) be mediated by the consequently diminished availability of other RelA-dependent NF-κB dimers. Second, potential competition in dimerization interactions between limited pools of NF-κB monomers may mediate cross-regulation in dimer generation when specific RHD polypeptide synthesis rates change. However, a primary binding partner for the TAD-containing RelA, RelB, and cRel proteins is p50, which is generally produced in excess by

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abundant synthesis and processing of p105, leading to system robustness. When p50 is lacking in *nfkb1*$^{-/-}$, then the mature *nfkb2* gene product p52 compensates. In this scenario, however, the demand for p52 depletes the pool of p100, which is required for the noncanonical IκBδ activity, thereby attenuating noncanonical signal responsiveness. This demonstrates the capacity for one means of cross talk regulation between the canonical and noncanonical pathways (Basak et al. 2008).

One motivation for understanding the regulation of the cell-type-specific NF-κB dimer repertoire is that disease-associated cells may well exhibit an altered equilibrium state. Such altered states may be caused by chronic exposure to external signals within the disease microenvironment. Inflammatory signals such as TNF, IL-1, IL-6, or IFNγ are associated with inflammatory conditions or tumors that can skew the NF-κB dimer repertoire and NF-κB responsiveness of macrophages. Such changes may mitigate or potentiate the pathology. In other pathological conditions, altered NF-κB dimer repertoires may be a cause of the pathology. In some subsets of B-cell lymphomas, an enhanced pro-proliferative NF-κB-cRel gene expression signature can be traced back to cRel gene amplifications (Shaffer et al. 2002). Enhanced cRel expression may in other subsets be the result of cell intrinsic alterations that are not immediately obvious, such as an altered chromatin homeostasis caused by misregulated HAT (histone acetyltransferase) and HDAC (histone deacetylase) activities. The effects of disease-associated altered NF-κB dimer repertoires continue to pose important questions for research.

Stimulus-responsive Alterations

As four RHD polypeptides are encoded by known NF-κB-RelA response genes, the NF-κB dimer repertoire is generally thought to be readily alterable in response to inflammatory stimulation or other RelA-dimer-inducing stimuli (Fig. 3). However, it is unclear how dynamic or reversible the resulting changes are, whether they are occurring within hours or days, or whether they are transient or long lasting. Most observations suggest that changes in the NF-κB dimer repertoire because of stimulus-induced transcription are occurring on long timescales and are long-lasting; we therefore suggest that transcriptional control of RHD polypeptide expression determines the homeostatic state of the NF-κB signaling system.

Stimulus-responsive alterations in the NF-κB dimer pool do, however, occur as a result of stimulus-responsive processing of p105 and p100 to p50 and p52. Both processing events occur cotranslationally or at least shortly following translation, before the precursors form higher order complexes (including IκBsomes) through homo- and heterotypic interactions that render them processing incompetent. Processing of p105 to p50 is mediated by long-term constitutive canonical IKK activity that ensures elevated expression of p50. The resulting p50:p50 homodimers have been observed in TLR-stimulated macrophages as well as other cell types, but their physiological role is unclear. Processing of p100 to p52 is mediated by noncanonical IKK activity and has been studied in detail. This processing event generates primarily the RelB:p52 dimer, which regulates expression of organogenic chemokines and is implicated in survival of certain differentiating and maturing cell types. Thus, noncanonical pathway activating stimuli, such as BAFF, LTβ, RANKL, and CD40L, can be thought of as altering the NF-κB dimer repertoire to mediate their functional effects (in addition to regulating the activities of NF-κB dimers via IκB regulation, as discussed later).

REGULATION OF NF-κB ACTIVITY VIA IκB DEGRADATION AND RESYNTHESIS

The three canonical IκB proteins, IκBα, IκBβ, and IκBε, and the noncanonical IκBδ activity bind and inhibit NF-κB dimers and thereby allow for their stimulus-responsive activation. The degradation and resynthesis of IκBs is coordinately regulated to produce dynamic NF-κB activities that are stimulus-specific. For example, the ubiquitous RelA:p50 dimer is bound and inhibited by the canonical IκB proteins (IκBα, IκBβ, and IκBε), as well as the

