IkBε Is a Key Regulator of B Cell Expansion by Providing Negative Feedback on cRel and RelA in a Stimulus-Specific Manner

Bryce N. Alves, Rachel Tsui, Jonathan Almaden, Maxim N. Shokhirev, Jeremy Davis-Turak, Jessica Fujimoto, Harry Birnbaum, Julia Ponomarenko and Alexander Hoffmann

*J Immunol* 2014; 192:3121-3132; Prepublished online 3 March 2014;
doi: 10.4049/jimmunol.1302351
http://www.jimmunol.org/content/192/7/3121

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/03/01/jimmunol.1302351.DCSupplemental

References
This article cites 48 articles, 28 of which you can access for free at:
http://www.jimmunol.org/content/192/7/3121.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IκBε Is a Key Regulator of B Cell Expansion by Providing Negative Feedback on cRel and RelA in a Stimulus-Specific Manner

Bryce N. Alves,* Rachel Tsui,*,† Jonathan Almaden,* Maxim N. Shokhirev,*,† Jeremy Davis-Turak,*,† Jessica Fujimoto,†,1 Harry Birnbaum,*,† Julia Ponomarenko,†,‡ and Alexander Hoffmann*,†,‡

The transcription factor NF-κB is a regulator of inflammatory and adaptive immune responses, yet only IκBε was shown to limit NF-κB activation and inflammatory responses. We investigated another negative feedback regulator, IκBε, in the regulation of B cell proliferation and survival. Loss of IκBε resulted in increased B cell proliferation and survival in response to both antigenic and innate stimulation. NF-κB activity was elevated during late-phase activation, but the dimer composition was stimulus specific. In response to IgM, cRel dimers were elevated in IκBε-deficient cells, yet in response to LPS, RelA dimers were also elevated. The corresponding dimer-specific sequences were found in the promoters of hyperactivated genes. Using a mathematical model of the NF-κB-signaling system in B cells, we demonstrated that kinetic considerations of IκB kinase–signaling input and IκBε’s interactions with RelA- and cRel-specific dimers could account for this stimulus specificity. cRel is known to be the key regulator of B cell expansion. We found that the RelA-specific phenotype in LPS-stimulated cells was physiologically relevant: unbiased interactions with RelA- and cRel-specific dimers could account for this stimulus specificity. cRel is known to be the key regulator of B cell expansion. We found that the RelA-specific phenotype in LPS-stimulated cells was physiologically relevant: unbiased interactions with RelA- and cRel-specific dimers could account for this stimulus specificity.

*Signaling Systems Laboratory, Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093; †San Diego Center for Systems Biology, University of California, San Diego, La Jolla, CA 92093; ‡San Diego Supercomputer Center, University of California, San Diego, La Jolla, CA 92093; and †Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90025

1Current address: New York Medical College, Valhalla, NY.

Received for publication September 3, 2013. Accepted for publication January 20, 2014.

This work was supported in part by National Institutes of Health Grants R01AI083453 and P50GM (to A.H.), National Institutes of Health/National Cancer Institute Grant T32 CA009523 (to B.N.A.), and the National Science Foundation Graduate Research Fellowship Program (to M.N.S. and R.T.).

Address correspondence and reprint requests to Dr. Alexander Hoffmann, University of California, San Diego, 2400 Urey Hall Addition, 9500 Gilman Drive, MC 0332, La Jolla, CA 92093. E-mail address: ahoffmann@ucsd.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: 7-AAD, 7-aminoactinomycin D; FO, follicular; FPKM, fragments per kb of exon per million fragments mapped; IKK, IκB kinase; MEF, mouse embryonic fibroblast; MZ, marginal zone; pF, progressor fraction; TdR0, time to death; TdR5, time to the first division.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/S16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1302351
of cRel-dependent expression of BAFF receptor and CD40 (25), but how it controls cRel-containing dimers or what other genes may be regulated remain unclear.

In this study, we investigated the role of IxBe in controlling NF-κB activity in B lymphocytes.

Our results indicate that the ablation of IxBe allows for increased proliferation and survival in B cells stimulated with either IgM or LPS. In fact, we found that IxBe had a role in limiting cRel- and RelA-containing dimers, albeit in a stimulus-specific manner, as evidenced by both biochemical data and DNA motif signatures in hyperregulated genes. Mathematical modeling was used to show that a consideration of known kinetic differences between these proteins provides a sufficient explanation. Further, we found that IxBe control of RelA in response to LPS was functionally relevant, because hyperinduction of IL-6 in IxBe-deficient B cells was shown to mediate hyperexpansion.

Materials and Methods
Cell isolation and culture
Spleens were harvested from C57Bl6 wild-type (WT) mice (The Jackson Laboratory, Bar Harbor, ME) and C57Bl6 IxBe−/− mice (2). The collected spleens were homogenized by grinding between frosted glass slides. For B cell isolation, homogenized splenocytes were incubated with anti-CD43 (Ly-48) microbeads for 15 min at room temperature. Following this incubation, cells were washed with HBSS (Life Technologies; cat. no. 14170) containing 1 mM EDTA (Thermo Fisher Scientific; cat. no. 15630) and 1% FCS (Sigma; cat. no. F2442) and separated over a magnetic column (LS column; Miltenyi Biotec; cat. no. 130-042-041). For B cells, purity was determined by flow cytometry using PE anti-B220 (cat. no. 12-0452-83), FITC anti-CD3 (cat. no. 11-0031-82), and PerCP anti-CD8 (cat. no. 46-0081-82; all from BD Biosciences). Gating of live B cells was determined by forward scatter and side scatter properties. Differences in cell proliferation were measured using FlowJo software (TreeStar).

Flow cytometry analysis of cell proliferation and survival
Purified B cells were stained with 5 μM CFSE (Invitrogen; cat. no. C1157) and cultured in complete media with previously mentioned stimuli (see above). The cultures were incubated at various time points and stained with 7-aminoactinomycin D (7-AAD; Invitrogen; cat. no. A1310). B cells were analyzed for proliferation and survival using a C6 Accuri flow cytometer (BD Biosciences). Gating of live B cells was determined by forward scatter and side scatter properties. Differences in cell proliferation were measured using FlowJo software (TreeStar), as well as FloMax (26), which determines maximum-likelihood parameter sets for fractions of responding cells, times to division, and times to death for generations 0 to 10.

EMSA and supershifts
Nuclear extracts were generated from B cells using high salt extraction. In this study, we investigated the role of IxBe in controlling NF-κB binding to a 3122 NEGATIVE FEEDBACK CONTROL BY IκB

Western blot analysis
Whole-cell lysates were prepared using RIPA buffer lysis of B cells. Cytoplasmic extracts and nuclear extracts were prepared as previously described (27, 28). The resulting lysates and extracts were run on either 10% SDS-PAGE gels or 5–14% Criterion Tris-HCl Gel (Bio-Rad). The following Abs were used to identify the protein of interest: p65, IκBα, cRel, and actin (all from Santa Cruz Biotechnology). The resulting proteins were detected using the Bio-Rad ChemiDoc XRS System and SuperSignal West Femto Substrate Maximum Sensitivity Substrate (Thermo Scientific) to detect chemiluminescence released by HRP-labeled secondary Abs.

Cytokine neutralization and receptor blocking
CFSE-labeled B cells isolated from either WT or IxBe−/− B cells were stimulated with either 10 μg/ml IgM or 10 μg/ml LPS in the presence of 2 μg/ml the following Abs: anti-mouse IL-1α/IL-1/IL-1F2 AF-400-NA, anti-mouse IL-1β/IL-1F2 AF-401-NA, anti-mouse IL-6 AF-406-NA, anti-mouse IL-1R AF-480-NA, and anti-mouse IL-6R AF1830 (all from R&D Systems). Cell proliferation was measured using a C6 Accuri flow cytometer (BD Biosciences). Differences in cell proliferation were measured using FlowJo software (TreeStar).

ELISAs
Isolated B cells were plated at a concentration of 2 million cells/ml in the presence of 10 μg/ml IgM or 10 μg/ml LPS. Cells were harvested at 2, 4, 8, 22, 34, and 48 h and spun down, and the supernatant was collected. The resulting supernatant was tested for measured release of IL-1α (Mouse IL-1α ELISA MAX Deluxe), IL-1β (Mouse IL-1β ELISA MAX Deluxe), and IL-6 (Mouse IL-6 ELISA MAX Deluxe; all from BioLegend).

Transcriptome analysis
Total RNA was isolated from IgM- or LPS-stimulated B cells isolated from WT or IxBe−/− B cells over a four-point time course. mRNA was extracted from 2 μg total RNA using oligo(dt) magnetic beads and fragmented at high temperature using divalent cations. Next, a CDNA library was generated using Illumina TruSeq kits, and quantitation was performed using the Roche LightCycler 480. Sequencing was performed on Illumina’s HiSeq 2000, according to the manufacturer’s recommendations, by the Biomedical Genomics Microarray Core facility at the University of California, San Diego. Reads were aligned to the mouse mm10 genome and RefSeq genes (PMID 12045153, PMID 12466850) with Tophat (PMID 19289445). Cufflinks and CummRbund were used to ascertain differential gene expression. Genes were identified as genes that have a 2-fold increase in expression above basal (0 h) in at least one time point. Hyperinduced is defined as genes in IxBe knockout B cells that have a ≥2-fold increase in expression above basal (0 h) in at least one time point. Using the R’s gplots package, heat maps were created for fold change for all genes that made the above induction cut-offs. Additionally, heat maps for latent genes, induced no more than 0.3 log base 2 above their corresponding WT control of RelA in response to LPS was measured using the NF-κB activity in B lymphocytes.

