Functional and Physical Associations between NF- κ B and C/EBP Family Members: a Rel Domain-bZIP Interaction

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Received 22 February 1993/Returned for modification 31 March 1993/Accepted 9 April 1993

NF- κ B and C/EBP represent distinct families of transcription factors that target unique DNA enhancer elements. The heterodimeric NF- κ B complex is composed of two subunits, a 50- and a 65-kDa protein. All members of the NF- κ B family, including the product of the proto-oncogene *c-rel*, are characterized by their highly homologous ~300-amino-acid N-terminal region. This Rel homology domain mediates DNA binding, dimerization, and nuclear targeting of these proteins. C/EBP contains the bZIP region, which is characterized by two motifs in the C-terminal half of the protein: a basic region involved in DNA binding and a leucine zipper motif involved in dimerization. The C/EBP family consists of several related proteins, C/EBP α , C/EBP β , C/EBP γ , and C/EBP δ , that form homodimers and that form heterodimers with each other. We now demonstrate the unexpected cross-coupling of members of the NF- κ B family with three members of the C/EBP family. NF- κ B p65, p50, and Rel functionally synergize with C/EBP α , C/EBP β , and C/EBP δ . This cross-coupling results in the inhibition of promoters with κ B enhancer motifs and in the synergistic stimulation of promoters with C/EBP binding sites. These studies demonstrate that NF- κ B augments gene expression mediated by a multimerized *c-fos* serum response element in the presence of C/EBP. We show a direct physical association of the bZIP region of C/EBP with the Rel homology domain of NF- κ B. The cross-coupling of NF- κ B with C/EBP highlights a mechanism of gene regulation involving an interaction between distinct transcription factor families.

Transcriptional regulation of gene expression is controlled by factors that bind to specific DNA sequences. Many of these factors can be classified into distinct families based on common structural motifs. These families include the homeo-domain family (30), the zinc finger proteins (26, 46), the leucine zipper family (49, 84), and the helix-loop-helix family (59, 60). Many of these transcription factors bind to their cognate DNA motifs as homodimers and, moreover, there are multiple examples of heterodimer formation within the same family of transcription factors. However, there is growing evidence that these dimerization motifs are also involved in interactions between transcription factors from different families. Examples are the interaction of the Jun leucine zipper region with the helix-loop-helix motif of MyoD (12) or with the DNA binding and ligand binding regions of the glucocorticoid receptor (24, 42, 74, 87). This cross-coupling between different families might lead to the formation of novel transcription factor complexes displaying altered biological activities.

The transcription factor NF- κ B binds to κ B enhancer motifs found in a variety of genes in response to various signals (5 and references therein, 35, 51). NF- κ B DNA binding activity typically involves two subunits, a 50-kDa protein (17, 31, 45) and a 65-kDa protein (63, 72). NF- κ B p50 and NF- κ B p65 share a highly homologous 300-residue N-terminal region. This region is also found in the products of the proto-oncogene *c-rel* and of the *dorsal* maternal effect gene of *Drosophila melanogaster* (16, 31, 32, 38, 80). The Rel homology region of these proteins mediates DNA binding, dimerization, nuclear targeting, and interactions with I κ B (9, 11, 17, 31, 33, 45, 63, 72).

The C/EBP family of transcription factors belongs, to-

gether with AP-1 and ATF/CREB, to a class of DNA binding proteins named bZIP proteins (84). These proteins are characterized by their leucine zipper structure and the adjacent DNA binding basic region, both located in the C-terminal half of the respective proteins. The originally characterized CCAAT/enhancer-binding protein C/EBP (41) is now named C/EBP α . C/EBP β (20) is the new term for LAP (23), IL6-DBP (67), CRP2 (86), AGP/EBP (21), and NF-IL6 (2). C/EBP γ replaces the old name Ig/EBP-1 (71). C/EBP α , C/EBP β , C/EBP γ , and C/EBP δ (20) form homodimers and heterodimers with each other in vitro and bind with a similar affinity to various C/EBP binding sites (20, 48). All members of the C/EBP family are encoded by different genes, and the amino acid sequences of the N-terminal two-thirds of these proteins are not related. C/EBP α expression is tissue restricted, with the highest expression occurring in liver, adipose, and intestinal tissue (14). C/EBP α is an activator of specific gene expression (22, 28, 29, 43, 65, 81). Furthermore, C/EBP α plays a general role in cellular growth inhibition (82) and has a hypothetical responsiveness to hormones that modulate energy balance (54). The *c-fos* serum response element (SRE) contains overlapping binding sites for the serum response factor and C/EBP β (56). Cyclic AMP (cAMP) stimulates phosphorylation and translocation of C/EBP β to the nucleus in PC12 cells. These events induce C/EBP β binding to the SRE and activate *c-fos* expression (55). Interestingly, several promoters of immune or acute-phase response genes, such as the interleukin-6 (IL-6) gene, the IL-8 gene, and the angiotensinogen gene, have adjacent or overlapping binding sites for C/EBP and NF- κ B (19, 40, 58).

In this study, we show that members of the NF- κ B/Rel family can interact with three members of the C/EBP family. This cross-coupling results in the inhibition of promoters

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with κ B enhancer motifs and the synergistic stimulation of promoters with C/EBP binding sites. Specifically, NF- κ B augments gene expression mediated by a multimerized *c-fos* SRE in the presence of C/EBP. A direct physical interaction between the bZIP region of C/EBP and the Rel homology domain of NF- κ B is demonstrated.