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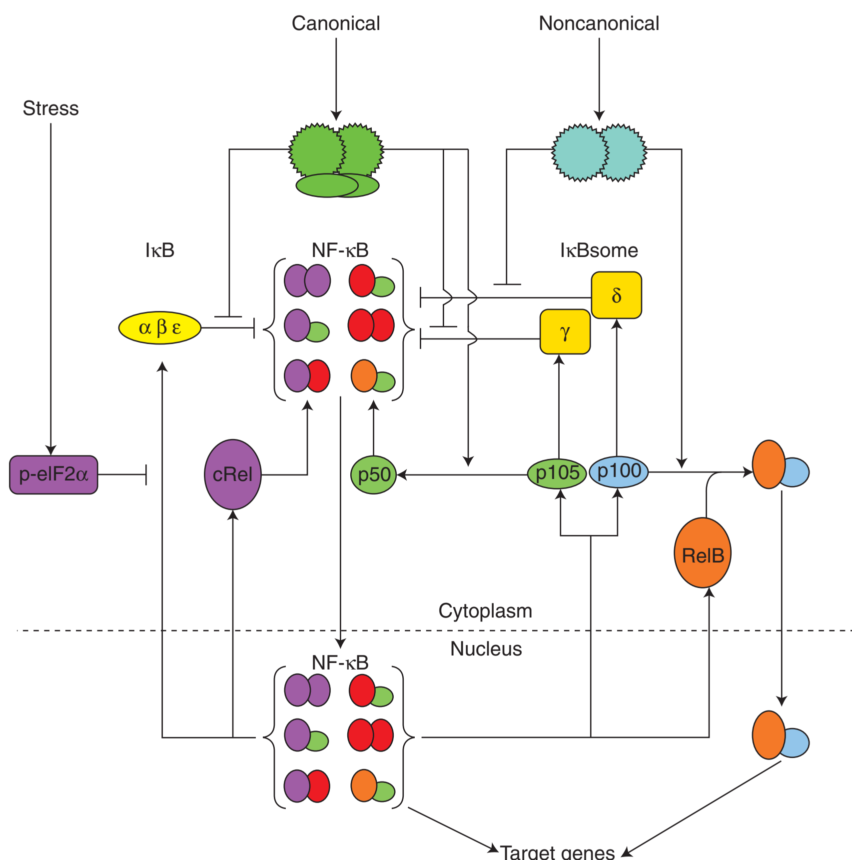

Figure 3. Mechanisms controlling stimulus-responsive NF-κB activities. Canonical signals activate NEMO-containing IKK complexes (green), which degrade the canonical IκB proteins (IκBα, β, and ε) and the IκBγ activity (composed of asymmetric p105 dimers) associated with NF-κB dimers. Released NF-κB dimers move to the nucleus to activate gene expression programs, including the expression of IκBα, IκBε, p105, p100, cRel, and RelB proteins. Noncanonical signals activate IKKα complexes, which degrade IκBδ complexes associated with NF-κB dimers. The resulting increase in synthesis of p100 and RelB, concomitant with IKKα activity, causes increased p100 processing to p52 and dimerization with RelB, to generate active RelB:p52 dimers to the nucleus. Stress signals can activate the eIF2α kinases, causing phosphorylation of eIF2α and resulting in inhibited translation. A block in IκB synthesis, in combination with constitutive IKK activity, results in the loss of IκB proteins and subsequent NF-κB dimer activation.

IκBδ activity. RelB-containing dimers, however, are known to have strong binding specificity only for IκBδ. In this manner, different IκB-NF-κB dimer associations allow for specific dimers to be activated in response to specific stimuli.

The regulated metabolism (synthesis and degradation) of the canonical IκB proteins, especially IκBα, has been studied in detail. Two degradation pathways control steady-state levels of canonical IκBs and NF-κB activity. When associated with NF-κB, IκBα, -β, and -ε are degraded through a canonical IKK-dependent mechanism. IκB proteins that are not associated with NF-κB, however, are intrinsically unstable with a 5–10 minute half-life, being degraded in an IKK- and ubiquitin-independent manner (O'Dea et al. 2007; Mathes et al. 2008). Accumulating evidence indicates that the 20S proteasome can mediate degradation of free IκBα, whereby weakly folded regions in carboxy-terminal ankyrin

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repeats and other carboxy-terminal sequences mediate its instability (Alvarez-Castelao and Castano 2005; Truhlar et al. 2006). NF-κB association triggers complete IκB protein folding and removes IκB from the 20S proteasome pathway. In addition to determining the IκB degradation pathway, NF-κB activity also drives the transcription of IκBα and IκBε, thus providing another self-inhibitory mechanism to prevent constitutive NF-κB activity.