Nuclear extracts were generated from B cells using high salt extraction. In brief, purified B cells were incubated with a low-salt buffer (110 mM HEPES (pH 7.9), Life Technologies), 10 mM KCl [Thermo Fisher Scientific; cat. no. P217], 0.1 mM EGTA [Sigma; E-4378], 0.1 mM EDTA [Thermo Fisher Scientific; cat. no. S312], 1 mM DTT [Thermo Fisher Scientific; BP172-5], 1 μM IκBα (Invitrogen; cat. no. P7626), 5 μg/ml apo-actinomycin D (7-AAD; Invitrogen; cat. no. A1310). B cells were analyzed for proliferation and survival using a C6 Accuri flow cytometer (BD Biosciences). Gating of live B cells was determined by forward scatter and side scatter properties. Differences in cell proliferation were measured using FlowJo software (TreeStar), as well as FloMax (26), which determines maximum-likelihood parameter sets for fractions of responding cells, times to division, and times to death for generations 0 to 10.

EMSA and supershifts
Nuclear extracts were generated from B cells using high salt extraction. In brief, purified B cells were incubated with a low-salt buffer (110 mM HEPES (pH 7.9), Life Technologies), 10 mM KCl [Thermo Fisher Scientific; cat. no. P217], 0.1 mM EGTA [Sigma; E-4378], 0.1 mM EDTA [Thermo Fisher Scientific; cat. no. S312], 1 mM DTT [Thermo Fisher Scientific; BP172-5], 1 μM IκBα (Invitrogen; cat. no. P7626), 5 μg/ml apo-actinomycin D (7-AAD; Invitrogen; cat. no. A1310). B cells were analyzed for proliferation and survival using a C6 Accuri flow cytometer (BD Biosciences). Gating of live B cells was determined by forward scatter and side scatter properties. Differences in cell proliferation were measured using FlowJo software (TreeStar), as well as FloMax (26), which determines maximum-likelihood parameter sets for fractions of responding cells, times to division, and times to death for generations 0 to 10.

EMSA and supershifts
Nuclear extracts were generated from B cells using high salt extraction. In brief, purified B cells were incubated with a low-salt buffer (110 mM HEPES (pH 7.9), Life Technologies), 10 mM KCl [Thermo Fisher Scientific; cat. no. P217], 0.1 mM EGTA [Sigma; E-4378], 0.1 mM EDTA [Thermo Fisher Scientific; cat. no. S312], 1 mM DTT [Thermo Fisher Scientific; BP172-5], 1 μM IκBα (Invitrogen; cat. no. P7626), 5 μg/ml apo-actinomycin D (7-AAD; Invitrogen; cat. no. A1310). B cells were analyzed for proliferation and survival using a C6 Accuri flow cytometer (BD Biosciences). Gating of live B cells was determined by forward scatter and side scatter properties. Differences in cell proliferation were measured using FlowJo software (TreeStar), as well as FloMax (26), which determines maximum-likelihood parameter sets for fractions of responding cells, times to division, and times to death for generations 0 to 10.
motifs. The percentage of genes from the identified gene lists that contained each motif versus the percentage of promoters containing the NF-εB motifs from genes identified as induced were graphed. Significance was determined using R’s stats package to perform a Pearson χ² test using the background counts as the expected values versus the percentage of induced or hyper-induced genes containing the NF-εB motifs.

**Computational modeling of NF-εB dimer activity**

The computational model appends the previously published IκB models with the reactions that govern the generation of RelA- and cRel-containing NF-εB dimers (Supplemental Fig. 3). The model contains 56 species and 146 reactions (Supplemental Model Equations) governed by 74 parameters (Supplemental Table I). Ordinary differential equations were solved numerically using MATLAB version R2013a (The MathWorks) with subroutine ode15s, a variable order, multistep solver. Prior to stimulation, the system was allowed to equilibrate from starting conditions to a steady-state, defined as showing no concentration changes > 1% over a period of 4000 min. Stimulus-induced perturbation from the steady-state was accomplished by direct modulation of IκB activity via a numerical input curve representing IgM or LPS stimulation (adapted from Ref. 31). MATLAB model codes are available upon request.

**Animal use**

The animal protocols for this study were approved by the University of California, San Diego Animal Care and Use Committee.

**Results**

**IkBε deficiency in B cell subsets results in increased stimulus-responsive proliferation and survival**

Because NF-εB controls B cell expansion, we sought to determine IκB regulators that limit NF-εB activity in B cells and, thus, B cell proliferation. Examining whole-cell extracts, we found that, although IκBε protein levels rapidly decrease upon B cell stimulation with IgM or LPS, IκBε protein levels decrease only slightly after stimulation and increase at late time points (Supplemental Fig. 1A), suggesting a role in the postinduction attenuation of NF-εB.

Using B cells magnetically purified from mixed splenocytes collected from WT or IκBε−/− mice, we examined B cell expansion following ex vivo stimulation with 10 μg/ml IgM or 10 μg/ml LPS using CFSE dye dilution. We found that B cells lacking IκBε displayed increased expansion with either stimulus compared with WT B cells (Fig. 1A). Several repeats of the CFSE experiments yielded highly reproducible results (Fig. 1B). To determine whether these differences were the result of a proliferation or survival defect, we used the computational phenotyping tool FloMax (26), which parameterizes a modified cyton model (icyton) to CFSE time courses and yields maximum likelihood nonredundant cellular parameters, such as the percentage of cells entering the proliferative program [progressor fraction (pF)], the time to the first division (Tdiv0), and the time to death (Tdie0) of cells not entering the proliferative program (Tdiv0), and the time to death (Tdie0) of cells not entering the proliferative program (Fig. 1C). Using FloMax, the CFSE data indicate that IκBε−/− B cells are more likely to respond to the stimulus (pF0) than are WT cells under the same conditions (Fig. 1D). In response to IgM, 51.7% of IκBε−/− B cells entered division compared with only 24% of WT B cells. Following LPS stimulation, 64% of the IκBε−/− B cells entered division compared with 52% of their WT counterparts. Because only nonresponding cells are susceptible to death (32–34), and the Tdie0 and Tdiv0 parameters showed little change in the knockout, this suggested that the death rates in IκBε−/− cells would be lower. Testing this prediction with 7-AAD staining of responding cells at 24 h, we indeed found lower percentages of dying cells in IκBε−/− cells than in WT populations (Fig. 1E, 1F; 33.3% versus 54.8% in response to IgM, and 21.9% versus 37.7% in response to LPS).

Examining the B cell subsets in the spleen, we observed a higher percentage of MZ B cells compared with FO B cells in IκBε−/− mice compared with WT controls (Fig. 2A). Because MZ B cell maturation is more sensitive to NF-εB activity than is FO B cell maturation, this observation is consistent with elevated NF-εB activity following IκBε ablation. It also prompted us to question whether the skewed distributions were responsible for the ex vivo expansion phenotype. To address this concern, we purified WT and IκBε−/− MZ and FO B cells away from each other, using allophycocyanin anti-CD9 staining in conjunction with FACS cell sorting (35). Purity was consistently between 90 and 100% (Fig. 2B). Purified FO B cells, whose physiological role is to respond to Ags, showed increased B cell expansion in response to IgM stimulation when derived from IκBε−/− mice. Similarly, MZ B cells, whose physiological role is to monitor for circulating endotoxin, showed increased proliferation when derived from IκBε−/− mice (compared with WT) and stimulated with LPS (Fig. 2C, 2D). These results indicate that the difference in proliferation and survival is a cell-intrinsic B cell phenotype rather than a result of developmental changes that result in differences in the distributions of B cell subpopulations.

**IkBε provides negative feedback on both RelA- and cRel-containing dimers, albeit stimulus specifically**

Given the B cell–proliferation phenotype of IκBε deficiency, we sought to determine how the loss of IκBε affects NF-εB dimer activation. In previous studies, IκBε was shown to bind preferentially to cRel (22, 24), suggesting that IκBε deficiency may preferentially affect cRel-containing dimer activity. We prepared nuclear extracts from WT and IκBε−/− B cells stimulated with IgM or LPS, as previously described, and used them for EMSAs with a κB site-containing probe. NF-εB heterodimers (all containing p50, but containing either RelA, cRel, or RelB; see below) were elevated in IκBε−/− B cells at later time points (18 and 24 h) compared with WT B cells (Fig. 3A), consistent with the high level of induction of this inhibitor seen in immunoblots of WT cells (Supplemental Fig. 1A). Supershift analysis of these B cells’ nuclear extracts using specific Abs for the three activation domain-containing Rel proteins, RelA, cRel, and RelB, was used to quantitate both the supershifted complex observed with one Ab, as well as the remaining nonsupershifted complex when two Abs were used to ablate the activities of their cognate Rel proteins. We found greatly increased levels of cRel:p50 dimer activity in IκBε−/− B cell extracts stimulated with either IgM or LPS (Fig. 3B, 3C). Interestingly, examining RelA activity, we found that RelA nuclear activity increased significantly in LPS-stimulated IκBε−/− B cells compared with WT B cells but not when stimulated with IgM (Fig. 3B, 3C). No difference was seen between the basal levels of RelA and cRel (Supplemental Fig. 1B), suggesting that this effect is induced during stimulation of the B cells. We used immunoblots of nuclear extracts to examine the results further. Following three biological repeats, we found statistically significant differences only for the hyperactivation of cRel in response to LPS, whereas other conditions showed the same trend as the EMSA results but did not achieve statistical significance (Fig. 3D). Together, these biochemical data suggest that IκBε provides a key function in limiting cRel-containing dimer activity via a negative-feedback loop, whereas it is critical for limiting RelA activity in response to some stimuli but not others. A mathematical model of RelA and cRel dynamics suggests a kinetic basis for IκBε’s stimulus-specific functions