MATERIALS AND METHODS

Plasmid constructs. The κ B-TATA-CAT and SRE-TATA-CAT reporter plasmids have been described elsewhere (42, 79). The C/EBP-TATA-CAT reporter was generated by cloning a single copy of an oligonucleotide with a C/EBP binding site derived from the *c-fos* SRE (5' AGCTTGATT AGGACATCG 3') (binding site printed in boldface type) into *Hind*III-*Bam*HI-cut TATA-CAT. The CRE-TK-CAT reporter contains a single copy of a CREB binding site (5' AGCTTGACGTCACCCG 3') (binding site printed in boldface type) cloned into *Hind*III-*Bam*HI-cut pBLCAT4 (79). The Myb-TK-CAT reporter contains five copies of a Myb binding site cloned in front of the herpes simplex virus thymidine kinase promoter (46a). The pCMV4T plasmid was derived from pCMV4 (3) by inserting an oligonucleotide with stop codons in all three reading frames into the *Sma*I site. The pCMV4T-p65 and pCMV4T-p50 plasmids contain cDNAs encoding human NF- κ B p65 (72) and NF- κ B p50 (amino acids 1 to 462) (45), respectively. Plasmid pCMV4T-p65mLZ was constructed by polymerase chain reaction-assisted site-directed mutagenesis of NF- κ B p65, resulting in the change of amino acids 436-LSEALLQLQFDDDEL-450 to 436-ASEAALQAQFDDEDA-450 (underlining highlights changes). Plasmid pCMV4T-p65 Δ 10 encodes an alternatively spliced form of NF- κ B p65 with an internal deletion of amino acid residues 222 to 231 (61, 73). Plasmid pCMV4T-p65Eco contains a cDNA encoding the Rel homology domain of NF- κ B p65 (amino acids 1 to 282). Expression vectors pCMV4T-vRel and pCMV4T-hcRel are as described previously (9). Plasmid pCMV4T-rC/EBP β contains a cDNA encoding rat C/EBP β (67) cloned into pCMV4T. Cytomegalovirus (CMV) enhancer-driven expression vectors for human C/EBP α , C/EBP β , and C/EBP δ were a gift of Steven L. McKnight. The mouse *c-Jun*, human *c-Fos*, and human CREB pCMV4T expression vectors contain full-length coding sequences of the corresponding T7 polymerase-driven vectors (13, 69, 70) cloned downstream of the CMV immediate-early-region promoter. The wild-type Myb expression vector (pRmb3SVneo) and the corresponding frameshift control pJTmyb-fs have been described elsewhere (25). The bacterial FLAG-heart muscle kinase (HMK) fusion protein expression vectors were constructed by cloning the cDNAs for NF- κ B p65, NF- κ B p50 (amino acids 1 to 462), and rat C/EBP β as polymerase chain reaction-amplified fragments into the *Eco*RI site of the T7 polymerase-driven FLAG-HMK vector (15). The bacterial glutathione *S*-transferase (GST) fusion protein expression vectors were constructed by cloning the cDNAs for rat C/EBP β and the bZIP truncation of rat C/EBP β (C/EBP β bZIP; encoding amino acids 187 to 297) as polymerase chain reaction-amplified fragments in frame into the *Eco*RI site of pGEX-1N (Amrad). Expression vectors for in vitro transcription were generated by cloning the cDNAs for NF- κ B p65, NF- κ B p65 Δ 10, and NF- κ B p50 (amino acids 1 to 462) into pGEM4 (Promega). In vitro translated proteins NF- κ B p65Eco (amino acids 1 to 282) and NF- κ B p65Bgl (amino acids 1 to 194) were generated by runoff transcription terminated at internal *Eco*RI and *Bgl*II sites, respectively.

Transfection of cells and analysis of CAT activity. All cell lines were cultured in Iscove's Dulbecco modified Eagle medium supplemented with 7.5% fetal calf serum and antibiotics. Mouse F9 embryonal carcinoma cells were transiently transfected by the calcium phosphate method (34). Monkey COS cells were transiently transfected with plasmid DNA by the DEAE-dextran method (44). Chloramphenicol acetyltransferase (CAT) enzymatic activity was assayed as previously described (62) and normalized for protein recovery (18).

Bacterial expression of proteins. Human C/EBP α bZIP, C/EBP β bZIP, and C/EBP δ bZIP proteins expressed in bacteria (20, 78) were a generous gift of Steven L. McKnight. The FLAG-HMK fusion proteins were expressed in *Escherichia coli* BL21 (LysS). The GST fusion proteins were expressed in *E. coli* JM109. Bacteria were grown overnight, diluted 1:10 and, after another hour of growth, stimulated with 0.4 mM isopropyl- β -D-thiogalactopyranoside (IPTG) for 3 h at 32°C. Bacteria were then harvested, resuspended in a 0.1 volume of phosphate-buffered saline (PBS), and sonicated for 1 min in PBS, and the extracts were cleared by centrifugation.

Purification and 32 P labeling of proteins. Cytosolic NF- κ B from HeLa cells was purified as previously described (9). Bacterially expressed human C/EBP proteins containing only the bZIP domain were purified by column chromatographic techniques as described previously (20, 78). Bacterially expressed FLAG-HMK fusion proteins were purified by low-pH elution from an M2 anti-FLAG antibody column (IBI-Kodak, New Haven, Conn.) in accordance with the manufacturer's instructions. FLAG-HMK fusion proteins were labeled in vitro with [γ - 32 P]ATP by use of HMK (Sigma) in buffer containing 20 mM Tris (pH 7.6), 100 mM NaCl, 12 mM MgCl₂, and 1 mM dithiothreitol for 60 min at 37°C (15). Whole-cell extracts from COS cells were prepared by a method used to prepare CAT assay extracts (4) 3 days posttransfection.

EMSA. Electrophoretic mobility shift assays (EMSA) were performed as previously described (79). The binding buffer contained 12 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH (pH 7.9), 4 mM Tris (pH 7.9), 60 mM KCl, 5 mM MgCl₂, 0.6 mM EDTA, 12% glycerol, and 5 mM dithiothreitol. The human immunodeficiency virus type 1 (HIV-1) κ B probe was as previously described (79). The C/EBP binding site found as a core sequence within the *c-fos* SRE was created by annealing the oligonucleotides 5' AGCTTGATTAGGACATCG 3' and 5' GATCCGATGTCCTAATCA 3' (binding site printed in boldface type).

Coimmunoprecipitation. For coimmunoprecipitation analyses, 0.6 μ l of 32 P-labeled FLAG-HMK fusion protein was combined with 5 μ l of unlabeled bacterial protein in EMSA binding buffer. After 15 min of incubation at room temperature (RT), the chemical cross-linker dithio-bis(succinimidylpropionate) (DSP) (Pierce) was added at a final concentration of 2 mM, and the mixture was incubated for 30 min at RT. The cross-linking reaction was squelched by the addition of 100 mM ethanolamine. After the volume was increased with RIPA buffer, normal rabbit serum and protein A-Sepharose were added, and the mixture was incubated for 1 h at 4°C. The immunoreaction was then cleared, specific antibody and protein A-Sepharose were added, and the mixture was incubated for 2 h at 4°C. After the immunocomplexes were washed, the cross-linked complexes were reduced with Laemmli sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (47) contain-