IκBδ activity is the result of the dimerization of two p100 molecules (through interaction between their RHDs), whereby the ARD of one p100 molecule folds back onto the RHD–RHD dimer interface to effect self-inhibition ("in *cis*"), leaving the ARD of the second p100 molecule capable of binding latent NF-κB dimers (primarily RelA:p50, but also RelB:p50) "in *trans*" (Basak and Hoffmann 2008). Subsequent studies with processing-defective mutants of p105 provided evidence for an analogous activity, termed IκBγ (Sriskantharajah et al. 2009). NF-κB also drives the synthesis of p105/IκBγ and p100/IκBδ, which may, like IκBα and IκBε, contribute to tight control over constitutive NF-κB activity. Recent studies revealed that these nonclassical IκB activities, IκBγ and IκBδ, reside within high molecular weight complexes, whose assembly is mediated by the helical dimerization domains present within p100 and p105 in addition to RHD dimerization and ARD interaction surfaces (Savinova et al. 2009). These complexes may be termed IκBsomes as they trap a variety of NF-κB dimers for slow homeostatic or stimulus-responsive release at later times.

IκB Signaling in Response to Inflammatory Signals

Endogenous inflammatory stimuli (e.g., cytokines-TNF, IL-1β) or pathogen-derived substances (e.g., LPS or CpG DNA) activate the canonical NF-κB pathway. Engagement of the TNF receptor (TNFR), IL-1β receptor (IL-1βR), or TLRs causes phosphorylation-dependent activation of a NEMO-containing kinase complex. Once activated, the canonical IKK complex phosphorylates IκBα, -β, and -ε at two specific serine residues, as a signal for K48-linked ubiquitination at two specific lysine residues, leading to degradation by the 26S proteasome (Karin and Ben-Neriah 2000). Degradation of IκB releases NF-κB, allowing it to localize to the nucleus to bind DNA and activate gene expression. Interestingly, IκBα and IκBε proteins are among the large number of NF-κB response genes, thus functioning as negative-feedback regulators. Indeed, NF-κB activity induced by inflammatory stimuli shows complex and diverse temporal or dynamic profiles.

A mathematical model of the IκB–NF-κB signaling module recapitulates the signaling events triggered by TNF stimulation observed experimentally in MEFs (Kearns and Hoffmann 2009; Cheong et al. 2008). Combined experimental and computational studies showed that the three IκB proteins IκBα, -β, and -ε each have distinct roles in the dynamic control of NF-κB activation and termination. In response to TNF, IκBα is rapidly degraded and then rapidly resynthesized in an NF-κB-dependent manner. Continued TNF stimulation propagates a cycle of synthesis and degradation of IκBα that can result in oscillations of nuclear NF-κB activity (Hoffmann et al. 2002). IκBε expression is also strongly induced by NF-κB activity, but with a distinct 40-minute delay (Kearns et al. 2006). As IκBε protein accumulates at later time points, this antiphase negative-feedback loop acts to dampen the IκBα-driven oscillations.

Recent evidence indicates that the noncanonical IκB, IκBδ provides negative feedback on canonical NF-κB signaling when it is induced by pathogen-triggered TLR signals that produce longer lasting canonical IKK activities than inflammatory cytokines (e.g., TNF or IL-1). The induction of NF-κB by either LPS or TNF drives increased synthesis of p100, but on a delayed and longer timescale than that of IκBα. The slowly accumulating p100 can form into IκBsomes, within which IκBδ traps NF-κB dimers (Shih et al. 2009). Whereas TNF-induced signaling wanes within 8 hours, LPS-induced NF-κB remains elevated at late time points. This late NF-κB activity becomes

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subject to attenuation by IκBδ, which is unresponsive to and cannot be degraded by canonical signals. Cells deficient in IκBδ activity (*nfkb2* gene knockout), show similar NF-κB activation profiles in responses to TNF, but show significantly higher LPS-induced late NF-κB activity as compared to wild-type (IκBδ containing) cells (Shih et al. 2009). This study also emphasized that the prominent IκBα negative feedback is actually specific for transient cytokine signals. Because its inhibition is reversible by canonical IKK activity, it plays little role in shaping NF-κB temporal profiles in response to long lasting TLR-initiated signals. In fact, perinatal lethality of the *iκbα*$^{-/-}$ mouse was rescued by compound deficiency with the *tnf* gene (Shih et al. 2009).