The observation that the signaling phenotypes are stimulus specific may suggest that there are underlying stimulus-specific biochemical mechanisms, such as a costimulatory signaling pathway, that are activated by one stimulus but not another. An alternative, more parsimonious explanation is that differential-signaling
FIGURE 1. *IκBε*−/− B cells have increased proliferation and survival in response to both antigenic and inflammatory signals. B cells were isolated and purified from whole splenocytes of WT and *IκBε*−/− cells using negative selection by CD43 magnetic beads. The separated B cells were stained with 1 nM CFSE and stimulated with either 10 μg/ml IgM or 10 μg/ml LPS. At designated time points, B cells were harvested and stained with 5 μg/ml 7-AAD and analyzed for proliferation and death using flow cytometry. (A) B cells from *IκBε*−/− mice displayed increased proliferation in response to both IgM and LPS at each time point. (B) The increased number of proliferating *IκBε*−/− B cells over that of WT B cells was measured using FlowJo software, and the cell numbers were graphed. (C) Diagram depicting the fcyton model. In this model, stimulated cells undergo death over time (Tdie0) or enter division (pF0, fraction entering division; Tdiv0, time to division). (D) CFSE-proliferation profiles of *IκBε*−/− and WT B cells stimulated with either IgM or LPS were analyzed using FlowMax, running the Fcyton model to predict pF0, Tdiv0, and Tdie0 cells. The fraction of responding B cells (pF0) was greatly increased in both IgM-and LPS-stimulated *IκBε*−/− B cells compared with WT B cells. (E) 7-AAD measurements of B cell death show that **Figure legend continues**
kinetics may account for the stimulus-specific phenotypes. Using a mathematical modeling approach, we sought to test the latter hypothesis. To begin, we summarized the known relative interactions between the two potential negative-feedback regulators IkBα and IkBe in terms of their interactions with RelA and cRel-containing dimers, their differential responsiveness to IKK-induced degradation, and the stimulus-specific dynamics of IKK activity (Fig. 4A). Interestingly, we found that, although NF-κB-responsive IkBe gene expression required RelA, NF-κB-responsive IkBe gene expression could be mediated by either RelA or cRel (Supplemental Fig. 2). Next, we constructed a mathematical model with these parameters by adapting a previously established mathematical model (3) to include the cRel dimers and to recapitulate B cell–specific dynamic control of RelA and cRel-containing dimers (Supplemental Mathematical Model Equations). Simulations of this model with the IkM-induced transient IKK activity showed that RelA:p50 dimer is barely affected by IkBe deficiency; however, in response to LPS-induced long-lasting IKK activity, RelA:p50 remains hyperactivated at late time points (Fig. 4B, upper panels). In contrast, cRel:p50 was hyperactivated under both conditions (Fig. 4B, lower panels). By quantitatively time the course at 24 h, the stimulus-agnostic effect on cRel and stimulus-specific effect on RelA are readily appreciated (Fig. 4C); in fact, this graph closely resembles the experimental results obtained biochemically (Fig. 3C).

These simulation results demonstrate that the kinetic argument is a sufficient explanation for the stimulus-specific phenotype seen in IkBe-deficient B cells. We can summarize the kinetic argument as follows: in response to transient IKK signals, IkBe is capable of providing postinduction repression on its high-affinity target RelA:p50 but less effectively on its low-affinity target cRel:p50, which requires IkBe for complete suppression. However, IkBe’s responsiveness to long-lasting IKK signals renders it effectively neutralized; thus, under these conditions, IkBe, which has lower responsiveness, plays an important role for its high-affinity target cRel:p50 and for its low-affinity target RelA:p50. Interestingly, the single specificity of the IkBe negative-feedback loop for RelA:p50 and the dual specificity of the IkBe negative-feedback loop for RelA:p50 and cRel:p50 are reflected in the dimer requirements for IkBe and IkBe inducible expression (Supplemental Fig. 1). We note that, although reported kinetic relationships (Supplemental Table 1) are consistent with this sufficiency argument, we cannot rule out that a stimulus-specific signaling pathway also plays a role in the described phenotype.

**IkBe**-/- B cells show increased expression of NF-κB target genes**

To further characterize the phenotype at the molecular level, we examined how the gene-expression programs induced by IgM or LPS were affected by the loss of IkBe. To this end, we used high-throughput sequencing of polyA RNA isolated from WT and IkBe(-/-) B cells at 0, 2, 8, and 24 h following stimulation with either IgM or LPS. Sequence data were converted into transcriptome levels using CummRbund (36), and these were normalized to the WT 0-h time point. We selected for genes induced in WT B cells by ≥2-fold by IgM and LPS in at least one time point. This resulted in 881 and 846 genes, respectively. We identified hyperinduced genes as those that showed a ≥2-fold change in IkBe(-/-) B cell data at two time points compared with their WT counterparts, resulting in 56 and 106 genes, respectively (Fig. 5A).

To determine whether the identified hyperinduced genes are under the control of NF-κB dimers, we used the HOMER motif discovery software adapted to perform searches of the known NF-κB dimer motifs identified previously (29) and summarized in this article (Fig. 5B, top panel). We found enrichment for these κB motifs within the promoter regions of genes hyperinduced in IkBe(-/-) B cells compared with controls that were not hyperinduced (Fig. 5B). Interestingly, we found that enrichment of the cRel:p50 motif in hyperinduced genes was statistically significant under both IgM and LPS conditions; however, RelA:p50 motif enrichment was statistically significant only when B cells were activated with LPS. These results reflect the biochemical data that showed that, although cRel:p50 is hyperactivated in IkBe(-/-) B cells under both IgM and LPS conditions, RelA:p50 hyperactivation occurs primarily in response to LPS (Fig. 3C).

**Increased expression of IL-6 mediates the enhanced proliferation of IkBe-deficient B cells**

Inflammatory IL-6, which was initially discovered as a B cell–stimulating and differentiation factor, was among the NF-κB target genes that were hyperinduced in LPS-stimulated IkBe(-/-) B cells (Supplemental Table I). Subsequent studies of IL-6’s effects on B cells found that it allows for increased proliferation, enhanced differentiation, and reduced apoptosis (37–40). The literature is unclear about whether IL-6’s expression is cRel or RelA dependent (41–44). In our stimulation conditions, we found that IL-6 is robustly induced in response to LPS in WT cells both at the level of mRNA (Fig. 6A) and secreted cytokine measured in supernatants (Fig. 6B). As expected, IkBe(-/-) B cells show hyperinduction at both early and late times, with the strongest effect at the protein level being at the late time point of 24 h. Interestingly, cRel-deficient B cells showed no mRNA reduction at early time points (2 and 8 h), although a significant deficiency was observed at 24 h. Further, IL-6 hyperinduction appeared to be LPS specific, correlating with RelA hyperactivity and prior observations in other cell types that pointed to RelA-dependent expression (41–43). Together, these data suggest that IL-6 induction is triggered by RelA and then may be enhanced by cRel-containing dimers.

We next asked whether the enhanced proliferation of LPS-stimulated IkBe(-/-) B cells is potentially mediated by increased autocrine IL-6 costimulation of these cells. To test this hypothesis, we isolated B cells from WT or IkBe(-/-) mice and stimulated them with LPS in the presence of either 2 μg/ml cytokine-neutralizing or 2 μg/ml receptor-blocking Abs for IL-6. We used Abs neutralizing IL-1α and IL-1β as controls, because these proproliferative cytokines were not found to be hyperinduced in our transcriptomic profiling. Effects on B cell proliferation were measured using CFSE staining. We found that Abs blocking IL-6 signaling had little effect on B cell expansion from WT mice; however, using B cells from IkBe(-/-) mice, we found a reduction in the B cell expansion to almost WT levels (Fig. 6C). Neither of the control Abs had an effect on the LPS-triggered expansion of WT or IkBe(-/-) B cells. These findings suggest that the increased production of IL-6 is responsible, at least in part, for the increased proliferative capacity of IkBe(-/-) B cells.