FIG. 1. Cross-coupling of NF- κ B p65 with C/EBP β . Mouse F9 embryonal carcinoma cells were transiently cotransfected with 8 μ g of the indicated CAT reporter plasmids (κ B-TATA-CAT, containing one copy of the duplicated HIV-1 κ B enhancer motif from the HIV-1 long terminal repeat [positions -105 to -79] linked to the TATA box of the *X. laevis* albumin gene; SRE-TATA-CAT, containing four copies of the SRE of the human *c-fos* promoter [-320 to -299]; and C/EBP-TATA-CAT, containing one copy of a C/EBP binding site and 0.1 μ g of pCMV4T-p65 (A), 1 μ g of pCMV4T-rC/EBP β (B and C), and increasing amounts (0 to 6 μ g) of pCMV4T-rC/EBP β (A) or pCMV4T-p65 (B and C). The total amount of transfected DNA was kept constant by the addition of the parental pCMV4T vector. At 6 h after transfection, CAT activity was determined. The mean levels of CAT activity determined in at least three independent experiments are presented as the fold induction relative to the activity obtained with transfected pCMV4T alone. The standard errors were always less than 20% of the means.

ing 15% β -mercaptoethanol, boiled, and subjected to SDS-PAGE.

GST fusion protein interaction assay. Glutathione-Sepharose 4B beads (Pharmacia) were equilibrated in PBS-1% Triton X-100 and then mixed with 20 volumes of bacterial GST fusion proteins on a rotary shaker for 20 min at RT. The beads were washed three times with PBS-1% Triton X-100 and then equilibrated in 20 mM HEPES-NaOH (pH 7.9)-25 mM NaCl-2.5 mM MgCl₂-0.1 mM EDTA-0.05% Nonidet P-40-1% Triton X-100 (LSBT). Five microliters of a 1:1 bead slurry in PBS was combined with 5 μ l of a ³⁵S-labeled reticulocyte lysate in a final volume of 200 μ l of LSBT-1 mM dithiothreitol-1 mM phenylmethylsulfonyl fluoride on a rotary shaker for 60 min at RT. The beads were then washed four times with LSBT and once with 50 mM Tris (pH 6.8). The bound proteins were eluted by boiling in Laemmli SDS-PAGE loading buffer (47) and subjected to SDS-PAGE.

Western blot (immunoblot) analysis. Nuclear extracts from $\sim 1 \times 10^6$ COS cells prepared 3 days after transfection as described previously (79) were fractionated by SDS-PAGE, blotted onto a nitrocellulose membrane, and immunostained by use of an Amersham enhanced chemiluminescence (ECL) kit in accordance with the manufacturer's instructions.

RESULTS

NF- κ B functionally acts in synergy with C/EBP-related proteins. NF- κ B activity is increased in response to IL-1 (64), and C/EBP β is activated by IL-6 (66). Interestingly, synergy between IL-1 and IL-6 in several physiological responses, including the acute-phase response, in T-cell activation, and in immunoglobulin secretion has been reported (1, 37, 83). To test for possible cross-coupling between the NF- κ B and C/EBP families of transcription factors, we performed transfection experiments with mouse F9

embryonal carcinoma cells. F9 cells were selected because of their exceedingly low levels of endogenous nuclear NF- κ B (39, 78a). F9 cells were transfected with a CAT reporter plasmid composed of the duplicated HIV-1 κ B enhancer motif linked to the TATA box of the *Xenopus laevis* albumin gene (κ B-TATA-CAT). This κ B enhancer motif is sufficient to mediate activation by various members of the NF- κ B transcription factor family (8, 78a). Unexpectedly, cotransfection of a rat C/EBP β expression vector strongly reduced the induction of κ B-TATA-CAT by NF- κ B p65 in a dose-dependent manner (Fig. 1A). Therefore, we were interested in whether C/EBP β functions as a positive regulator of gene expression in F9 cells when tested on reporter plasmids containing C/EBP binding sites. SRE-TATA-CAT is a reporter plasmid in which the κ B enhancer motifs are replaced with four repeats of the *c-fos* SRE that contains in its core a C/EBP binding site (55, 56). Expression of rat C/EBP β in F9 cells caused dose-dependent activation of this reporter plasmid (data not shown and Fig. 1B). To analyze whether a single C/EBP binding site is sufficient to mediate the stimulation of CAT activity by C/EBP, we transfected F9 cells with C/EBP-TATA-CAT, a reporter plasmid with one copy of a core C/EBP binding site. As expected, cotransfection of a rat C/EBP β expression vector led to the strong activation of this reporter plasmid in a dose-dependent manner (data not shown). These results also indicate that the inhibitory effect of C/EBP β on κ B-TATA-CAT gene transcription was dependent on the κ B enhancer motifs. We then transfected a combination of NF- κ B p65 and rat C/EBP β and observed strong synergistic stimulation of SRE-TATA-CAT (Fig. 1B), while NF- κ B p65 by itself was unable to affect the expression of SRE-TATA-CAT (data not shown). Similar results were obtained when the C/EBP-TATA-CAT reporter plasmid with a single C/EBP binding site was used (Fig. 1C). To detect possible differences in

FIG. 2. Cross-coupling of NF- κ B p65 with C/EBP-related proteins. (A) Mouse F9 embryonal carcinoma cells were transiently cotransfected with 8 μ g of CAT reporter plasmid κ B-TATA-CAT (containing one copy of the duplicated HIV-1 κ B enhancer motif from the HIV-1 long terminal repeat [positions -105 to -79] linked to the TATA box of the *X. laevis* albumin gene), 0.1 μ g of pCMV4T-p65, and 6 μ g of pCMV4T or CMV expression vectors for rat C/EBP β or human C/EBP α , C/EBP β , and C/EBP δ . CAT activity is presented as percent inhibition relative to 100% activity obtained with transfected pCMV4T alone. (B) Mouse F9 embryonal carcinoma cells were transiently cotransfected with 8 μ g of CAT reporter plasmid SRE-TATA-CAT (containing four copies of the SRE of the human *c-fos* promoter [-320 to -299]) and 1 μ g of CMV expression vectors for rat C/EBP β or human C/EBP α , C/EBP β , and C/EBP δ in the presence or absence of 3 μ g of pCMV4T-p65. The total amount of transfected DNA was kept constant by the addition of the parental pCMV4T vector. At 6 h after transfection, CAT activity was determined. CAT activity is presented as the fold induction relative to the activity obtained with transfected pCMV4T alone. (C) Monkey COS cells were transiently transfected with 12 μ g of pCMV4T (lanes 1, 6, 9, and 12); a combination of 400 ng of pCMV4T-p65 and 12 μ g of pCMV4T, pCMV-C/EBP α , pCMV-C/EBP β , or pCMV-C/EBP δ (lanes 2 to 5); a combination of 4 μ g of pCMV-C/EBP α and 12 μ g of pCMV4T or pCMV4T-p65 (lanes 7 and 8); a combination of 4 μ g of pCMV-C/EBP β and 12 μ g of pCMV4T or pCMV4T-p65 (lanes 10 and 11); or a combination of 4 μ g of pCMV-C/EBP δ and 12 μ g of pCMV4T or pCMV4T-p65 (lanes 13 and 14). The SDS-PAGE-fractionated and blotted nuclear proteins prepared from cells 3 days after transfection were immunostained with antibodies specific for NF- κ B p65 (lanes 1 to 5), C/EBP α (lanes 6 to 8), C/EBP β (lanes 9 to 11), and C/EBP δ (lanes 12 to 14). The arrowheads indicate the positions of the expressed proteins.

