Interactions between NF-κB and SP3 Connect Inflammatory Signaling with Reduced FGF-10 Expression*

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Background: Inflammation and NF-κB activation inhibit FGF-10 expression and lung development by an unknown mechanism.

Results: In the presence of NF-κB activation, SP3 functions as a transcriptional repressor, inhibiting SP1-mediated FGF-10 transcription.

Conclusion: NF-κB can regulate developmental gene expression by recruiting inhibitory transcription factors to gene promoters.

Significance: Interaction between NF-κB and Sp proteins identifies a mechanism by which inflammation inhibits normal developmental programs.

Inflammation inhibits normal lung morphogenesis in preterm infants. Soluble inflammatory mediators present in the lungs of patients developing bronchopulmonary dysplasia disrupt expression of multiple genes critical for development. However, the mechanisms linking innate immune signaling and developmental programs are not clear. NF-κB activation inhibits expression of the critical morphogen FGF-10. Here, we show that interactions between the RELA subunit of NF-κB and SP3 suppress SP1-mediated FGF-10 expression. SP3 co-expression reduced SP1-mediated Fgf-10 promoter activity, suggesting antagonistic interactions between SP1 and SP3. Chromatin immunoprecipitation of LPS-treated primary mouse fetal lung mesenchymal cells detected increased interactions between SP3, RELA, and the Fgf-10 promoter. Expression of a constitutively active IκB kinase β mutant not only decreased Fgf-10 promoter activity but also increased RELA-SP3 nuclear interactions. Expression of a dominant-negative IκB, which blocks NF-κB nuclear translocation, prevented inhibition of FGF-10 by SP3. The inhibitory functions of SP3 required sequences located in the N-terminal region of the protein. These data suggested that inhibition of FGF-10 by inflammatory signaling involves the NF-κB-dependent interactions between RELA, SP3, and the Fgf-10 promoter. NF-κB activation may therefore lead to reduced gene expression by recruiting inhibitory factors to specific gene promoters following exposure to inflammatory stimuli.

During fetal lung development, spatially restricted expression of mesenchymal growth factors stimulates cell proliferation, elongation of epithelial tubes, and expansion of newly formed airways (1). FGF-10 is expressed in the lung mesenchyme from the earliest stages of development and is critical for lung formation. By activating its receptor FGFR2b on adjacent epithelial cells, FGF-10 stimulates both proliferation and airway branching (2, 3). Mice lacking either FGF-10 or FGFR2b develop only rudimentary lung structures containing tracheas but lacking bronchial airways (2, 4). During later stages of lung development, transgenic expression of an FGF-10 antagonist or addition of inhibitory antibodies disrupts normal airway elongation and branching (5–7). Although many genes play important roles in lung development, data clearly implicate FGF-10 as a major regulator of lung morphogenesis.

Defects in Fgf-10 expression contribute to lung disease in both children and adults. Preterm infants with severe bronchopulmonary dysplasia have reduced saccular airway and alveolar duct formation and have lower FGF-10 expression in their lungs (8, 9). In adult patients, Fgf-10 haploinsufficiency can lead to abnormal pulmonary function and chronic obstructive pulmonary disease (10). In both chronic obstructive pulmonary disease and bronchopulmonary dysplasia, lung inflammation plays a key role in disease pathogenesis and also inhibits FGF-10 expression (11–13). Inflammatory signaling may therefore interfere with the mechanisms regulating FGF-10 expression and formation and maintenance of normal lung architecture.

Microbial products and inflammatory mediators stimulate lung inflammation by binding pattern recognition receptors on the surface of cells (14, 15). Although the expression of multiple receptors provides a diverse detection repertoire at the cell surface, many of these receptors signal through overlapping intracellular pathways that activate the transcription factor NF-κB (10, 16). In quiescent cells, NF-κB resides in the cytoplasm bound to IκB. When cell surface receptors detect inflammatory
Sp3 and NF-κB Suppress FGF-10

stimuli, IκB kinase β (IKKβ)2 phosphorylates IκB, displacing it from NF-κB and leading to IκB degradation (17, 18). NF-κB is then free to traffic into the nucleus, where it regulates gene transcription. In addition to driving the acute innate immune response, the NF-κB signaling pathway also influences wound repair, tumor formation, and tissue morphogenesis (19–23).

Because inflammation plays a major role in the pathogenesis of bronchopulmonary dysplasia, we previously investigated how innate immunity and NF-κB activation in the fetal lung affects expression of genes important for normal development. NF-κB activation by microbial products or inflammatory mediators inhibits FGF-10 expression in the fetal mouse lung, leading to alterations in normal airway formation (13).

