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# The p50 subunit of NF-κB associates with the NF-IL6 transcription factor

KENNETH P. LECLAIR*†, MICHAEL A. BLANAR‡, AND PHILLIP A. SHARP*

*Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‡Hormone Research Institute, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0534

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**ABSTRACT** **The NF-κB-p50 polypeptide, a member of the Rel family of transcription factors, was produced as a fusion protein containing amino-terminal peptide additions that facilitate purification and detection with a monoclonal antibody and specific radiolabeling by phosphorylation *in vitro*. The $^{32}$P-labeled NF-κB-p50 fusion polypeptide was used as the probe in Western blotting experiments and in screenings of a bacteriophage expression library to isolate cDNAs encoding interacting protein domains. As expected, cDNAs encoding proteins of the Rel family were identified. Surprisingly, the $^{32}$P-labeled NF-κB protein also specifically bound to proteins encoded by cDNAs for the human NF-IL6 transcription factor. The NF-κB-p50 and NF-IL6 proteins directly interact, and the Rel homology domain and leucine-zipper motif, respectively, are important for this interaction. Since induction of the NF-κB and NF-IL6 factors are important events in immune and acute-phase responses, this interaction could permit coregulation of genes.**

The NF-κB complex was characterized as a critical transcription factor in B-lymphoid cell development (1, 2). Further analysis revealed that it is important in controlling the expression of many inducible genes and that several viruses, including the simian virus 40, cytomegalovirus, and human immunodeficiency viruses, contain at least one copy of an NF-κB binding site in their enhancers (reviewed in ref. 3). Biochemical purification and subsequent molecular cloning experiments demonstrated that the NF-κB transcription factor is a complex of two proteins, NF-κB-p50 and NF-κB-p65, which are members of a larger protein family.

Analysis of the protein encoded by the human NF-κB-p50 cDNA revealed that it is initially synthesized as a 105-kDa polypeptide, which contains several ankyrin repeat domains in the carboxyl-terminal portion (4). Posttranslational processing generates the mature DNA-binding NF-κB-p50 form by removal of the segment containing the ankyrin repeats (5). The processed protein contains an ≈300-amino acid region (the Rel domain) with sequence similarity to proteins encoded by the *rel* oncogene and the dorsal gene of *Drosophila* (reviewed in ref. 6). The Rel domain of NF-κB-p50 is required for DNA binding and for the ability to form homodimers and heterodimers with other Rel family proteins (4, 7). Studies of the c-Rel protein and the retrovirus-transduced form v-Rel indicated that these proteins associate with other cellular proteins (8–10). It is not apparent whether all of the cellular proteins that associate with these Rel proteins are themselves members of the Rel protein family.

To study the interactions between NF-κB-p50 and other cellular proteins, a form of NF-κB-p50 was generated that has a unique antigenic determinant for purification and detection and a separate element that enables labeling *in vitro* by phosphorylation with [γ-$^{32}$P]ATP. When $^{32}$P-labeled NF-κB-p50 was used as a molecular probe in Western blots and in λgt11 expression library screenings, interactions with other members of the Rel protein family were observed. In addition, these analyses indicated that the NF-κB-p50 protein also associates with other cellular proteins that are not members of the Rel family, including the NF-IL6 protein, a transcription factor of the basic domain, leucine-zipper class.

## MATERIALS AND METHODS

**Bacterial Expression.** The NF-κB-p50 cDNA was obtained by the polymerase chain reaction using human Jurkat T-cell cDNA and oligonucleotide primers based on the NF-κB-p50 sequence (4). The construction of the T7 polymerase expression vector (referred to as the FLAG-HMK vector) has been described (11).

The 343-amino acid NF-κB-Spe protein was generated by cleaving and religating the NF-κB cDNA at a unique *Spe* I site. The human c-*rel* cDNA (12) and the NF-IL6 cDNA fragments in phages B and C were subcloned to the FLAG-HMK expression vector to produce the c-Rel, NF-IL6-B, and NF-IL6-C proteins. Recombinant proteins were expressed in the BL21 pLysS bacterial strain after isopropyl β-D-thiogalactoside induction (13).