transcriptional activity among three members of the C/EBP family, we compared the effect of rat C/EBP β and human C/EBP α , C/EBP β , and C/EBP δ on the induction of κ B-TATA-CAT by NF- κ B p65 in F9 cells. All three isoforms of human C/EBP were able to strongly inhibit a κ B enhancer-dependent promoter (Fig. 2A). Similarly, NF- κ B p65 was able to synergistically activate SRE-TATA-CAT in the presence of human C/EBP α , C/EBP β , or C/EBP δ (Fig. 2B).

Western blot analyses of nuclear extracts from cells transfected with combinations of NF- κ B p65 and C/EBP showed that the decreased activation of κ B-TATA-CAT by NF- κ B p65 in the presence of cotransfected C/EBP expression

vectors was not the result of an inhibition of NF- κ B p65 expression. Vice versa, the expression of C/EBP family members was not increased by the cotransfected NF- κ B p65 expression vector (Fig. 2C).

Several members of the NF- κ B/Rel family cross-couple with C/EBP β . The functional interaction between the NF- κ B/Rel and C/EBP transcription factor families was further confirmed by cotransfection of plasmid κ B-TATA-CAT with expression vectors for different members of the NF- κ B family (Table 1) in the absence or presence of rat C/EBP β . In addition, we also tested a modified form of NF- κ B p65, NF- κ B p65mLZ, containing a point mutation in a recently

TABLE 1. Inhibition of NF- κ B Rel transcription factors by C/EBP β ^a

Transcription factor	Inhibition by rat C/EBP β (% of induced level) with:	
	4 μ g of vector	6 μ g of vector
NF- κ B p65	29.4 $\pm$ 6.1	18.6 $\pm$ 3.5
NF- κ B p65mLZ	37.5 $\pm$ 3.2	25.5 $\pm$ 6.3
NF- κ B p50	91.3 $\pm$ 27.8	65.2 $\pm$ 1.2
v-Rel	58.4 $\pm$ 20.3	40.5 $\pm$ 18.6

^a Mouse F9 embryonal carcinoma cells were transiently cotransfected with 8 μ g of κ B-TATA-CAT (containing one copy of the κ B enhancer from the HIV-1 long terminal repeat [positions -105 to -79] linked to the TATA box of the *X. laevis* albumin gene), different amounts of expression vectors for NF- κ B p65, NF- κ B p65mLZ, NF- κ B p50, and v-Rel, and different amounts (0, 4, and 6 μ g) of the pCMV4T-rC/EBP β expression vector. The total amount of transfected DNA was kept constant by the addition of the parental pCMV4T vector. At 6 h after transfection, CAT activity was determined. The mean levels of CAT activity and standard errors determined in at least three independent experiments are presented as percent inhibition relative to 100% activity obtained with transfected pCMV4T alone.

described leucine zipper-like structure (73). For comparison of the effects of rat C/EBP β on κ B enhancer-dependent gene expression, the amounts of transfected expression vectors for NF- κ B proteins were adjusted to obtain similar levels of induced CAT activity. As shown in Table 1, rat C/EBP β inhibited induction by NF- κ B p65, NF- κ B p65mLZ, and v-Rel. The v-Rel oncoprotein functions as a κ B-specific transcriptional activator in the undifferentiated F9 cells used for these transfection analyses (85). NF- κ B p50 was a very weak transcriptional activator, even when we transfected large amounts of expression vector. This result and the absence of endogenous NF- κ B activity in F9 cells explains why we obtained only weak inhibition of promoter activity by rat C/EBP β when we used NF- κ B p50.

Next, we were interested in whether these members of the NF- κ B/Rel family of transcription factors can synergistically stimulate the SRE-TATA-CAT reporter in the presence of rat C/EBP β . While transfecting a small amount of the rat C/EBP β expression plasmid with the control vector pCMV4T yielded about 20-fold stimulation of CAT activity, cotransfection of NF- κ B p65, NF- κ B p65mLZ, NF- κ B p50, v-Rel, or human c-Rel resulted in 3- to 7-fold higher levels of gene expression (Table 2). Interestingly, NF- κ B p65 Δ 10, a naturally occurring variant of NF- κ B p65 that has an internal 10-amino-acid deletion and that can dimerize only very weakly (61, 78a), and NF- κ B p65Eco, a C-terminal truncation encompassing only the Rel homology domain and not the C-terminal transactivation domain, were not able to efficiently act in synergy with rat C/EBP β (Table 2). Both proteins were expressed at levels comparable to those of NF- κ B p65, as shown by Western blot analysis (data not shown).

The synergistic stimulation of C/EBP is limited to members of the NF- κ B/Rel family. Several members of other transcription factor families, such as c-Jun, c-Fos, CREB, and Myb, were not able to synergistically stimulate the SRE-TATA-CAT reporter in the presence of rat C/EBP β (Table 2). These transcription factors were expressed at levels similar to that of NF- κ B p65 (as shown by Western blot analysis; data not shown) and were able to transactivate promoter-CAT constructs with their cognate binding sites (Table 3 and data not shown). The specificity of the cross-coupling between NF- κ B and C/EBP was further shown by

TABLE 2. Synergistic stimulation of C/EBP β by NF- κ B Rel transcription factors^a