The ability of the IκB-NF-κB signaling module to mediate complex temporal control over NF-κB activity has led to research into the functionality of NF-κB dynamics. Different inflammatory stimuli elicit different IKK activation profiles, which induce distinct temporal profiles of NF-κB activity. For example, TNF stimulation induces a transient spike in IKK activity in which the second phase is rather small because of the short TNF half-life and the inhibitory functions of the deubiquitinase A20 (Werner et al. 2008). In contrast, LPS activates gene expression of cytokines that provide for positive autocrine feedback, amplifying late IKK activity (Werner et al. 2005). These studies have demonstrated that temporal control of NF-κB activity is important for stimulus-specific gene expression programs, yet it remains unclear how gene promoters decipher temporal profiles. If the temporal profile of NF-κB activity conveys information about the stimulus and therefore represents a code (i.e., "temporal code"), it remains unclear how this information is decoded. Also, given that temporal control depends on kinetic rate constants, it is likely that different cells encode stimulus information differently. Further experimental investigation and expansion of the current NF-κB mathematical model to include other cell types will be necessary to decipher how NF-κB dynamics plays a role in mammalian physiology.

IκB Signaling in Response to Developmental Signals

A group of noninflammatory stimuli have been shown to activate NF-κB through the noncanonical NF-κB signaling pathway. These developmental signals of the TNF-receptor super-family such as B-cell activation factor receptor (BAFFR), lymphotoxin β receptor (LTβR), and receptor activator of NF-κB (RANK), have been described to activate NF-κB activity at a low level for hours or days. The noncanonical pathway is not transduced by a canonical NEMO-containing kinase complex, but rather by an IKKα-containing complex, whose activity is also dependent on NIK. In addition to the noncanonical NIK/IKKα-dependent NF-κB activation, these stimuli may also, in certain cellular conditions and contexts, activate the canonical NEMO/IKKβ-dependent NF-κB activation pathway (Pomerantz and Baltimore 2002).

Initial studies of NF-κB activation by these developmental stimuli focused on the generation of the RelB:p52 dimer by cotranslational proteolytic processing of de novo synthesized p100 to p52. The p100 protein contains carboxy-terminal serines whose phosphorylation by IKKα is critical for stimulus-responsive processing. More recently, it was shown that the same pathway also activates latent RelA:p50 NF-κB complexes not through NEMO-dependent degradation of classical IκB, but rather via the degradation of IκBδ activity.

IκBδ activity and the IκBsomes, which mediate it, were first discovered in cells lacking all three classical IκB proteins, IκBα, -β, and -ε (Tergaonkar et al. 2005; Basak et al. 2007). In these cells, the ubiquitous RelA:p50 dimer was shown to be associated with cytoplasmic p100 proteins. Further biochemical analysis showed that all cells contain significant amounts of NF-κB associated with high molecular weight complexes of p100 and p105 that mediate IκBγ and IκBδ activities (Savinova et al. 2009). The functional importance of these complexes was revealed by the discovery that noncanonical signals, through NIK- and IKKα-dependent degradation of IκBδ, release associated RelA:p50

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and RelB:p50 to the nucleus (Basak et al. 2007). Furthermore, conditions or specific stimuli that control the abundance of IκBδ are therefore able to tune the responsiveness of RelA:p50 activation through noncanonical signals. When IκBδ is highly abundant, usually weak developmental signals that engage the noncanonical pathway are able to activate inflammatory genes via strong RelA:p50 activation.

IκB Signaling in Response to Cellular Stresses

Recent work has uncovered homeostatic mechanisms of regulation of NF-κB in resting cells (i.e., in the absence of a stimulus) that predetermine the responsiveness of the NF-κB system to inducers, including stress stimuli.