**Discussion**

In this study, we identified IkBe as a key negative-feedback regulator of cRel-containing NF-κB dimers in B cells, which limits an increased percentage of B cells in the WT population undergoing apoptotic death compared with IkBe(-/-) B cells. (F) The percentage of 7AAD+ B cells from several experiments was measured using FlowJo software, and the percentages of 7AAD+ cells were graphed. Data in (A), (B), (D), (E), and (F) are representative of at least four independent experiments. **p < 0.01, ***p < 0.005, ****p < 0.001, unpaired t test.
B cell proliferation in response to mitogenic stimulation. In contrast to our understanding based on fibroblast studies, we found that IkBe also plays a nonredundant role in limiting the ubiquitous RelA-containing NF-κB, albeit in a stimulus-specific manner. This result led to two insights: first from a physiological perspective we found that limiting RelA activation is relevant for controlling

![Image of figure 2](image-url)

**Figure 2.** FO and MZ IkBe−/− B cells show increased proliferation. FO and MZ B cell populations were analyzed from whole splenocyte populations by flow cytometry using anti-B220, anti-CD21, and anti-CD23. (A) IkBe−/− B cells were composed of 67% FO B cells and 21.9% MZ B cells, whereas WT B cells were composed of 80.1% FO B cells and 8.75% MZ B cells. (B) MZ and FO B cells were separated by FACS sorting of anti-CD9. Purity of the separated CD9+ and CD9− B cell populations was between 90 and 100%. (C) IkBe−/− FO and MZ B cells showed increased proliferation over WT FO and MZ B cells. (D) The increased number of proliferating IkBe−/− B cells over that of WT B cells was measured using FlowJo software, and the cell numbers were graphed. All data are representative of two independent experiments.
B cell expansion, because neutralizing the expression of the RelA target gene IL-6 mitigated the IkBε-deficient phenotype. Second, considering the NF-κB system as a dynamic one, we conclude that the stimulus-specific functions of IkBε negative feedback are based on kinetics rather than the engagement of a stimulus-specific mechanism or pathway.

Members of the IkB protein family were found to preferentially bind different NF-κB members (22, 23, 45–47). Unlike IkBα, which is known to bind and regulate RelA-containing dimers, IkBε was shown to bind with cRel homodimers and cRel:p50 heterodimers, although RelA-containing dimers may also be bound (22, 23). Interestingly, we found that these binding specificities are also

---

**FIGURE 3.** NF-κB activity is increased in IkBe−/− B cells. Purified B cells were collected and extracted into cytoplasmic and nuclear fractions at various time points. Nuclear extracts were tested for total NF-κB activity using EMSAs. (A) IkBe−/− B cells stimulated with either IgM or LPS exhibited increased NF-κB activity at 18 and 24 h following stimulation. (B) The 24-h nuclear extracts were incubated with Abs directed toward anti-RelA (αRelA), anti-RelB (αRelB), anti-cRel (αcRel), as well as combinations of these Abs (αRelA/αRelB, αRelA/αcRel, and αRelB/αcRel). Following a 20-min incubation, 32P-labeled probe was added and allowed to incubate for an additional 15 min. The resulting samples were run on a 5% nonreducing acrylamide gel. (C) The resulting supershifts were quantitated using ImageJ software and graphed below each shift. Both IgM and LPS stimulation resulted in increases in cRel/p50 activity in IkBe−/− B cell extracts compared with extracts of WT B cells stimulated under the same conditions. LPS-stimulated IkBe−/− B cell extracts had increased RelA activity. (D) Nuclear Western blots for RelA and cRel were run and quantitated to determine whether the increased cRel activity observed in the supershifts was the result of increased RelA and cRel levels in the IkBe−/− B cells. Increased cRel protein levels were observed in the nuclear extracts from IkBe−/− B cells compared with WT B cell extracts. Similar levels of RelA were found in WT and IkBe−/− B cell nuclear extracts. Data shown in (A) are representative of two independent experiments. Data shown in (B), (C), and (D) are representative of three independent experiments (n = 3, error bars represent SD). *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001, unpaired t test.
reflected in the specificity of their NF-κB-responsive expression; although IkBα expression is highly NF-κB inducible in a RelA-dependent manner, NF-κB–inducible expression of IkBe may be mediated by RelA or cRel. Although IkBo is a negative-feedback regulator dedicated to RelA dimers, IkBe can be effective for both cRel and RelA dimers. As a regulator of two NF-κB dimers, IkBe may also mediate cross-regulation between them; the physiological relevance of this remains to be explored.

Although RelA is a critical regulator of the inflammatory response in tissue cells and macrophages, cRel is required for B cell proliferation (16–19). In this study, we revealed that IkBe−/− B cells showed increased expansion in CFSE dye–dilution studies. Using the computational phenotyping tool FloMax revealed that IkBe−/− B cells stimulated with either IgM or LPS showed an increase in the percentage of B cells responding to stimulation (pF0), with little change in the time to division or death parameters. Because responding cells are protected from undergoing apoptosis, we also found an increase in survival by 7-AAD staining. In a previous study (25), IkBe−/− B cells exhibited enhanced survival and enhanced expression of cRel in unstimulated conditions, but the functions of IkBe within the dynamical context of B cell expansion was not investigated. However, IkBe−/− B cells is only elevated after LPS stimulation in IkBe-deficient B cells.

**FIGURE 4.** Computation modeling of IkBe−/− B cells’ NF-κB activity identifies IkBe as the dominant regulator of cRel:p50 dimers. Hypothetical model of IkBo and IkBe control of NF-κB within B cells following IgM or LPS stimulation. (A) IgM and LPS stimulation results in two different IKK-activity profiles; IgM stimulation causes transient IKK activity, whereas LPS results in lasting IKK activity. Activation of IKK results in the degradation of IkBs and can release NF-κB dimers into the nucleus. From previous data, we infer that IkBe has a very strong affinity for cRel:p50, whereas IkBo has a strong affinity for RelA:p50 but not as strong as IkBe-cRel:p50. IkBe has a very low affinity for RelA:p50, whereas IkBo has a weak affinity for cRel:p50, although the affinity between IkBo-cRel:p50 is stronger than IkBe-cRel:p50. (B) Data from our computation model illustrate the NF-κB activity profiles for both WT and IkBe-deficient B cells under IgM and LPS stimulation. (C) The model is able to recapitulate the experimental late time points, in which IkBe-deficient B cells have elevated cRel:p50 after both LPS and IgM stimulation. However, RelA:p50 is only elevated after LPS stimulation in IkBe-deficient B cells.
FIGURE 5. IκBε<sup>−/−</sup> B cells have an enrichment of NF-κB–dependent gene expression. Total RNA gene expression was obtained from RNA sequencing of both WT and IκBε<sup>−/−</sup> B cells at 0 h and after 2, 8, and 24 h of stimulation with IgM or LPS. Hyperinduced genes were identified as those having a 2-fold induction in at least one time point in WT B cells and an additional >2-fold increase in induction at two time points in the IκBε<sup>−/−</sup> B cells. (A) The genes identified as hyperinduced over WT were displayed as heat maps for IgM and LPS stimulation. (B) Motifs for the NF-κB dimers were loaded and used in Homer motif discovery software to search the promoter sequences of the identified induced and hyperinduced genes of IκBε<sup>−/−</sup> samples for occurrences of the listed NF-κB motifs. The resulting genes containing one of these NF-κB motifs were graphed as percentages over the total genes found to be hyperinduced within the IκBε<sup>−/−</sup> samples. *p < 0.05, **p < 0.01.
a known activator of B cell proliferation, as being misregulated in response to both BCR and TLR stimulation. Interestingly, we also identified the RelA:p50 dimer as being misregulated, albeit only in cells responding to LPS, and not BCR, stimulation. NF-κB dimers may be distinguished biochemically and with immunological tools; however, given their somewhat different binding sequence preferences, they also may be distinguished, to some degree, by the binding motifs present in downstream target genes. We used this approach by identifying hyperinduced genes in the IkBε−/− B cells using RNA sequencing and screening their regulatory regions using Homer motif discovery software modified to search for NF-κB dimer motifs identified by Bulyk and colleagues (29).

Intriguingly, the cRel:p50 motif was statistically significantly overrepresented in hyperexpressed genes in response to both stimuli, but the RelA:p50 motif only achieved statistical significance in response to LPS. Thus, two lines of evidence support the
conclusion that NF-kB dimers under the control of IκBε are a function of the initiating stimulation.

Given the established role of cRel in B cell expansion, we examined whether RelA misregulation is functionally relevant. The cytokine IL-6 is one LPS-specific misregulated target gene whose NF-kB binding site conforms to the RelA-dimer motif. Remarkably, both the direct neutralization of IL-6 and the blocking of IL-6R reduced IκBε−/− B cell proliferation to near WT levels, while only slightly affecting WT B cell proliferation. These results suggest that the autocrine stimulation of IL-6 is responsible, at least in part, for the enhanced proliferation of IκBε−/− B cells in response to LPS and that hyperactivation of RelA is indeed functionally relevant. Our investigation of the proliferative phenotype observed in IgM-stimulated IκBε−/− B cells found IL-6 expression to be lacking, yet the IgM-stimulated IκBε−/− B cells still displayed increased proliferation compared with WT B cells. This difference between IgM- and LPS-stimulated IκBε−/− B cells could be explained by the differences in the downstream-stimulation pathways of IgM and LPS. The RNA sequencing data displayed in our study (Fig. 5A) demonstrate that there is very little overlap of the genes upregulated by each pathway. We propose that stimulation with LPS leads to a proliferative phenotype that is dependent on IL-6 upregulation and stimulation, yet, in the case of IgM stimulation, upregulation of a different set of proliferative genes occurs.

How may the same negative-feedback regulator target different signal transducers in response to different stimuli? By adapting an established mathematical model of the NF-kB-cIκB-signaling module to B cells, we showed in this study that a kinetic explanation is sufficient to account for the observations. Specifically, differences in the interaction parameters of IκBκ and IκBε with RelA and cRel dimers, in conjunction with IκBκ’s differential responsiveness to IKK activities, whose temporal profiles are, in turn, stimulus specific, could reproduce the stimulus-specific control of the RelA dimer. Thus, the present study is an extension of previous work that demonstrated that kinetic differences between IκBκ and IκBε could impede them with stimulus-specific functions: although IκBκ is critical for turning off NF-kB activity following transient activation signals, IκBε limits NF-kB activity when the activation signals are long lasting (3). As our understanding of signaling systems improves and mathematical modeling is adopted more widely, we may expect to find an increasing number of examples in which consideration of the kinetics is critical for an understanding of the specificity of observed phenomena.