Transcription factor	Fold activation by rat C/EBP β
pCMV4T.....	20.4 $\pm$ 2.6
NF- κ B p65.....	133.6 $\pm$ 26.4
NF- κ B p65mLZ.....	113.2 $\pm$ 26.8
NF- κ B p65 Δ 10.....	39.1 $\pm$ 10.1
NF- κ B p65Eco.....	11.1 $\pm$ 2.3
NF- κ B p50.....	82.7 $\pm$ 12.8
v-Rel.....	67.8 $\pm$ 11.1
hc-Rel.....	70.0 $\pm$ 8.3
c-Jun.....	30.7 $\pm$ 4.6
c-Fos.....	33.9 $\pm$ 7.5
CREB.....	29.9 $\pm$ 8.5
Myb.....	39.3 $\pm$ 3.5

^a Mouse F9 embryonal carcinoma cells were transiently cotransfected with 8 μ g of SRE-TATA-CAT (containing four copies of the SRE of the human *c-fos* promoter [positions -320 to -299]), 1 μ g of pCMV4T-rC/EBP β , and 3 μ g of pCMV4T or expression vectors for NF- κ B p65, NF- κ B p65mLZ, NF- κ B p65 Δ 10, NF- κ B p65Eco, NF- κ B p50, v-Rel, hc-Rel, c-Jun, c-Fos, CREB, and Myb. The total amount of transfected DNA was kept constant by the addition of the parental pCMV4T vector. At 6 h after transfection, CAT activity was determined. The mean levels of CAT activity and standard errors determined in at least three independent experiments are presented as the fold induction relative to the activity obtained with transfected pCMV4T alone.

the lack of synergistic stimulation of the CRE-TK-CAT reporter by NF- κ B p65 in the presence of CREB and of the Myb-TK-CAT reporter in the presence of Myb (Table 3).

Requirement of the Rel homology domain and the bZIP region for alterations in the DNA binding of NF- κ B and C/EBP family members. Our *in vivo* experiments showed a functional interplay between two transcription factor families, NF- κ B/Rel and C/EBP. This interplay resulted in the synergistic stimulation of reporter plasmids with C/EBP binding sites and in the inhibition of reporter plasmids with κ B enhancer motifs. Thus, these data raised the possibility that NF- κ B and C/EBP might physically interact to form a novel transcription factor complex that exhibits decreased DNA binding activity and/or transcriptional activity when tested with κ B enhancer motifs but displays increased DNA

TABLE 3. Specificity for C/EBP of synergistic activation by NF- κ B p65^a

Expt (CAT reporter)	Transcription factor	Relative CAT activity
A (CRE-TK-CAT)	pCMV4T	1.0
	CREB	3.7 $\pm$ 1.4
	NF- κ B p65	1.6 $\pm$ 0.3
	NF- κ B p65 + CREB	1.7 $\pm$ 0.5
B (Myb-TK-CAT)	Control	1.0
	Myb	8.4 $\pm$ 2.5
	NF- κ B p65	1.1 $\pm$ 0.8
	NF- κ B p65 + Myb	8.6 $\pm$ 3.4

^a Mouse F9 embryonal carcinoma cells were transiently cotransfected in experiment A with 8 μ g of CRE-TK-CAT (containing one copy of the fibronectin promoter CREB binding site [positions -174 to -162]), 2 μ g of pCMV4T-CREB, and/or 1 μ g of pCMV4T-p65 and in experiment B with 8 μ g of Myb-TK-CAT (containing five copies of a Myb binding site), 0.8 μ g of RSV-Myb, and/or 1 μ g of pCMV4T-p65. The total amount of transfected DNA was kept constant by the addition of the corresponding parental vectors. At 6 h after transfection, CAT activity was determined. The mean levels of CAT activity and standard errors determined in at least three independent experiments are presented as the fold induction relative to the activity obtained with transfected pCMV4T alone.

binding activity and/or transcriptional activity when tested with C/EBP binding sites. We expressed NF- κ B p65 and NF- κ B p65mLZ in bacteria by using the FLAG-HMK expression vector system (15). This system produces fusion proteins with two additional elements at the N terminus. The first element is an 8-amino-acid antigenic epitope recognized by the M2 anti-FLAG antibody. The second element is a 5-amino-acid recognition site for HMK. These elements allow for the purification of the expressed proteins over an M2 anti-FLAG antibody affinity column and for labeling in vitro with [γ - 32 P]ATP. Partially purified bacterial NF- κ B p65 and NF- κ B p65mLZ are composed of two major species, full-length p65 and p65mLZ, respectively, and an ~46-kDa truncated protein (a degradation product or an internal translation stop). We identified this 46-kDa protein as a C-terminally truncated protein because it can be labeled with [γ - 32 P]ATP, indicating the presence of the N-terminal HMK peptide, and by use of antibodies directed against an N-terminal or a C-terminal peptide of NF- κ B p65 (data not shown).

We were interested in whether NF- κ B p65 is able to change the binding of C/EBP to its cognate binding site. EMSA were performed with bacterially expressed and partially purified human C/EBP. The binding of full-length C/EBP β and the bZIP regions of C/EBP α , C/EBP β , and C/EBP δ to a C/EBP binding site was strongly enhanced by the addition of bacterially expressed NF- κ B p65 (Fig. 3), although no change in the mobility of the DNA-protein complex was observed (see Discussion). The amount of added bacterial protein was kept constant by the addition of a bacterial extract expressing only the FLAG-HMK peptide. NF- κ B p65 was not able to bind to a C/EBP binding site by itself but bound to a κ B binding site (data not shown). The increase in DNA binding activity might have depended on the basic DNA binding region of NF- κ B p65, since basic polypeptides, such as poly-L-lysine, also enhanced C/EBP DNA binding activity (10) (data not shown) (see Discussion).

Furthermore, the addition of increasing amounts of bacterially expressed, partially purified rat FLAG-HMK-C/EBP β inhibited the DNA binding of bacterially expressed NF- κ B p65 and NF- κ B p65mLZ as well as NF- κ B p65 and NF- κ B p50 expressed in vivo in COS cells (data not shown). Of note, we were unable to detect any effects of bacterially expressed human C/EBP β on the DNA binding of NF- κ B p65 (see Discussion).