NF-κB most commonly acts as a transcriptional activator; the mechanisms by which NF-κB can reduce gene transcription are less well characterized. Inhibition of FGF-10 does not involve direct interaction between NF-κB and canonical DNA binding elements in the Fgf-10 promoter (24). We hypothesized that NF-κB might therefore inhibit FGF-10 expression by regulating the activity of other transcription factors.

The Fgf-10 promoter lacks a TATA box sequence but contains multiple conserved GC-rich regions predicted to bind Sp proteins. SP1 is a potent activator of TATA-less gene transcription. The related Sp family member SP3 can act as both a transcriptional activator and repressor, depending on cellular context (25). We previously showed that SP1 drives FGF-10 transcription and that this effect can be inhibited by NF-κB activation (8). However, it was not clear how NF-κB might inhibit SP1-mediated transcription in the absence of conserved predicted NF-κB binding sites. Here, we show that NF-κB activation recruits Sp3 to the Fgf-10 promoter, where it functions as a transcriptional inhibitor. This novel mechanism may provide new insight into how inflammation can alter expression of developmentally important genes.

EXPERIMENTAL PROCEDURES

Reagents—Gel-purified Escherichia coli LPS (O55:B5) was purchased from Sigma-Aldrich. Recombinant IL-1β was purchased from R&D Systems. The IKKβ inhibitor BMS-345541 was purchased from EMD Biosciences (San Diego, CA). Antibodies against RELA, SP1, and SP3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-FLAG was purchased from Sigma. Rabbit anti-GFP was purchased from Millipore (Bedford, MA). Rat anti-E-cadherin and Alexa Fluor-conjugated secondary antibodies were procured from Invitrogen. Alexa Fluor-conjugated antibodies against RELA, SP1, and SP3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). This kit was generously supplied by Benoit Bruneau (Gladstone Institute of Cardiovascular Disease, San Francisco, CA). This apoptosis assay was used as a positive control. Wells without nuclear extract were used as negative controls.

DNA-based ELISA—Quantitative protein assay for SP1 and SP3 was performed using TransAm transcription factor ELISA (Active Motif, Carlsbad, CA). Control and LPS-treated 3T3 cells were grown in culture to 90% confluency, harvested, and lysed by hypotonic buffer and 0.5% Nonidet P-40. Nuclei were pelleted, and equal amounts of nuclear proteins were applied to wells coated with oligonucleotide containing consensus binding sequences for SP1 or SP3. After incubation with primary antibodies that recognize SP1 and SP3 only in their DNA-bound states, an HRP-conjugated secondary antibody provided quantitative detection of SP1 and SP3 by spectrophotometry. 5 μg of nuclear extract from MCF-7 cells, supplied by the manufacturer, was used as a positive control. Wells without nuclear extract were used as negative controls.

In Situ Proximity Ligation Assay—CHO cells were transfected with plasmids expressing FLAG-tagged RELA and constitutively active IKKβ mutant (cIKKβ), dnIκB, or empty vector. Cells were fixed 24 h after transfected in 4% paraformaldehyde before being permeabilized with 0.1% Triton X-100 (Pierce) and blocked with normal donkey serum (Sigma). Interactions between FLAG-RELA and SP3 were measured using the Red Duolink II in situ proximity ligation assay (PLA) kit (OLink Bioscience, Uppsala, Sweden). Cells were incubated with anti-FLAG and anti-SP3 primary antibodies overnight. After wash-
ing with PBS, the cells were incubated with PLA anti-mouse MINUS and PLA anti-rabbit PLUS for 1 h at 37 °C in a humidified chamber. After washing, the ligation, polymerization, and hybridization steps of the Duolink II protocol were carried out as instructed by the manufacturer. Slides were mounted in medium containing DAPI. Control cells were not incubated with the anti-FLAG antibody. Mounted cells were imaged using an inverted Olympus BX-81 fluorescence microscope and analyzed using SlideBook software.