Disclosures
The authors have no financial conflicts of interest.

References
The authors have no financial conflicts of interest.


Supplementary Figure 1. (A) Whole cell extracts from wild type and IκBε⁻/⁻ B cells stimulated with 10 μg/ml IgM or 10 μg/ml LPS were collected for western blot analysis. Time course of IκBα, IκBβ and IκBε degradation in wild type B cells stimulated with either IgM or LPS was run to establish a baseline of IκB protein expression and degradation. (B) Whole cell extracts of unstimulated IκBε⁻/⁻ and wild type B cells were generated and the levels of RelA, cRel and p50 were tested using western blots.
Supplementary Figure 2. Total cellular RNA was isolated from confluent and serum-starved fibroblasts stimulated with TNF-α using Trizol. Transcript levels were monitored with α-[32P] UTP-labeled probes using a RiboQuant kit (BD Biosciences) according to the manufacturer’s instructions. Data was obtained using a storage phosphor screen (GE Healthcare) and a variable mode Imager (Typhoon 9400; GE Healthcare). NFκB-responsive IkBα gene expression required RelA. NFκB-responsive IkBε gene expression could be mediated by either RelA or cRel, but the loss of both cRel and RelA dramatically hinders IkBε expression.
Supplementary Figure 3. Wiring diagram of the full multi-dimer model containing RelA, p50, and cRel monomers forming indicated RelA:50, cRel:p50, and p50:p50 dimers, and corresponding complexes with \(\text{I}_\kappa\text{B}\alpha\), \(-\beta\), and \(-\epsilon\). Based on our data, this model allows for RelA:p50-responsive induction of \(\text{I}_\kappa\text{B}\alpha\), and RelA:p50 and cRel:p50-responsive induction of \(\text{I}_\kappa\text{B}\epsilon\). The model calculates the activity dynamics of NF\(\kappa\)B dimers RelA:p50 and cRel:p50 in response to experimentally determined IKK activities following IgM and LPS stimulation of B-cells.
Supplemental Mathematical Model Equations

NFκB monomer and IκB transcript reactions

\[ \frac{dRelA}{dt} = k_t - trdeg(\text{RelA}(t)) \]
\[ \frac{dtcRel}{dt} = k_t - trdeg(\text{cRel}(t)) \]
\[ \frac{dtp50}{dt} = k_t - trdeg(p50(t)) \]
\[ \frac{dtIkBa}{dt} = k_t - trdeg(tIkBa(t)) \]
\[ \frac{dtIkBb}{dt} = k_t - trdeg(tIkBb(t)) \]
\[ \frac{dtIkBe}{dt} = k_t - trdeg(tIkBe(t)) \]

\[ kt = \frac{ktc \left( 1 + \sum w \left( \frac{d}{K_d} \right)^3 \right)}{(1 + \sum \left( \frac{d}{K_d} \right)^3)} \]

kte = constitutive basal mRNA synthesis rate
w = inducible multiplier
d = NFκB dimer concentration
Kd = Hill Kd (NFκB induced)

NFκB monomer reactions

Cytoplasmic Reactions
\[ \frac{dRelA}{dt} = tr(tRelA(t)) - ka(\text{RelA}(t))(p50(t)) - ka(\text{RelA}(t))(\text{RelA}(t)) + \]
\[ kd(\text{RelA}:A(t)) + kd(\text{RelA:p50}(t)) - \text{deg(\text{RelA}(t))} \]
\[ \frac{dcRel}{dt} = tr(tcRel(t)) - ka(\text{cRel}(t))(p50(t)) + kd(\text{cRel:p50}(t)) - \text{deg(\text{cRel}(t))} \]

\[ \frac{dp50}{dt} = tr(tp50(t)) - ka(\text{RelA}(t))(p50(t)) - ka(p50(t))(p50(t)) + \]
\[ kd(p50:p50(t)) + kd(\text{RelA:p50}(t)) - \text{deg(p50(t))} \]

Nuclear Reactions
\[ \frac{dRelA}{dt} = tr(tRelA(t)) - ka(\text{RelA}(t))(p50(t)) - ka(\text{RelA}(t))(\text{RelA}(t)) + \]
$$kd(\text{RelA: } A(t)) + kd(\text{RelA: } p50(t)) - \text{deg}(\text{RelA}(t))$$

$$\frac{dcRel}{dt} = \text{tr}(tcRel(t)) - ka(cRel(t))(p50(t)) + kd(cRel: p50(t)) - \text{deg}(cRel(t))$$

$$\frac{dp50}{dt} = \text{tr}(tp50(t)) - ka(\text{RelA}(t))(p50(t)) - ka(p50(t))(p50(t)) +$$

$$kd(p50: p50(t)) + kd(\text{RelA: } p50(t)) - \text{deg}(p50(t))$$

**IkB reactions**

**Cytoplasmic Reactions**

$$\frac{dlkBa}{dt} = \text{tr}(tIkBa(t)) - ikkdeg(IkBa(t))(IKK) - \text{ideg}(IkBa(t)) - \text{imp}(IkBa(t)) +$$

$$\exp(IkBa(t)) - ka(\text{RelA: } A(t))(IkBa(t)) - ka(\text{RelA: } p50(t))(IkBa(t))$$

$$- ka(cRel: p50(t))(IkBa(t)) + kd(IkBa_RelA: A(t) + kd(IkBa_RelA: p50(t))$$

$$+ kd(IkBa_cRel: p50(t)) + \text{ndeg}(IkBa_RelA: A(t)) + \text{ndeg}(IkBa_RelA: p50(t))$$

$$+ \text{ndeg}(IkBa_cRel: p50(t))$$

$$\frac{dlkBb}{dt} = \text{tr}(tIkBb(t)) - ikkdeg(IkBb(t))(IKK) - \text{ideg}(IkBb(t)) - \text{imp}(IkBb(t)) +$$

$$\exp(IkBb(t)) - ka(\text{RelA: } A(t))(IkBb(t)) - ka(\text{RelA: } p50(t))(IkBb(t))$$

$$- ka(cRel: p50(t))(IkBb(t)) + kd(IkBb_RelA: A(t) + kd(IkBb_RelA: p50(t))$$

$$+ kd(IkBb_cRel: p50(t)) + \text{ndeg}(IkBb_RelA: A(t)) + \text{ndeg}(IkBb_RelA: p50(t))$$

$$+ \text{ndeg}(IkBb_cRel: p50(t))$$

**Nuclear Reactions**

$$\frac{dlkBa}{dt} = \text{tr}(tIkBa(t)) - ikkdeg(IkBa(t))(IKK) - \text{ideg}(IkBa(t)) + \text{imp}(IkBa(t)) -$$

$$\exp(IkBa(t)) - ka(\text{RelA: } A(t))(IkBa(t)) - ka(\text{RelA: } p50(t))(IkBa(t))$$

$$- ka(cRel: p50(t))(IkBa(t)) + kd(IkBa_RelA: A(t) + kd(IkBa_RelA: p50(t))$$

$$+ kd(IkBa_cRel: p50(t)) + \text{ndeg}(IkBa_RelA: A(t)) + \text{ndeg}(IkBa_RelA: p50(t))$$

$$+ \text{ndeg}(IkBa_cRel: p50(t))$$

$$\frac{dlkBb}{dt} = \text{tr}(tIkBb(t)) - ikkdeg(IkBb(t))(IKK) - \text{ideg}(IkBb(t)) + \text{imp}(IkBb(t)) -$$
\[ \frac{d\text{RelA}}{dt} = \text{tr(tIkBe(t))} - \text{ikkdeg(IkBb(t))(IKK)} - \text{ideg(IkBb(t))} + \text{imp(IkBb(t))} - \\
\exp(IkBb(t)) - \text{ka(RelA: A(t))(IkBa(t))} - \text{ka(RelA: A(t))(IkBb(t))} \\
- \text{ka(cRel: p50(t))(IkBa(t))} + \text{kd(IkBb_RelA: A(t))} + \text{kd(IkBb_RelA: p50(t))} \\
+ \text{kd(IkBb_cRel: p50(t))} + \text{ndeg(IkBb_RelA: A(t))} + \text{ndeg(IkBb_RelA: p50(t))} \\
+ \text{ndeg(IkBb_cRel: p50(t))} \\
\]