In vitro association of the Rel homology domain of NF- κ B with the bZIP region of C/EBP β . Studies were performed to determine whether NF- κ B and C/EBP β can associate in vitro in the absence of DNA. For these studies, NF- κ B p65, NF- κ B p65mLZ, and NF- κ B p50 were expressed in bacteria by use of the FLAG-HMK expression vector system, partially purified, and labeled in vitro with [γ - 32 P]ATP. The radioactively labeled NF- κ B p65 and NF- κ B p65mLZ proteins were separately combined with bacterially expressed NF- κ B p50, rat C/EBP β , human C/EBP β bZIP, or bacterially expressed FLAG-HMK peptide. The potentially formed heteromeric protein complexes were chemically cross-linked by use of the reversible cross-linker DSP to stabilize the complexes during immunoprecipitation. Before the samples were subjected to SDS-PAGE, the cross-linked complexes were cleaved under reducing conditions. The full-length and C-terminally truncated 32 P-labeled NF- κ B p65 proteins (see above) were efficiently coimmunoprecipitated with anti-p50 antibodies through their association with NF- κ B p50 (Fig. 4, compare lanes 1 and 2). Similarly, both forms of 32 P-labeled NF- κ B p65 protein were coimmunoprecipitated with anti-

FIG. 3. NF- κ B stimulates DNA binding of C/EBP proteins. Bacterially expressed, purified human C/EBP β or human C/EBP β bZIP, C/EBP α bZIP, and C/EBP δ bZIP (truncated proteins that contain only the DNA binding region and leucine zipper) were incubated with increasing amounts (0, 1, and 5 μ l) of bacterially expressed, partially purified FLAG-HMK-NF- κ B p65 or affinity-purified NF- κ B prepared from the cytosol of HeLa cells (5 μ l) for 15 min at RT in EMSA buffer with 0.1 μ g of poly(dI-dC) and 5 μ g of bovine serum albumin. After the addition of a 32 P-labeled probe (C/EBP) and incubation at RT for 30 min, DNA-protein complexes were analyzed by EMSA with 0.25 $\times$ Tris-borate-EDTA native gels. All shifted DNA-protein complexes were shown to be specific by competition with an unlabeled oligonucleotide (data not shown).

C/EBP β antibodies in the presence of full-length C/EBP β (Fig. 4, compare lanes 3 and 4). To determine which portion of C/EBP β associated with NF- κ B p65, we combined 32 P-NF- κ B p65mLZ with full-length rat C/EBP β and the truncated form of human C/EBP β bZIP. Both proteins interacted with NF- κ B p65, as shown by coimmunoprecipitations with antibodies directed against a C-terminal peptide of human C/EBP β (Fig. 4, lanes 6 to 9). Likewise, we showed an interaction between NF- κ B p50 and rat C/EBP β (Fig. 4, lanes 10 and 11).

We also used an alternative approach to show an interaction between NF- κ B and C/EBP β . The coding regions of rat C/EBP β or an N-terminal truncation of rat C/EBP β encoding only the bZIP region (C/EBP β bZIP) were fused in frame to the GST gene. GST fusion proteins were expressed in bacteria and bound to glutathione-Sepharose 4B beads. Wild-type NF- κ B p65 and NF- κ B p50 and several mutant cDNAs of NF- κ B p65 were transcribed in vitro and translated in a rabbit reticulocyte lysate in the presence of [35 S]methionine or [35 S]cysteine. All radiolabeled proteins were expressed at similar levels (data not shown). The radiolabeled proteins were incubated separately with control GST beads, GST-C/EBP β beads, or GST-C/EBP β bZIP beads. After being extensively washed, the bound proteins were eluted and analyzed by SDS-PAGE. The radiolabeled

FIG. 4. In vitro association of NF- κ B with C/EBP β . Bacterially expressed, purified proteins were incubated as indicated with 32 P-labeled bacterial NF- κ B p65 (lanes 1 to 5), NF- κ B p65mLZ (lanes 6 to 9), and NF- κ B p50 (lanes 10 to 12). After being cross-linked with DSP, the protein complexes were immunoprecipitated with antibodies directed against NF- κ B p50 (lanes 1, 2, and 12), anti-C/EBP β (lanes 3, 4, and 6 to 11), and antibodies directed against NF- κ B p65 (lane 5). The immunocomplexes were reduced and subjected to SDS-PAGE. The arrowheads indicate the positions of the 32 P-labeled proteins. Numbers to the left of the gel are in kilodaltons.

proteins bound only weakly or not at all to the control GST beads (Fig. 5A, lanes 2 to 5). As expected, full-length NF- κ B p65 and a C-terminal truncation of NF- κ B p65 encompassing only the Rel homology domain (NF- κ B p65Eco) bound efficiently to GST-C/EBP β (Fig. 5A, lanes 6 and 8) or to GST-C/EBP β bZIP (Fig. 5A, lanes 10 and 11). A shorter truncation of NF- κ B p65 encompassing only a portion of the Rel homology domain (NF- κ B p65Bgl) was unable to bind to GST-C/EBP β (Fig. 5A, lane 9) or to GST-C/EBP β bZIP (Fig. 5A, lane 12). These results identify the cross-coupling of C/EBP β with NF- κ B as a bZIP-Rel homology domain interaction. The alternatively spliced form of NF- κ B p65 (NF- κ B p65 Δ 10), which had only weak effects on C/EBP β transactivation in vivo (Table 2), was not able to efficiently interact with C/EBP β in vitro (Fig. 5A, lane 7). We also demonstrated the physical association of NF- κ B p50 with GST-C/EBP β and GST-C/EBP β bZIP (data not shown).

The observed physical interaction between NF- κ B p65 and C/EBP β indicates it to be of an affinity similar to that of the homodimer formation between 35 S-radiolabeled NF- κ B p65 and GST-NF- κ B p65 beads (compare Fig. 5A, lanes 6 and 8, with Fig. 5B, lanes 4 and 5). The specificity of this interaction was further proven by mixing 35 S-radiolabeled NF- κ B p65Eco and c-Fos proteins and applying this mixture to GST-C/EBP β beads. As expected, NF- κ B p65Eco bound specifically to GST-C/EBP β and GST-C/EBP β bZIP (Fig. 5C, lanes 3 to 5), while c-Fos, in accordance with our in vivo data (Table 2), did not specifically interact with C/EBP β .