RESULTS

SP3 Is Expressed throughout Development in the Fetal Lung—We hypothesized that NF-κB inhibits FGF-10 transcription by interfering with other transcriptional activators. Because SP1 interacts with GC-rich sequences, such as those found in the Fgf-10 promoter (Fig. 1C) and stimulates Fgf-10 promoter activity, we first tested whether SP1 and SP3 might both regulate FGF-10. SP3 binds similar GC-rich regions as SP1 but can function as a transcriptional inhibitor. Similar to SP1, SP3 was expressed throughout fetal mouse lung development in both epithelial and mesenchymal cell populations (Fig. 1A). Expression appeared heterogenous, with higher levels of nuclear staining in some individual cells compared with others. Immunoblotting confirmed expression in the fetal mouse lung with the four previously identified SP3 isoforms present in fetal lung homogenate and in primary fetal lung mesenchymal cell lysates (Fig. 1B).

SP3 Decreases SP1-mediated Fgf-10 Promoter Activity—To test whether SP3 could inhibit Fgf-10 transcription, we employed an FGF-10 luciferase reporter plasmid containing a 6-kb region of the mouse Fgf10 gene that included the transcriptional start site and 5′-UTR. In transfected CHO cells, expression of increasing amounts of mouse Sp3 cDNA did not change basal FGF-10 luciferase activity (Fig. 2A). As we demonstrated previously, Sp1 increased FGF-10 luciferase expression, but co-expression with increasing Sp3 inhibited this activation in a concentration-dependent manner, returning reporter expression to basal levels (Fig. 2A). These data suggested that SP1 and SP3 have opposing functions on Fgf-10 transcription.

NF-κB Activation Recruits SP3 to the Fgf-10 Promoter—Because LPS and inflammatory mediators that signal through NF-κB reduce FGF-10 expression, we tested whether NF-κB activation might increase the amount of SP3 present at the Fgf-10 promoter. We treated primary fetal lung mesenchymal cells with LPS and performed ChIP analysis using antibodies against SP1, SP3, and the NF-κB subunit RELA (Fig. 2B). In control cells, we detected SP1-FGF-10 interactions; faint bands were also detected in samples precipitated with antibodies against SP3 and RELA. However, in LPS-treated cells, interactions between all three proteins and the Fgf-10 promoter region were strongly detected. We obtained similar results using a molecular approach to increase NF-κB activation. Expressing a cIKKβ in which serines 177/181 are replaced with negatively charged glutamate residues (27) increased SP3-FGF-10 interactions in CHO cells (Fig. 2C), further supporting the possible connection between SP3 and NF-κB in regulating the Fgf-10

FIGURE 1. SP1 and SP3 expression in the fetal mouse lung. A, immunostaining of lung sections from embryonal day 13 (E13) through postnatal day 1 (P1) mice using antibodies against SP1 (top panels) or SP3 (bottom panels). Airway epithelial cells were immunostained using an antibody against E-cadherin (E-Cad), and nuclei were labeled with DAPI. B, immunoblot of total fetal mouse lung homogenate using antibodies against SP1 (left) and SP3 (right). C, sequence comparison of murine and human Fgf-10 promoter regions, showing conserved GC-rich regions upstream of the transcriptional start site.
We next tested the functional effects of both SP3 and increased IKKβ activity. SP3 and cIκKβ separately inhibited SP1-mediated Fgf-10 promoter activity and co-expressing SP3 and cIκKβ appeared to have an additive inhibitory effect (Fig. 2C). Collectively, the ChIP and co-expression data suggested that SP3 might play a role in NF-κB-dependent Fgf-10 inhibition.

**SP1 and SP3 Levels Are Not Altered by NF-κB Activation**—LPS and NF-κB activation could reduce Fgf-10 transcription by changing the relative levels of SP1 and SP3. As seen in Fig. 3, activation of NF-κB by treating fetal lung mesenchyme with LPS (Fig. 3A) or CHO cells with recombinant IL-1β (Fig. 3B) did not change the amount of SP3 in nuclear extracts based on immunoblotting. Importantly, we also did not observe any differences in the relative abundance of each of the four naturally occurring SP3 isoforms. Treating CHO cells with recombinant IL-1β also did not cause changes in SP1 or SP3 protein concentration in nuclear extracts as measured by DNA-based ELISA (Fig. 3C). We next tested this hypothesis by immunostaining control and LPS-treated lung explants from embryonic day 15 fetal mice. In Fig. 3D, SP1 and SP3 expression appear similar in control and LPS-treated explants. Quantification of nuclear fluorescence intensity in cells located in the mesenchyme showed that LPS caused small changes in both SP1 and SP3 promoter. We next tested the functional effects of both SP3 and increased IKKβ activity. SP3 and cIκKβ separately inhibited SP1-mediated Fgf-10 promoter activity and co-expressing SP3 and cIκKβ appeared to have an additive inhibitory effect (Fig. 2C). Collectively, the ChIP and co-expression data suggested that SP3 might play a role in NF-κB-dependent Fgf-10 inhibition.