**Purification and Labeling of Proteins.** NF-κB and NF-IL6 proteins were purified by conventional chromatography or low pH elution from M2 anti-FLAG antibody resin (provided by William Brizzard, IBI–Kodak, New Haven, CT). Proteins were labeled *in vitro* with [γ-$^{32}$P]ATP (NEG-002Z; DuPont/NEN) by using protein kinase (P-2645; Sigma) as described (11).

**Western Blotting and λgt11 Library Screening.** Eukaryotic RIPA extracts (14) and nuclear extracts (15) were prepared, electrophoresed, and transferred to nitrocellulose. Transferred proteins were denatured in 6 M guanidine hydrochloride at 4°C, renatured, and then incubated with $^{32}$P-labeled protein for 4 hr at 4°C in buffer of 1% Nonidet P-40, 50 mM Hepes (pH 7.5), 5 mM DTT, 1 mM EDTA, 1 mM PMSF, and 5 mM $MgCl_2$, with the NaCl concentration indicated in the figure legends.

A λgt11 library constructed from poly(A)$^+$ RNA from γ-interferon-treated HeLa cells (Stratagene) was plated and screened (16, 17) with $^{32}$P-labeled NF-κB-p50 protein as described (11).

**Transcription and Translation *in Vitro*.** The NF-κB-p50 and NF-IL6-C cDNA inserts were subcloned into the Bluescript vector (Stratagene). To generate a shorter form of the NF-κB-p50 protein, the DNA template was digested at the *Spe* I site (18). The NF-IL6 cDNA was linearized at a *Sac* I site to produce a form of the NF-IL6-C protein (NF-IL6-Sac) that terminates at amino acid 306 (19). Linearized DNAs were

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Abbreviations: DTT, dithiothreitol; HMK, heart muscle kinase; IL, interleukin; PMSF, phenylmethylsulfonyl fluoride.

†Present address: Division of Immunology, Department of Medicine, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

transcribed in the presence of $m^7G(5')ppp(5')G$ cap (Pharmacia) using T3 or T7 polymerase (Stratagene). RNA was translated in the presence of [$^{35}$S]methionine (NEG-009A; DuPont/NEN) using rabbit reticulocyte lysate (Promega).

**Immunoprecipitation.** For immunoprecipitation analysis, $^{35}$S-labeled proteins were combined with control bacterial extract (BL21) or with extracts containing NF-κB-p50 or NF-IL6-C proteins in an immunoprecipitation buffer of phosphate-buffered saline with 10% glycerol, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 0.1% Nonidet P-40, 1 mM DTT, 1 mM PMSF, and trypsin inhibitor at 0.02 mg/ml. After addition of M2 anti-FLAG antibody (IBI–Kodak) and protein G beads (Pharmacia), immune complexes were precipitated by centrifugation. Immunoprecipitated proteins were electrophoresed on 12% polyacrylamide/SDS gels and then treated with EN$^3$HANCE solution (DuPont/NEN) for autoradiography.

## RESULTS

**Synthesis of Human NF-κB-p50 with an Antigenic Tag and Phosphorylation Element.** The NF-κB-p50 polypeptide used in these studies terminated after amino acid 438, which is in the immediate vicinity of the suggested site (amino acid 435) for processing (5). A T7 polymerase-driven bacterial expression vector (13) was modified (11) to allow production of an NF-κB-p50 fusion protein with two additions to the amino terminus (Fig. 1*A*). The first element forms an antigenic determinant recognized by the M2 anti-FLAG monoclonal antibody. Immediately following are five amino acids that form the recognition element for HMK (20), which allows *in vitro* labeling with [$\gamma$-$^{32}$P]ATP.

NF-κB-p50 fusion protein, before and after phosphorylation with [$\gamma$-$^{32}$P]ATP by HMK, was tested for its ability to bind NF-κB and related DNA sequence elements in a gel-shift analysis (Fig. 1*B*). Both the standard (lanes 1 and 2) and "reverse-label" (lanes 3–6) gel-shift analyses show that the NF-κB-p50 fusion protein has a higher affinity for the palindromic major histocompatibility complex class I element than it has for the NF-κB element, a property of the native NF-κB-p50 polypeptide (4, 21).