**NFkB dimer reactions**

**Cytoplasmic Reactions**

\[ \frac{d\text{RelA}: A}{dt} = \text{ka(RelA: A(t))(RelA: A(t))} - \text{kd(RelA: A(t))} - \text{imp(RelA: A(t))} + \\
\exp(RelA: A(t)) - \text{deg(RelA: A(t))} - \text{ka(RelA: A(t))(IkBa(t))} - \text{ka(RelA: A(t))(IkBb(t))} \\
- \text{ka(RelA: p50(t))(IkBe(t))} + \text{kd(IkBa_RelA: A(t))} + \text{kd(IkBb_RelA: A(t))} \\
+ \text{bidg(IkBa_RelA: A(t))} + \text{bidg(IkBb_RelA: A(t))} + \text{bidg(IkBb_RelA: A(t))} \\
+ \text{bikdeg(IkBb_RelA: A(t))} + \text{bikkdeg(IkBb_RelA: A(t))(IKK))} \\
+ \text{bikkdeg(IkBb_RelA: p50(t))(IKK))} + \text{bikkdeg(IkBb_RelA: p50(t))(IKK))} \\
\]

\[ \frac{d\text{RelA}: p50}{dt} = \text{ka(RelA: p50(t))(RelA: p50(t))} - \text{kd(RelA: p50(t))} - \text{imp(RelA: p50(t))} + \\
\exp(RelA: p50(t)) - \text{deg(RelA: p50(t))} - \text{ka(RelA: p50(t))(IkBa(t))} \\
- \text{ka(RelA: p50(t))(IkBb(t))} - \text{ka(RelA: p50(t))(IkBe(t))} + \text{kd(IkBa_RelA: p50(t))} \\
+ \text{kd(IkBb_RelA: p50(t))} + \text{kd(IkBb_RelA: p50(t))} + \text{bidg(IkBa_RelA: p50(t))} \\
+ \text{bidg(IkBb_RelA: p50(t))} + \text{bidg(IkBb_RelA: p50(t))} \\
+ \text{bikdeg(IkBa_RelA: p50(t))(IKK))} + \text{bikkdeg(IkBb_RelA: p50(t))(IKK))} \\
+ \text{bikkdeg(IkBb_RelA: p50(t))(IKK))} \\
\]

\[ \frac{dcRel:p50}{dt} = \text{ka(cRel(t))(p50(t)) - kd(cRel:p50(t)) - imp(cRel:p50(t)) + \\
\exp(cRel:p50(t)) - deg(cRel:p50(t)) - ka(cRel:p50(t))(IkBa(t)) \\
- ka(cRel:p50(t))(IkBb(t)) - ka(cRel:p50(t))(IkBe(t)) + kd(IkBa_cRel:p50(t)) \\
+ kd(IkBb_cRel:p50(t)) + kd(IkBb_cRel:p50(t)) + bidg(IkBa_cRel:p50(t)) \\
+ bidg(IkBb_cRel:p50(t)) + bidg(IkBb_cRel:p50(t)) \\
+ bikkdeg(IkBa_cRel:p50(t))(IKK)) + bikkdeg(IkBb_cRel:p50(t))(IKK)) \\
+ bikkdeg(IkBb_cRel:p50(t))(IKK)) \\
\]

\[ \frac{dp50:p50}{dt} = \text{ka(p50(t))(p50(t)) - kd(p50:p50(t)) - imp(p50:p50(t)) +} \]
\[
\exp(p50:\ p50(t)) - \text{deg}(p50:\ p50(t))
\]

**Nuclear Reactions**

\[
\frac{d\text{RelA}:A}{dt} = ka(\text{RelA}(t))(\text{RelA}(t)) - kd(\text{RelA}:A(t)) + \text{imp}(\text{RelA}:A(t)) - \\
\exp(\text{RelA}:A(t)) - \text{deg}(\text{RelA}:A(t)) - ka(\text{RelA}:A(t))(IkBa(t)) - ka(\text{RelA}:A(t))(IkBb(t)) - \\
- ka(\text{RelA}:A(t))(IkBe(t)) + kd(IkBa_{\text{RelA}}:A(t)) + kd(IkBb_{\text{RelA}}:A(t)) + \\
+ kd(IkB_{\text{RelA}}:p50(t)) + kd(IkB_{\text{RelA}}:p50(t)) + bideg(IkBa_{\text{RelA}}:p50(t)) + \\
+ bideg(IkBb_{\text{RelA}}:p50(t)) + bideg(IkB_{\text{RelA}}:p50(t)) + bideg(IkB_{\text{RelA}}:p50(t))(IKK) + \\
+ bideg(IkB_{\text{RelA}}:p50(t))(IKK) + bideg(IkBb_{\text{RelA}}:p50(t))(IKK) + \\
+ bideg(IkB_{\text{RelA}}:p50(t))(IKK) + bideg(IkBb_{\text{RelA}}:p50(t))(IKK) + \\
+ bideg(IkB_{\text{RelA}}:p50(t))(IKK)
\]

\[
\frac{d\text{cRel}:p50}{dt} = ka(\text{cRel}(t))(p50(t)) - kd(\text{cRel}:p50(t)) + \text{imp}(\text{cRel}:p50(t)) - \\
\exp(\text{cRel}:p50(t)) - \text{deg}(\text{cRel}:p50(t)) - ka(\text{cRel}:p50(t))(IkBa(t)) - \\
- ka(\text{cRel}:p50(t))(IkBb(t)) - ka(\text{cRel}:p50(t))(IkBe(t)) + kd(IkBa_cRel:p50(t)) + \\
+ kd(IkBb_cRel:p50(t)) + kd(IkBcRel:p50(t)) + bideg(IkBa_cRel:p50(t)) + \\
+ bideg(IkBb_cRel:p50(t)) + bideg(IkBcRel:p50(t)) + bideg(IkBcRel:p50(t))(IKK) + \\
+ bideg(IkBcRel:p50(t))(IKK) + bideg(IkBcRel:p50(t))(IKK) + \\
+ bideg(IkBcRel:p50(t))(IKK) + bideg(IkBcRel:p50(t))(IKK) + \\
+ bideg(IkBcRel:p50(t))(IKK)
\]

\[
\frac{dp50:p50}{dt} = ka(p50(t))(p50(t)) - kd(p50:p50(t)) + \text{imp}(p50:p50(t)) - \\
\exp(p50:p50(t)) - \text{deg}(p50:p50(t))
\]

**IkB-NFkB reactions**

**Cytoplasmic reactions**

\[
\frac{dIkBa_{\text{RelA}}:A}{dt} = ka(\text{RelA}:A(t))(IkBa(t)) - kd(IkBa_{\text{RelA}}:A(t)) - \\
\text{imp}(IkBa_{\text{RelA}}:A(t)) + \exp(IkBa_{\text{RelA}}:A(t)) - bideg(IkBa_{\text{RelA}}:A(t))(IKK) + \\
- bideg(IkBa_{\text{RelA}}:A(t)) - \text{deg}(IkBa_{\text{RelA}}:A(t))
\]
\[
\frac{dlkBb_{RelA}:A}{dt} = ka(\text{RelA:}A(t))(IkBb(t)) - kd(IkBb_{RelA}:A(t)) - \\
\text{imp}(IkBb_{RelA}:A(t)) + \exp(IkBb_{RelA}:A(t)) - bikkdeg(IkBb_{RelA}:A(t))(IKK) - \\
bideg(IkBb_{RelA}:A(t)) - \deg(IkBb_{RelA}:A(t))
\]

\[
\frac{dlkBe_{RelA}:A}{dt} = ka(\text{RelA:}A(t))(IkBe(t)) - kd(IkBe_{RelA}:A(t)) - \\
\text{imp}(IkBe_{RelA}:A(t)) + \exp(IkBe_{RelA}:A(t)) - bikkdeg(IkBe_{RelA}:A(t))(IKK) - \\
bideg(IkBe_{RelA}:A(t)) - \deg(IkBe_{RelA}:A(t))
\]

\[
\frac{dlkBa_{RelA}:p50}{dt} = ka(\text{RelA:p50}(t))(IkBa(t)) - kd(IkBa_{RelA}:p50(t)) - \\
\text{imp}(IkBa_{RelA}:p50(t)) + \exp(IkBa_{RelA}:p50(t)) - bikkdeg(IkBa_{RelA}:p50(t))(IKK) - \\
bideg(IkBa_{RelA}:p50(t)) - \deg(IkBa_{RelA}:p50(t))
\]

\[
\frac{dlkBb_{RelA}:p50}{dt} = ka(\text{RelA:p50}(t))(IkBb(t)) - kd(IkBb_{RelA}:p50(t)) - \\
\text{imp}(IkBb_{RelA}:p50(t)) + \exp(IkBb_{RelA}:p50(t)) - bikkdeg(IkBb_{RelA}:p50(t))(IKK) - \\
bideg(IkBb_{RelA}:p50(t)) - \deg(IkBb_{RelA}:p50(t))
\]

\[
\frac{dlkBe_{RelA}:p50}{dt} = ka(\text{RelA:p50}(t))(IkBe(t)) - kd(IkBe_{RelA}:p50(t)) - \\
\text{imp}(IkBe_{RelA}:p50(t)) + \exp(IkBe_{RelA}:p50(t)) - bikkdeg(IkBe_{RelA}:p50(t))(IKK) - \\
bideg(IkBe_{RelA}:p50(t)) - \deg(IkBe_{RelA}:p50(t))
\]

\[
\frac{dlkBa_cRel:p50}{dt} = ka(\text{cRel:p50}(t))(IkBa(t)) - kd(IkBa_cRel:p50(t)) - \\
\text{imp}(IkBa_cRel:p50(t)) + \exp(IkBa_cRel:p50(t)) - bikkdeg(IkBa_cRel:p50(t))(IKK) - \\
bideg(IkBa_cRel:p50(t)) - \deg(IkBa_cRel:p50(t))
\]

\[
\frac{dlkBb_cRel:p50}{dt} = ka(\text{cRel:p50}(t))(IkBb(t)) - kd(IkBb_cRel:p50(t)) - \\
\text{imp}(IkBb_cRel:p50(t)) + \exp(IkBb_cRel:p50(t)) - bikkdeg(IkBb_cRel:p50(t))(IKK) - \\
bideg(IkBb_cRel:p50(t)) - \deg(IkBb_cRel:p50(t))
\]

\[
\frac{dlkBc_cRel:p50}{dt} = ka(\text{cRel:p50}(t))(IkBe(t)) - kd(IkBc_cRel:p50(t)) - \\
\text{imp}(IkBc_cRel:p50(t)) + \exp(IkBc_cRel:p50(t)) - bikkdeg(IkBc_cRel:p50(t))(IKK) - \\
bideg(IkBc_cRel:p50(t)) - \deg(IkBc_cRel:p50(t))
\]