**FIGURE 2.** SP1 and SP3 antagonistically regulate Fgf-10 promoter activity. A, CHO cells were transfected with FGF-10 luciferase and increasing concentrations of Sp3 cDNA in the absence or presence of Sp1 cDNA. SP1 expression increased FGF-10 luciferase activity, and this effect was inhibited by SP3. #, p < 0.01 compared with control; *, p < 0.01 compared with SP1 only (n = 6). B, ChIP analysis of the Fgf-10 promoter in primary fetal mouse lung mesenchymal cells. Cells were cultured in the absence or presence of LPS (250 ng/ml) for 4 h. DNA-protein complexes were immunoprecipitated with antibodies against RELA, SP1, SP3, or rabbit IgG. The immunoprecipitated DNA was amplified by PCR using primers flanking 350 bp of the Fgf-10 promoter immediately upstream of the transcriptional start site. C, chromatin immunoprecipitation from CHO cells expressing either cIκKβ or GFP control plasmid. Anti-SP3 antibodies used as in B. Input DNA are included on the right. D, expression of either SP3 or cIκKβ (black bars) inhibited SP1-activated FGF-10 luciferase activity. Co-expression of both SP3 and cIκKβ (gray bar) had an additve inhibitory effect. #, p < 0.01 compared with control (n = 4).

**FIGURE 3.** NF-κB inhibition increases SP3 expression. A and B, representative SP3 immunoblot from control (ctrl) and LPS-treated mouse fetal lung mesenchymal cells (A) and control and IL-1β-treated CHO cells (B), showing no differences in SP3 expression level or relative isoform abundance. C, the protein levels of SP1 (left) and SP3 (right) in control and IL-1β-treated CHO cell nuclei were measured using DNA-binding ELISA. D–F, control and LPS-treated embryonic day 15 fetal mouse lung explants were immunolabeled with antibodies against SP1 and SP3. Cell nuclei within the mesenchyme were identified by confocal microscopy. Sum fluorescence intensity in ~700 cell nuclei was quantified in control and LPS-treated samples. LPS increased both SP1 and SP3 expression similarly (E; *, p < 0.01) and had no net effect on the SP1/SP3 ratio. epi, epithelial cells. G, NF-κB activation did not alter SP3 subcellular localization. Primary fetal mouse lung mesenchymal cells were immunolabeled with antibodies against endogenous SP3 following treatment with the IKKβ inhibitor BMS345541 or LPS. Cells were counterstained with phalloidin (red) and DAPI (blue). rel. u., relative units.
We next tested the effect of expressing a dominant-negative mutant of FGF-10, we co-transfected Sp3 and dnIκB into CHO cells with an FGF-10 luciferase reporter. When NF-κB activation was inhibited with dnIkB expression, Sp3 did not decrease Fgf-10 promoter activity but instead activated FGF-10 expression (Fig. 5F). These data suggest that nuclear import of activated RELA recruits Sp3 to the FGF-10 promoter where it then inhibits FGF-10.

The N-terminal Region of Sp3 Is Required for FGF-10 Inhibition—We next determined whether each of the four translation-dependent isoforms of Sp3 were equally able to inhibit FGF-10 expression. The ATG start sites in Sp3 were mutated so that each plasmid expressed only a single Sp3 isoform. Each isoform had similar nuclear localization and was expressed at the appropriate molecular weight (Fig. 6, A and B). Although expression of the longest isoform reduced Fgf-10 promoter activity, the shortest Sp3 isoform, which lacks the N-terminal region, had no effect even at the highest cDNA concentration (Fig. 6C). We next tested two additional N-terminal Sp3 mutants. Sp3 lacking the N-terminal 149 amino acids (Sp32.5) and Sp3 lacking the conserved Sp region located between amino acids 44–90 (Sp3KSPBox) localized to the cell nucleus. However, both Sp32.5 and Sp3KSPBox had reduced ability to inhibit Sp1-mediated Fgf-10 promoter activity (Fig. 6, D and E). These data suggest that the inhibitory function of Sp3 requires structural elements located at the N terminus of the protein.