DISCUSSION

In this report, we demonstrate the functional and physical associations of members of two important transcription factor families, NF- κ B and C/EBP. The functional associations are characterized by (i) the inhibition of NF- κ B-induced expression of promoters containing NF- κ B binding sites by the expression of C/EBP family members and (ii) the positive synergistic action of NF- κ B family members with C/EBP family members on promoters containing C/EBP binding sites. Evidence is presented that NF- κ B subunits

FIG. 5. The Rel homology domain of NF- κ B interacts with the bZIP region of C/EBP β in vitro. (A) Radiolabeled, rabbit reticulocyte lysate-translated proteins (5 μ l) were incubated as indicated with bacterially expressed GST (lanes 2 to 5), GST-C/EBP β (lanes 6 to 9), and GST-C/EBP β bZIP (lanes 10 to 12) immobilized on glutathione-Sepharose 4B beads. (B) Radiolabeled NF- κ B p65 or NF- κ B p65Eco (5 μ l) was incubated separately with control GST beads (lanes 2 and 3) or GST-NF- κ B p65 beads (lanes 4 and 5). (C) Radiolabeled c-Fos and NF- κ B p65Eco (5 μ l each) were mixed and incubated with control GST beads, GST-C/EBP β beads, or GST-C/EBP β bZIP beads. Lanes 1 and 2 show one-fifth of the input amounts used for lanes 3 to 5. After being extensively washed, the bound proteins were eluted and analyzed by SDS-PAGE. Equal amounts of bacterially expressed fusion proteins were bound to glutathione-Sepharose 4B beads. All radiolabeled proteins were expressed in the rabbit reticulocyte lysate at similar levels (data not shown). Numbers to the left of the gels are in kilodaltons.

strongly act in synergy with members of the C/EBP family of transcription factors in regulating gene expression mediated by the multimerized c-fos SRE. In addition, we demonstrate a physical interaction between the two transcription factor families through contacts on the Rel homology domain of NF- κ B and on the bZIP region of C/EBP. The experiments that revealed that several functional NF- κ B subunits (p50, p65, and c-Rel) interact with three different isoforms of C/EBP (α , β , and δ) indicated a general interaction between these families and not merely a limited interaction between individual members. Interestingly, and supporting our data, the work of LeClair et al. (50) demonstrated that screening of a λ gt11 expression library with radiolabeled NF- κ B p50 identified a cDNA encoding C/EBP β . The functional synergy was limited to members of the NF- κ B and C/EBP families, since other transcription factors, such as c-Jun, c-Fos, CREB, and Myb, were not able to act in synergy with C/EBP and NF- κ B p65 did not act in synergy with CREB

and Myb (Tables 2 and 3). Our data also suggest an inhibition of CREB-induced promoter activity by NF- κ B p65.

The positive synergy observed between the NF- κ B and C/EBP families of transcription factors in regulating gene expression (Fig. 1B and C, Fig. 2B, and Table 2) presumably is based on the direct physical interaction between these factors. We found that NF- κ B p65 increases the formation of complexes between C/EBP and its DNA binding site (Fig. 3). It was of interest that the mobility of the C/EBP-DNA complexes was not altered by the addition of NF- κ B p65, suggesting that NF- κ B p65 is not part of the complexes detected by EMSA. Thus, it is possible that NF- κ B p65 functions to stabilize the C/EBP-DNA interaction by forming a ternary complex in solution but that any NF- κ B p65 associated with this complex becomes separated during electrophoresis. A similar observation was reported recently for the interaction between the homeodomain protein Phox1 and the serum response factor (36). Another possibility is that NF- κ B p65 transiently interacts with C/EBP and causes a conformational change in C/EBP that then results in increased DNA binding of C/EBP accompanied by the release of NF- κ B p65 from the complex. A similar mechanism has been postulated for the increase in MyoD1 DNA binding by HSP90 (75). Furthermore, it has been shown that C/EBP DNA binding is also increased by small basic polypeptides, such as poly-L-lysine (10). Therefore, the increase in C/EBP DNA binding by NF- κ B p65 might depend on the basic DNA binding region of NF- κ B p65. Experiments with a truncated form of NF- κ B missing the C-terminal transactivation domain (NF- κ B p65Eco; Table 2) indicated that the functional synergy in vivo not only was a result of increased DNA binding of the C/EBP-NF- κ B p65 complex but also depended on the transactivation domain of NF- κ B p65.

Our experiments clearly demonstrate the direct physical association between NF- κ B and C/EBP through an interaction of the Rel homology domain and the bZIP region (Fig. 4 and 5). This interaction is detected in the absence of binding site DNA. A truncated form of NF- κ B p65 expressing only the Rel homology domain (NF- κ B p65Eco) efficiently associates with a truncated form of C/EBP β expressing only the bZIP region (C/EBP β bZIP) (Fig. 5A). Further deletion of the NF- κ B p65 Rel homology region (NF- κ B p65Bgl) or an internal deletion of 10 amino acids within the Rel homology region (NF- κ B p65 Δ 10) results in a loss of the interaction with C/EBP β (Fig. 5A). This result leads to the conclusion that the same motifs that play a role in dimer formation within the NF- κ B and C/EBP families of transcription factors are also used for complex formation between the C/EBP and NF- κ B families. Alternatively, since these mutations in the Rel homology domain also prevent homodimer formation by NF- κ B p65, it is possible that a monomeric form of NF- κ B p65 does not interact with C/EBP β . Chimeric proteins containing the GAL4 DNA binding and dimerization domain (amino acids 1 to 147) fused in frame to NF- κ B p65Eco or NF- κ B p65Bgl are able to form homodimers through the GAL4 domain, but only GAL4-NF- κ B p65Eco is able to interact with C/EBP (data not shown). This result suggests either that amino acids 195 to 282 of NF- κ B p65, missing in GAL4-NF- κ B p65Bgl, are the target for the physical interaction between these two families or that deletion of the C-terminal portion of the Rel homology domain changes the conformation of the residual protein, preventing an interaction with C/EBP.

If NF- κ B and C/EBP physically interact in vitro and synergistically activate promoters in vivo, then they may also physically interact in vivo. We have not detected an in

vivo interaction, probably because of the very low intracellular concentrations of NF- κ B and C/EBP family members. Furthermore, the interaction appears to be stabilized by binding site DNA (see below). The specificity of the physical interaction between NF- κ B and C/EBP is also evident from our protein mixing experiments proving that c-Fos, which does not cross-couple with C/EBP, is not able to bind to GST-C/EBP β beads (Fig. 5C) and from experiments showing that NF- κ B p65 binds with similar affinities to GST-NF- κ B p65 beads and to GST-C/EBP β beads (Fig. 5B).