Nuclear Reactions
\[
\frac{dlkBa_{RelA}:A}{dt} = ka(\text{RelA:}A(t))(IkBa(t)) - kd(IkBa_{RelA}:A(t)) +
\]
imp(IkBa_RelA: A(t)) – exp(IkBa_RelA: A(t)) – bikkdeg(IkBa_RelA: A(t))(IKK) 
– bideg(IkBa_RelA: A(t)) – deg(IkBa_RelA: A(t))

dlkBb_RelA: A \( \frac{dt}{d} \) = ka(RelA: A(t))(IkBb(t)) – kd(IkBb_RelA: A(t)) +
imp(IkBb_RelA: A(t)) – exp(IkBb_RelA: A(t)) – bikkdeg(IkBb_RelA: A(t))(IKK) 
– bideg(IkBb_RelA: A(t)) – deg(IkBb_RelA: A(t))

dlkBe_RelA: A \( \frac{dt}{d} \) = ka(RelA: A(t))(IkBe(t)) – kd(IkBb_RelA: A(t)) +
imp(IkBb_RelA: A(t)) – exp(IkBb_RelA: A(t)) – bikkdeg(IkBb_RelA: A(t))(IKK) 
– bideg(IkBb_RelA: A(t)) – deg(IkBb_RelA: A(t))

dlkBa_RelA: p50 \( \frac{dt}{d} \) = ka(RelA: p50(t))(IkBa(t)) – kd(IkBa_RelA: p50(t)) +
imp(IkBa_RelA: p50(t)) – exp(IkBa_RelA: p50(t)) – bikkdeg(IkBa_RelA: p50(t))(IKK) 
– bideg(IkBa_RelA: p50(t)) – deg(IkBa_RelA: p50(t))

dlkBb_RelA: p50 \( \frac{dt}{d} \) = ka(RelA: p50(t))(IkBb(t)) – kd(IkBb_RelA: p50(t)) +
imp(IkBb_RelA: p50(t)) – exp(IkBb_RelA: p50(t)) – bikkdeg(IkBb_RelA: p50(t))(IKK) 
– bideg(IkBb_RelA: p50(t)) – deg(IkBb_RelA: p50(t))

dlkBe_RelA: p50 \( \frac{dt}{d} \) = ka(RelA: p50(t))(IkBe(t)) – kd(IkBb_RelA: p50(t)) +
imp(IkBb_RelA: p50(t)) – exp(IkBb_RelA: p50(t)) – bikkdeg(IkBb_RelA: p50(t))(IKK) 
– bideg(IkBb_RelA: p50(t)) – deg(IkBb_RelA: p50(t))

dlkBa_cRel: p50 \( \frac{dt}{d} \) = ka(cRel: p50(t))(IkBa(t)) – kd(IkBa_cRel: p50(t)) +
imp(IkBa_cRel: p50(t)) – exp(IkBa_cRel: p50(t)) – bikkdeg(IkBa_cRel: p50(t))(IKK) 
– bideg(IkBa_cRel: p50(t)) – deg(IkBa_cRel: p50(t))

dlkBb_cRel: p50 \( \frac{dt}{d} \) = ka(cRel: p50(t))(IkBb(t)) – kd(IkBb_cRel: p50(t)) +
imp(IkBb_cRel: p50(t)) – exp(IkBb_cRel: p50(t)) – bikkdeg(IkBb_cRel: p50(t))(IKK) 
– bideg(IkBb_cRel: p50(t)) – deg(IkBb_cRel: p50(t))

dlkBe_cRel: p50 \( \frac{dt}{d} \) = ka(cRel: p50(t))(IkBe(t)) – kd(IkBb_cRel: p50(t)) +
imp(IkBb_cRel: p50(t)) – exp(IkBb_cRel: p50(t)) – bikkdeg(IkBb_cRel: p50(t))(IKK) 
– bideg(IkBb_cRel: p50(t)) – deg(IkBb_cRel: p50(t))
**Supplemental Table 1**

Reactions, parameters and their value, represented in the mathematical model.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Category</th>
<th>Location</th>
<th>Param ID no</th>
<th>Parameter Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>=&gt; tIkBα (basal)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>1</td>
<td>1.5e-5 nM sec⁻¹</td>
<td>Set to fit measured mRNA and protein expression profiles (Werner et al., 2008) and a Hill function where Hill coefficient = 1.1</td>
</tr>
<tr>
<td>=&gt; tIkBβ (basal)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>2</td>
<td>1e-5 nM sec⁻¹</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tIkBε (basal)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>3</td>
<td>1.2e-6 nM sec⁻¹</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tRelA (basal)</td>
<td>NFκB Synth.</td>
<td>Nucleus</td>
<td>4</td>
<td>1.2e-6 nM sec⁻¹</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; cRel (basal)</td>
<td>NFκB Synth.</td>
<td>Nucleus</td>
<td>5</td>
<td>1.2e-6 nM sec⁻¹</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tp50 (basal)</td>
<td>NFκB Synth.</td>
<td>Nucleus</td>
<td>6</td>
<td>1.2e-6 nM sec⁻¹</td>
<td>As in #1</td>
</tr>
</tbody>
</table>

**IkB Reactions**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Category</th>
<th>Location</th>
<th>Param ID no</th>
<th>Parameter Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA =&gt; mRNA + protein</td>
<td>Translation</td>
<td>Nuc -&gt; Cyt</td>
<td>7</td>
<td>0.2 proteins/ mRNA sec⁻¹</td>
<td>Derived from the elongation rate of the ribosome and corrected for the nucleotide spacing between adjacent ribosomes on the same transcript: 30 nt sec⁻¹ / 150 nt = 0.2 sec⁻¹</td>
</tr>
<tr>
<td>=&gt; tIkBα (A50-induced)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>8</td>
<td>100 fold over constitutive</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tIkBβ (A50-induced, 45 min. delay)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>9</td>
<td>1 fold over constitutive</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tIkBε (A50-induced, 45 min. delay)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>10</td>
<td>25 fold over constitutive</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tIkBα (C50-induced)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>11</td>
<td>1 fold over constitutive</td>
<td>As in #1</td>
</tr>
<tr>
<td>=&gt; tIkBβ (C50-induced, 45 min. delay)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>12</td>
<td>1 fold over constitutive</td>
<td>this study (adapted from Tsui et al. in preparation)</td>
</tr>
<tr>
<td>=&gt; tIkBε (C50-induced, 45 min. delay)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>13</td>
<td>125 fold over constitutive</td>
<td>this study (adapted from Tsui et al. in preparation)</td>
</tr>
<tr>
<td>IkBα Hill Kᵦ (NFκB-induced)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>14</td>
<td>150 nM</td>
<td>As in #1</td>
</tr>
<tr>
<td>IkBβ Hill Kᵦ (NFκB-induced)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>15</td>
<td>150 nM</td>
<td>As in #1</td>
</tr>
<tr>
<td>IkBε Hill Kᵦ (NFκB-induced)</td>
<td>IkB Synth.</td>
<td>Nucleus</td>
<td>16</td>
<td>150 nM</td>
<td>As in #1</td>
</tr>
</tbody>
</table>