FIGURE 4. Mutation of Sp3 SUMOylation sites does not alter ability of Sp3 to inhibit Fgf-10 promoter activity. CHO cells were transfected with Sp3 mutants containing single amino acid mutations (K120R and K551R) or mutation of both SUMOylation sites (KKRR). A, Sp3-GFP and Sp3K000-GFP localized to the cell nucleus when expressed in CHO cells. B, immunoblot of transfected CHO cells showed that each Sp3 SUMOylation mutant was expressed in CHO cells at the predicted molecular weight. C, mutation of SUMOylated lysine residues in Sp3 did not affect the ability of Sp3 to inhibit Sp1-mediated Fgf-10 promoter activity (n = 8).

DISCUSSION

Our data show that NF-κB activation decreases FGF-10 expression by altering Sp protein-mediated transcription. We determined that NF-κB activation increases interactions between the transcription factor Sp3 and the Fgf-10 promoter, decreasing FGF-10 expression. Sp3 is incapable of down-regulating FGF-10 without NF-κB nuclear translocation, and the N terminus of the Sp3 protein is required for this effect. These findings identify an important molecular mechanism regulating FGF-10 expression.

In extremely preterm infants that develop bronchopulmonary dysplasia, inflammation disrupts the normal developmental programs that control lung morphogenesis. Although the connections between inflammatory signals and altered development have long been suggested, the mechanisms linking immunity and development have been less clear. Within the developing lung mesenchyme, microbial products and cytokines activate NF-κB and inhibit FGF-10 expression and prevent normal epithelial-mesenchymal interactions during airway formation (8, 13). Our results here reveal a novel mechanism linking inflammation-mediated NF-κB activation and abnormal transcriptional regulation of Fgf-10.

The NF-κB subunit RELA interacts with Sp3 to inhibit FGF-10 transcription. This inhibitory function could occur in one of several ways. RELA could act as a molecular chaperone within the nuclear microenvironment, recruiting Sp3 to the Fgf-10 locus. In cells expressing FGF-10 in the absence of inflammatory stimuli, RELA is mostly cytoplasmic. Interaction of Sp3 with the Fgf-10 promoter is minimal and FGF-10 expression is high due to the stimulatory function of Sp1. However, when cells are exposed to inflammatory signals, the nuclear transport of RELA increases Sp3 levels at the Fgf-10 promoter, inhibiting Sp1-mediated transcription. As part of this model, RELA–Sp3 complexes could specifically interact with the Fgf-10 promoter (as well as other Sp-regulated genes also inhibited by NF-κB).
Alternatively, SP3-RELA interactions could cause functional switching. SP3 appeared to activate Fgf-10 transcription when NF-κB was inhibited. Therefore, interacting with RELA may turn SP3 into a transcriptional repressor. Additionally, NF-κB activation and binding of RELA to the Fgf-10 gene could change the structure or modification of the Fgf-10 promoter-enhancer region, leading to increased SP3 binding and perhaps preventing SP1 from promoting transcription. These types of interactions may help explain how transcription factors such as NF-κB can function as both activators and repressors given different genetic contexts.

Examples of transcription factors functioning both as activators and repressors are found throughout molecular biology (29). The retinoic acid receptor family of retinoid x receptor/retinoic acid receptor complexes normally functions as co-repressors, inhibiting transcription. In the presence of ligand, additional co-activators are recruited and promote gene expression. In this example, the retinoid x receptor/retinoic acid receptor acts somewhat as a molecular scaffold, allowing ligand-dependent recruitment of additional factors (30, 31). For genes regulated by Wnt and β-catenin signaling, activated β-catenin can switch T cell factor family transcription factors from repressors to activators (32, 33). β-Catenin also promotes exchange of repressor T cell factor proteins for activating family members (34). This exchange of activating and inhibiting transcription factors has similarities to the one we propose here. Activated RELA increases the localization of the repressor SP3 at the Fgf-10 promoter that is occupied primarily by SP1 in the absence of inflammatory stimuli. As many genes contain GC boxes in their promoter regions, transcriptional repression by RELA-SP3 complexes could target more genes than just FGF-10.

The inhibitory function of SP3 requires the N-terminal peptide region of the protein. Expression of SP3 isoforms lacking the amino-terminal 302 amino acids did not inhibit Fgf-10 promoter activity, and a smaller truncation of the 149 N-terminal region resulted in an intermediate inhibitory ability of SP3. Within the SP3 N-terminal domain, deletion of the conserved Sp box reduced but did not completely abolish inhibitory function. The differential function of the various SP3 isoforms is particularly interesting. Expressed from a single, full-length mRNA, internal ATG start sites produce peptides of 60, 62, 100, and 102 kDa (35). As each isoform appears to be expressed in all cell types examined to date, the differential roles of short and long SP3 peptides are unknown (36, 37). As Sp box domains are described in all Sp family members, the potentially unique properties of the SP3 Sp box are not clear (38). Future experiments will better define this region and how it might regulate interactions between SP3 and other transcription factors, including RELA and SP1.