Homeostatic regulation of IκB synthesis and degradation has emerged as an important characteristic that renders the NF-κB signaling system surprisingly insensitive to a variety of perturbations. First, the differential degradation rates of free and bound IκB allows for compensation between IκBs, evident by studies in IκB knockout cells (O'Dea et al. 2007). The loss of IκBα, for example, causes excess NF-κB to bind and thus stabilize IκBε, in addition to enhancing its synthesis. Second, the very short half-life of free IκB necessitates a high rate of constitutive IκB synthesis to maintain the small cellular free IκB pool (estimated at 15% of total [Rice and Ernst 1993]) that is critical for keeping basal NF-κB activity levels low. One consequence of the very high constitutive IκB synthesis/degradation flux is that the NF-κB system is remarkably resistant to transient alterations in translation rates that are a hallmark of metabolic stress agents. Indeed, UV, UPR (unfolded protein response), or other ribotoxic stress that cause partial inhibition of IκB synthesis rates were found to activate NF-κB only modestly (O'Dea et al. 2008).

Understanding homeostatic control of the IκB-NF-κB system also resolved some seemingly conflicting observations with regards to NF-κB activation by UV. Although UV does not induce IKK activity, basal IKK activity was found to be required (Huang et al. 2002; O'Dea et al. 2008). The systems model revealed how basal IKK activity is critical for slowly degrading NF-κB-bound IκBα, allowing for accumulation of nuclear NF-κB in response to translational inhibition. Whereas stimuli that induce canonical IKK activity cause the degradation of NF-κB-bound IκB proteins, UV irradiation was shown to cause the depletion of the free IκB pool. Indeed, mutations that stabilize free IκB (but allow for IKK-dependent degradation of NF-κB-bound IκB) abolished activation of NF-κB by UV (O'Dea et al. 2008). UV-induced CK2 activity and phosphorylation of the carboxyl terminus of IκBα may further accelerate IκBα degradation but the molecular mechanism of this pathway remain unclear (Kato et al. 2003).

DNA damage, caused by irradiation or chemotherapeutic drugs, however, has been reported to induce IKK activation. Until recently, it was unclear how a nuclear signal could relay back to the cytoplasm. It was found that DNA damage not only initiates the activation of the nuclear kinase ataxia telangiectasia mutated (ATM), the primary regulator of the tumor suppressor and transcription factor p53, but it also initiates the sumoylation of NEMO by the sumo ligase PIASy, promoting its nuclear localization (Mabb et al. 2006). It was known that activated ATM was required for NF-κB activation by DNA damage, and recent work has uncovered the connection between ATM activity and IKK activation. Wu et al. (2006) showed that nuclear sumoylated NEMO associates with and is phosphorylated by the activated ATM, promoting mono-ubiquitination of NEMO that triggers its export to the cytoplasm. The ATM-NEMO complex associates with the protein ELKS, facilitating ATM-dependent activation of the canonical IKK complex, leading to IκBα degradation and NF-κB activation (Wu et al. 2006).

THE NF-κB SIGNALING SYSTEM AS AN INTEGRATOR OF SIGNALS

The NF-κB signaling system mediates the physiological effects of a variety of signals. It does so via a large number of biochemical reactions

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determining the abundance and kinetic regulation of two families of proteins (NF-κB and IκB) and their interactions with each other. Many NF-κB-inducing stimuli control the activity of the two IKK complexes, but others impact many other reactions to alter NF-κB activity, or modulate the responsiveness of the NF-κB signaling system. When stimuli affect different reaction rates within the same signaling system, there is potential for signaling cross talk. As such, the NF-κB signaling system has great potential for integrating diverse signals. In the following section, we summarize just a few of many possible examples.

Integrating Signals that Control IκB Degradation and Synthesis

Ribotoxic Stresses and Inflammatory Signals Cross Talk via IκBα

Whereas ribotoxic stress-inducing stimuli such as UV irradiation or the unfolded protein response (UPR) that act to inhibit the translation of IκBα can rapidly deplete the cellular pool of free, intrinsically unstable IκBα, NF-κB-bound IκBα remains associated with NF-κB until either constitutive or induced canonical IKK-mediated phosphorylation triggers its degradation. The concept of this differential degradation of IκBα, combined with computational simulations suggesting that the level of constitutive IKK activity predetermines the responsiveness of NF-κB activity to translational inhibition, led to the prediction that cells chronically exposed to low levels of inflammatory signals may have significantly enhanced responses to stress stimuli that inhibit protein synthesis (O'Dea et al. 2008).