**Hill Coefficient for inducible txn of tIkB by NFκB**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Category</th>
<th>Location</th>
<th>Param ID no</th>
<th>Parameter Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>IkBα(n) =&gt; IkBα(n)</td>
<td>Transport</td>
<td>Cyt -&gt; Nuc</td>
<td>18</td>
<td>1e-3 sec⁻¹</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>IkBβ(n) =&gt; IkBβ(n)</td>
<td>Transport</td>
<td>Nuc</td>
<td>19</td>
<td>1.5e-4 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td>IkBε(n) =&gt; IkBε(n)</td>
<td>Transport</td>
<td>Cyt -&gt; Nuc</td>
<td>20</td>
<td>7.5e-4 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td>IkBα/β/ε(n) =&gt; IkBα/β/ε(n)</td>
<td>Transport</td>
<td>Nuc -&gt; Cyt</td>
<td>21</td>
<td>2e-4 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td>tIkBα =&gt; IkB Deg.</td>
<td>Cyt, Nuc</td>
<td>22</td>
<td>7.3e-4 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td>tIkBβ =&gt; IkB Deg.</td>
<td>Cyt, Nuc</td>
<td>23</td>
<td>4.8e-5 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td>tIkBε =&gt; IkB Deg.</td>
<td>Cyt, Nuc</td>
<td>24</td>
<td>6.4e-5 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td>Reaction Type</td>
<td>Direction</td>
<td>Location</td>
<td>Concentration</td>
<td>Half-life</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>----------</td>
<td>---------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td><strong>IκBα =&gt; IκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>25 2e-3 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBβ =&gt; IκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>26 2e-3 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBε =&gt; IκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>27 1.925e-4 sec⁻¹</td>
<td>Schuernberg and Hoffmann unpublished results</td>
<td></td>
</tr>
<tr>
<td><strong>IκBα/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>28 4e-6 sec⁻¹</td>
<td>Based on estimated 48 hour half-life</td>
<td></td>
</tr>
<tr>
<td><strong>IκBβ/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>29 2.25e-5 nM sec⁻¹</td>
<td>Based on measured IκB degradation timecourses given numerical input curves</td>
<td></td>
</tr>
<tr>
<td><strong>IκBε/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>30 7.5e-6 nM sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBα/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>31 7.5e-6 nM sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBε/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>32 1.5e-5 nM sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBα/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>33 1.5e-5 nM sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBβ/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>34 4.8e-5 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>IκBε/NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>35 4.8e-5 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>NFκB α/β/ε =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>36 4.8e-5 sec⁻¹</td>
<td>As in #21</td>
<td></td>
</tr>
<tr>
<td><strong>NFκB α/β/ε =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cytosol</td>
<td>37 4.8e-5 sec⁻¹</td>
<td>Based on Shih et al. 2009</td>
<td></td>
</tr>
<tr>
<td><strong>RelA + p50 =&gt; RelA:p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>41 3.16e-5 nM sec⁻¹</td>
<td>Tsui et al. in preparation</td>
<td></td>
</tr>
<tr>
<td><strong>p50 + p50 =&gt; p50:p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>42 3e-5 nM sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>cRel + p50 =&gt; cRel:p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>43 3.16e-5 nM sec⁻¹</td>
<td>As in #47</td>
<td></td>
</tr>
<tr>
<td><strong>RelA + p50 =&gt; RelA:p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>44 3.16e-5 nM sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>p50 + p50 =&gt; p50:p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>45 3e-5 nM sec⁻¹</td>
<td>As in #47</td>
<td></td>
</tr>
<tr>
<td><strong>cRel + p50 =&gt; cRel:p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>46 3.16e-5 nM sec⁻¹</td>
<td>As in #47</td>
<td></td>
</tr>
<tr>
<td><strong>RelA:p50 =&gt; RelA + p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>47 3.16e-5 sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>p50:p50 =&gt; p50 + p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>48 9e-5 sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>cRel:p50 =&gt; cRel + p50</strong></td>
<td>Synthesis</td>
<td>Nucleus</td>
<td>49 3.16e-5 nM⁻¹ sec⁻¹</td>
<td>As in #47</td>
<td></td>
</tr>
<tr>
<td><strong>RelA:p50 =&gt; RelA + p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>50 3.16e-4 sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>p50:p50 =&gt; p50 + p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>51 9e-4 sec⁻¹</td>
<td>As in #47</td>
<td></td>
</tr>
<tr>
<td><strong>cRel:p50 =&gt; cRel + p50</strong></td>
<td>Synthesis</td>
<td>Cytosol</td>
<td>52 3.16e-5 nM⁻¹ sec⁻¹</td>
<td>As in #44</td>
<td></td>
</tr>
<tr>
<td><strong>NFκB (c) =&gt; NFκB (n)</strong></td>
<td>Transport</td>
<td>Cyto -&gt; Nuc</td>
<td>53 9e-2 sec⁻¹</td>
<td>(Shih et al., 2009)</td>
<td></td>
</tr>
<tr>
<td><strong>NFκB (n) =&gt; NFκB (c)</strong></td>
<td>Transport</td>
<td>Nuc -&gt; Cyto</td>
<td>54 8e-5 sec⁻¹</td>
<td>(Shih et al., 2009)</td>
<td></td>
</tr>
<tr>
<td><strong>NFκB =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>55 4e-6 sec⁻¹</td>
<td>Based on estimated 48 hour half-life</td>
<td></td>
</tr>
<tr>
<td><strong>IκBα/β/ε =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>56 2e-6 sec⁻¹</td>
<td>As in #31</td>
<td></td>
</tr>
</tbody>
</table>

**NFκB reactions**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Direction</th>
<th>Location</th>
<th>Concentration</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>tRelA =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Nucleus</td>
<td>35 4.8e-5 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td><strong>tp50 =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Nucleus</td>
<td>36 4.8e-5 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td><strong>tcRel =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Nucleus</td>
<td>37 4.8e-5 sec⁻¹</td>
<td>Based on Shih et al. 2009</td>
</tr>
<tr>
<td><strong>RelA =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>38 3.85e-4 sec⁻¹</td>
<td>Based on estimated 30 min half-life of NFκB monomers</td>
</tr>
<tr>
<td><strong>p50 =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>39 3.85e-4 sec⁻¹</td>
<td>As in #21</td>
</tr>
<tr>
<td><strong>cRel =&gt; NFκB</strong></td>
<td>Degradation</td>
<td>Cyt, Nuc</td>
<td>40 3.85e-4 sec⁻¹</td>
<td>As in #21</td>
</tr>
</tbody>
</table>

**IκBα + RelA => IκBα**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Direction</th>
<th>Location</th>
<th>Concentration</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IκBα + RelA =&gt; IκBα</strong></td>
<td>Interaction</td>
<td>Cyt, Nuc</td>
<td>57 8.01e-5 nM sec⁻¹</td>
<td>As in #44</td>
</tr>
<tr>
<td><strong>IκBβ + RelA =&gt; IκBβ</strong></td>
<td>Interaction</td>
<td>Cyt, Nuc</td>
<td>58 3.55e-6 nM sec⁻¹</td>
<td>As in #44</td>
</tr>
<tr>
<td><strong>IκBε + RelA =&gt; IκBε</strong></td>
<td>Interaction</td>
<td>Cyt, Nuc</td>
<td>59 2.24e-5 nM sec⁻¹</td>
<td>As in #44</td>
</tr>
<tr>
<td>Reaction</td>
<td>Protein State</td>
<td>Cyt, Nuc</td>
<td>Rate Constant</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------</td>
<td>---------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>$\text{IkBa} + \text{cRel:p50} \rightarrow \text{IkBa-NFkB}$</td>
<td>Cyt-Nuc</td>
<td>60</td>
<td>$5.01 \times 10^{-5} \text{M}^{-1} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\beta + \text{cRel:p50} \rightarrow \text{IkB}\beta$</td>
<td>Cyt-Nuc</td>
<td>61</td>
<td>$3.55 \times 10^{-6} \text{M}^{-1} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\epsilon + \text{cRel:p50} \rightarrow \text{IkB}\epsilon$</td>
<td>Cyt-Nuc</td>
<td>62</td>
<td>$2.24 \times 10^{-5} \text{M}^{-1} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkBa-RelA:p50} \rightarrow \text{IkBa} + \text{RelA:p50}$</td>
<td>Cyt-Nuc</td>
<td>63</td>
<td>$1.0 \times 10^{-5} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\beta-\text{RelA:p50} \rightarrow \text{IkB}\beta$</td>
<td>Cyt-Nuc</td>
<td>64</td>
<td>$2.82 \times 10^{-4} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\epsilon-\text{RelA:p50} \rightarrow \text{IkB}\epsilon$</td>
<td>Cyt-Nuc</td>
<td>65</td>
<td>$1.0 \times 10^{-4} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\alpha-\text{cRel:p50} \rightarrow \text{IkB}\alpha$</td>
<td>Cyt-Nuc</td>
<td>66</td>
<td>$8.0 \times 10^{-5} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\alpha-\text{NFkB(c)} \rightarrow \text{IkB}\alpha-\text{NFkB(n)}$</td>
<td>Transport</td>
<td>67</td>
<td>$2.82 \times 10^{-4} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\beta-\text{NFkB(c)} \rightarrow \text{IkB}\beta-\text{NFkB(n)}$</td>
<td>Transport</td>
<td>68</td>
<td>$4.47 \times 10^{-8} \text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\text{IkB}\epsilon-\text{NFkB(c)} \rightarrow \text{IkB}\epsilon-\text{NFkB(n)}$</td>
<td>Transport</td>
<td>69</td>
<td>$4.60 \times 10^{-3} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>$\text{IkB}\alpha-\text{NFkB(n)} \rightarrow \text{IkB}\alpha-\text{NFkB(c)}$</td>
<td>Transport</td>
<td>70</td>
<td>$4.60 \times 10^{-3} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>$\text{IkB}\beta-\text{NFkB(n)} \rightarrow \text{IkB}\beta-\text{NFkB(c)}$</td>
<td>Transport</td>
<td>71</td>
<td>$2.30 \times 10^{-3} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>$\text{IkB}\epsilon-\text{NFkB(n)} \rightarrow \text{IkB}\epsilon-\text{NFkB(c)}$</td>
<td>Transport</td>
<td>72</td>
<td>$1.40 \times 10^{-2} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>$\text{IkB}\beta-\text{NFkB(n)} \rightarrow \text{IkB}\beta-\text{NFkB(c)}$</td>
<td>Transport</td>
<td>73</td>
<td>$7.0 \times 10^{-3} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
<tr>
<td>$\text{IkB}\epsilon-\text{NFkB(n)} \rightarrow \text{IkB}\epsilon-\text{NFkB(c)}$</td>
<td>Transport</td>
<td>74</td>
<td>$7.0 \times 10^{-3} \text{sec}^{-1}$</td>
<td>(Shih et al., 2009)</td>
</tr>
</tbody>
</table>