NF-κB can activate and inhibit gene expression by distinct mechanisms. For activation of innate immunity genes, NF-κB directly binds to the gene promoter via a consensus NF-κB binding sequence (39). In addition, members of the NF-κB family possess a transactivating domain that can recruit additional transcription factors to discrete regulatory sites (40). NF-κB
mutations were expressed in CHO cells. SP3LONG and SP3SHORT correspond to
the longest and shortest SP3 isoforms that are expressed from the full-length
mRNA. SP32.5 lacks the 149 N-terminal amino acids. SP3
activates when expressed in CHO cells.

NF-κB signaling therefore regulates developmental processes in addi-
tion to the innate immune response. By interacting with other
developmental transcription factors, NF-κB provides an addi-
tional level of expression control. These molecular mechanisms
may provide unique insight into the connections between
inflammation, immunity, and the pathogenesis of developmen-
tal diseases.

FIGURE 6. The N terminus of SP3 is required for inhibition of SP1-mediated
Fgf-10 expression. A, SP3-GFP fusion proteins containing N-terminal
mutations were expressed in CHO cells. SP3LONG and SP3SHORT correspond to
the longest and shortest SP3 isoforms that are expressed from the full-length
mRNA. SP32.5 lacks the 149 N-terminal amino acids. SP3LONG lacks the SP
box domain located between amino acids 44–90. Immunoblotting for GFP
confirms that each mutant SP3 construct was expressed at the expected
molecular weight. B, SP3-GFP mutant isoforms localized to the cell nucleus
when expressed in CHO cells. C, increasing amounts (0.2, 0.4, 0.8 μg) of full-
length SP3 (SP3LONG, gray bars) inhibited SP1-mediated Fgf-10 promoter
activity (*, p < 0.05 compared with SP1 alone; n = 3). The shortest SP3 isoform
(SP3SHORT, black bars) failed to inhibit SP1-mediated Fgf-10 promoter activity.
D and E, SP3 mutants lacking either the 149 N-terminal amino acids (D) or the
Sp box (E) were less effective at inhibiting SP1-mediated Fgf-10 promoter
activity than wild type SP3 (*, p compared with SP1 alone; #, p compared with
wild type SP3; n = 6).

NF-κB also represses the expression of a smaller subset of genes, many
of which are involved in development and repair (41–43). The
mechanisms responsible for suppression by NF-κB are less well
understood. NF-κB can stimulate the expression of transcrip-
tional regulators that then inhibit downstream target genes. For
instance, NF-κB interacts with histone deacetylases to guide
these enzymes to targets of NF-κB suppression (44). This
particular mechanism is thought to transcriptionally silence spe-
cific genes, such as the proapoptotic gene Bnip3 in cardiomyo-
cytes (45), as well as tempering activation of NF-
κB targets, including TNFα and IL-8 (44). NF-κB directly inhibits expres-
sion of intestinal trefoil factor (TFF3) (46), E-cadherin (42), and
collagen 1A1 (41). These inhibitory functions of NF-κB suggest
a regulatory role in development.

Many components of the NF-κB signaling pathway are
required for normal fetal development and tissue morphogen-
esis. Mice deficient for IκKα die shortly after birth with pro-
found developmental defects in skin, skeleton, and hematopo-
etic system (47–49). In addition to its role in lymphocyte
survival, NF-κB prevents apoptosis during development. Mice
null for RELA die in utero with massive liver apoptosis (50). An
emerging body of literature also implicates NF-κB in regulating
neural repair and neural synapse integrity. NF-κB influences
neuronal excitability in the amygdala, protecting neurons in
this critical region of the brain from excitotoxicity while simulta-
neously promoting long term potentiation (51, 52). NF-κB has
also been implicated in promoting neuronal regeneration and

neural synapse pruning in the hippocampus (52–55). NF-κB
signaling therefore regulates developmental processes in addi-
tion to the innate immune response. By interacting with other
developmental transcription factors, NF-κB provides an addi-
tional level of expression control. These molecular mechanisms
may provide unique insight into the connections between
inflammation, immunity, and the pathogenesis of developmen-
tal diseases.

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