The basis for the inhibition of NF- κ B transactivation of gene expression by C/EBP (Fig. 1A, Fig. 2A, and Table 1) is not known. One possibility is that C/EBP blocks the ability of NF- κ B to interact with a critical transcriptional cofactor. This process is termed "squenching" and has been described elsewhere (57, 68). If this hypothesis is true, it is curious why squenching was not observed when a C/EBP-responsive reporter plasmid was used in the presence of the two transcription factors. A second explanation is the possible induction of an inhibitor of NF- κ B function by the expression of C/EBP in F9 cells. Although we cannot rule out these mechanisms, we favor a third explanation: the formation between C/EBP and NF- κ B of a novel transcription factor complex with reduced transcriptional activity at κ B enhancers. The latter could be manifested by a reduced affinity for κ B binding sites or the inability of the complex to stimulate transcriptional activity. We have observed that bacterially expressed rat C/EBP β inhibits the ability of NF- κ B p65 and NF- κ B p50 to bind to its cognate sequence, yet bacterially expressed human C/EBP β does not alter the ability of NF- κ B to bind to DNA (data not shown). It is not clear whether this result is caused by the presence of the N-terminal FLAG-HMK region on the rat protein or whether it reflects differences in the concentrations or state of purification of these proteins. It is important to note that three isoforms of human C/EBP (α , β , and δ) are able, like rat C/EBP β , to inhibit NF- κ B-induced gene activation (Fig. 2A).

The nature of the molecular complex formed between NF- κ B and C/EBP is not clear, but evidence points to an interaction between the dimer forms of the two factors. First, a mutation in a putative NF- κ B p65 leucine zipper does not block the functional or physical interaction between this protein and C/EBP (Tables 1 and 2 and Fig. 4). In addition, no leucine zipper-like structure has been reported in NF- κ B p50, ν -Rel, or c-Rel, yet these proteins functionally and physically interact with C/EBP family members (Tables 1 and 2 and Fig. 4). Second, an alternatively spliced form of NF- κ B p65 (NF- κ B p65 Δ 10) and a C-terminally truncated form (NF- κ B p65Bgl) that cannot efficiently dimerize (61, 78a) do not functionally or physically interact with C/EBP subunits (Table 2 and Fig. 5). This result suggests that the same motif that is necessary for dimer formation is also involved in cross-dimerization between these two families. Of particular importance is the observation that a single C/EBP binding site is sufficient to function as a target for the functional synergy between NF- κ B and C/EBP (Fig. 1C). Thus, it is unlikely that NF- κ B makes DNA contacts on the single C/EBP binding site. This idea supports the model of a dimer of a C/EBP family member binding to DNA and interacting through its bZIP region with the Rel homology region of an NF- κ B family member. Presumably, an altered configuration of the transcription factor complex is stabilized and ultimately is more efficient in interacting with the basic transcriptional machinery.

What is the functional significance of the interactions

observed between NF- κ B and C/EBP? Of particular interest is the observation that NF- κ B can augment gene expression mediated through the multimerized *c-fos* SRE in the presence of C/EBP. Thus, NF- κ B subunits, which are strongly implicated in the control of cellular proliferation and are induced in the G₀-to-G₁ transition (7), may functionally stimulate *c-fos* gene expression when C/EBP is present. C/EBP is induced by compounds that activate cAMP (55), and evidence has been presented that increased cAMP levels may induce preexisting NF- κ B as well (76, 77). Thus, an increase in cAMP levels may coincide C/EBP and NF- κ B, allowing for enhanced expression from C/EBP binding sites and, potentially, a decrease in gene expression mediated by NF- κ B binding sites. A basis for the functional interaction of NF- κ B and C/EBP is also supported by studies with IL-1 and IL-6. IL-1 is known to induce NF- κ B (64), and IL-6 activates C/EBP β (66). The physical and functional interactions observed between NF- κ B and C/EBP β may explain the functional synergy observed between IL-1 and IL-6 in several physiological responses, including the acute-phase response, in T-cell activation, and in immunoglobulin secretion (1, 37, 83). In this regard, the IL-6 promoter contains both an NF- κ B binding site and a C/EBP binding site (37, 40, 52). Both sites have been shown to be targets for IL-1 and tumor necrosis factor α stimulation (37); thus, it is likely that the induction of NF- κ B by IL-1 and tumor necrosis factor α functions to stimulate gene expression from each site. Furthermore, the IL-8 promoter contains adjacent binding sites for C/EBP and NF- κ B that cooperate in gene activation (58), and the angiotensinogen promoter contains overlapping binding sites for these two transcription factors (19). Preliminary data obtained with the IL-8 promoter show cooperative binding of NF- κ B and C/EBP to their adjacent binding sites, resulting in a dose-dependent synergistic activation of the IL-8 promoter (78a). Functional interactions between C/EBP and NF- κ B subunits could have profound influences on cellular growth control. It has been demonstrated that C/EBP α is growth inhibitory and likely functions in the terminal differentiation of certain cells (27, 82). The expression of C/EBP isoforms could alter the ability of NF- κ B subunits to control cellular proliferation.

There is growing evidence that the regulation of gene expression is not mediated solely by the presence or absence of a particular set of transcription factors. The function of a transcription factor can be inhibited by the presence of a specific inhibitor that blocks the ability of the transcription factor to bind to DNA (6). In addition, the interplay of transcription factors themselves likely functions as a critical regulatory component of gene expression. Physical and functional interactions, usually dimerization, of factors within a particular family are well documented. However, recent reports indicate cross-family interactions of specific transcription factors. One example is the interaction between the glucocorticoid receptor and the AP-1 proteins Fos and Jun (24, 42, 53, 74, 87). A second example is the recent report of the interaction between the leucine zipper protein Jun and the helix-loop-helix protein MyoD (12). Our data demonstrate that the important transcription factor families C/EBP and NF- κ B functionally and physically interact. This observation, along with those made previously, highlights a unique mechanism by which the levels of one transcription factor can strongly modulate the functional activity of another.

ACKNOWLEDGMENTS

We thank Dean Ballard, Michael Blonar, John Cogswell, Michael Gilman, Alain Israël, Steven McKnight, Michael Kuehl, Valeria Poli, David Ron, Craig Rosen, and Inder Verma for valuable reagents. We also thank Amer Beg, Adrienne Brown, and Shannon Kenney for helpful discussions and Kenneth LeClair and Phillip Sharp for communicating results prior to publication.

This research was supported by a grant (to A.S.B.) from the National Institutes of Health (CA 52515), by a March of Dimes Basil O'Connor Research Award, by an RJR-Nabisco Scholar Award in Immunology, and by an ACS Junior Faculty Research Award (JFRA-309).

ADDENDUM IN PROOF

The plasmids described as encoding human C/EBP forms as well as the recombinant proteins encoded by their respective cDNAs were erroneously named and are actually rat (C/EBP α) or mouse (C/EBP β and C/EBP δ). This does not alter the interpretations of any of the data.

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