Indeed, it was found that cells treated with low doses of inflammatory signals that only marginally enhance IKK and NF-κB activities, synergize with UV or ER stress agents that inhibit protein synthesis (via eIF2α phosphorylation) to produce a super-additive increase in NF-κB activity and NF-κB-dependent inflammatory gene expression (O'Dea et al. 2008). The combined effect of the metabolic stress reaction (eIF2α phosphorylation) and IKK activation in response to inflammatory signals or immune receptors may be a more common mechanism to activating NF-κB than previously recognized. A potential function of super-activating NF-κB during metabolic stresses may be to override the concomitant decrease in translation rates for NF-κB controlled survival and immune response genes.

Inflammatory Signals and Developmental Signals Cross Talk via IκBδ

Inflammatory signaling can interact and coordinate responses with developmental cues. For example, lymph node development and homeostasis appears to require not only the developmental regulator lymphotoxin β (LTβ) but also the inflammatory cytokine TNF (Rennert et al. 1998). Conversely, inflammation can derail normal developmental or cellular homeostasis and be a major factor in cancer progression (Karin and Greten 2005). Over the last few years, it has become apparent that the NF-κB signaling system may be a primary integrator of these diverse signals to mediate cross talk in both physiological and pathological processes.

Following the identification of the fourth IκBδ activity that mediates noncanonical signals, its NF-κB-induced expression by canonical signals was explored as a cross talk mechanism (Basak et al. 2007). Inflammatory stimuli drive the expression of p100, which mediates inhibition of RelA:p50 complexes through its IκBδ activity. Because IκBδ is not degraded by canonical NEMO-mediated signals, the cellular pool of RelA:p50 bound to IκBδ increases, thereby amplifying RelA:p50 responsiveness to subsequent developmental signals (which degrade IκBδ) to the extent that developmental stimuli may cause inflammatory gene expression. Combined computational modeling and experimental studies demonstrated the potential for developmental signals such as LTβ to result in inflammatory responses. This cross talk mediated by IκBδ has been speculated to play a role in regulating cancer-associated inflammation (Basak and Hoffmann 2008).

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Integrating Signals that Control NF-κB Synthesis and IκB Degradation

Several observations suggest that there may be synergistic cross talk between canonical and noncanonical IKK pathways via the NF-κB signaling system. RelA-containing dimers are known to increase the synthesis of RelB and p100/p52 polypeptides (www.nf-kb.org). Indeed, basal RelA NF-κB activity was shown to be required for LTβ-responsive RelB:p52 dimer generation (Basak et al. 2008). In B-cells, basal or tonic canonical IKK activity was shown to control the generation of the p100 substrate for noncanonical BAFFR signals (Stadanlick et al. 2008). By the same rationale and molecular mechanism, inflammatory signals such as TNF and LPS may potentially enhance RelB:p52 generation in the presence of developmental stimuli. However, at this time, no compelling physiological or molecular evidence has been presented, and the functional significance of the RelB versus RelA dependent gene expression remains to be elucidated.

CONCLUDING REMARKS

The regulatory logic of the NF-κB signaling system suggests regulation at two levels. The first pertains to the generation of the transcriptional activators, the NF-κB dimers, via monomer expression and dimerization reactions. The second is the regulation of the metabolism of the IκB inhibitors via synthesis and degradation control. Inflammatory signals largely involve the latter, and produce complex dynamic control of NF-κB activity. Homeostatic and cell differentiation associated mechanisms control NF-κB dimer generation. Interestingly, signals engaging the noncanonical IKK complex engage both mechanistic levels; such developmental signals are able to produce inflammatory responses (though generally weakly) and they affect the available NF-κB dimer repertoire. Recognizing the two levels also suggests that interesting, nonadditive or nonlinear signaling may result when the two interface. With multiple interdependencies apparent within the NF-κB signaling system, these cross-regulatory connections must be further characterized mechanistically and quantified to allow for a better understanding of physiological regulation and pathological misregulation of the NF-κB signaling system. Biophysical descriptions at the molecular and cellular level are likely to result in further insights about a signaling system that has tremendous human health relevance.

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