**Western Blots with $^{32}$P-Labeled NF-κB-p50.** The NF-κB-p50 protein forms homodimers and heterodimers with other Rel family proteins (10, 22). To test whether these and other specific interactions could be detected, nitrocellulose membranes containing immobilized proteins were probed with $^{32}$P-labeled NF-κB-p50 protein. Extracts from bacteria expressing the human NF-κB-p50 and c-Rel proteins were included as positive controls along with samples of whole-cell and nuclear extracts from HeLa and Jurkat cells. Because variations in salt concentration can affect protein–protein interactions, the Western blotted membranes were incubated with NF-κB protein probe at two different ionic strengths. At both 250 mM and 150 mM NaCl, the $^{32}$P-labeled NF-κB-p50 protein specifically bound bacterially expressed p50 (κB) and c-Rel (REL) proteins (Fig. 2). When the nitrocellulose filter was probed in 250 mM NaCl, interactions with only a few proteins in whole-cell and nuclear extracts were observed (Fig. 2*A*). Specifically, a strong interaction with a protein of ≈75 kDa, present in all three whole cell extracts, but only in the nuclear extract of activated Jurkat cells, was observed. At the lower salt concentration (150 mM NaCl), the $^{32}$P-labeled p50 protein interacted strongly with an ≈75-kDa protein, as well as with several low molecular mass proteins (≈10 kDa and 35 kDa) and with a larger (≈105 kDa) protein. Speculation as to the identity of such interacting proteins is presented in the *Discussion*.

**λgt11 Library Screening.** The ability of $^{32}$P-labeled protein to specifically associate with domains of other proteins provided the rationale for screening a λgt11 library. Nitrocellulose replicas of recombinant phage plaques were incubated with $^{32}$P-labeled NF-κB-p50 protein with 250 mM NaCl in the incubation buffer to identify two positive phage. One of the cDNA inserts encoded the c-Rel protein and the other encoded a portion of the NF-κB-p105 protein.

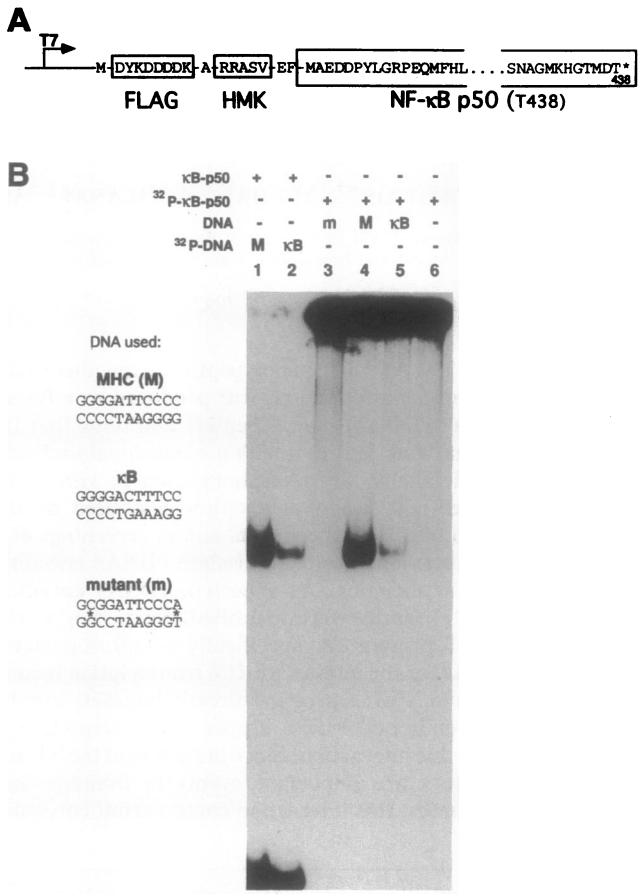


FIG. 1. (*A*) NF-κB-p50 expression vector. A diagram of the bacterial expression vector showing the T7 polymerase transcription initiation site. The initiator methionine is followed by the antigenic (FLAG) epitope and the heart muscle kinase (HMK) labeling site. The protein sequence of the NF-κB-p50 protein shows the translation stop signal (*) introduced after amino acid 438. (*B*) Electrophoretic mobility gel shift of the NF-κB-p50 fusion protein. Lanes 1 and 2 show the results of mixing $^{32}$P-labeled DNA with unlabeled NF-κB-p50 protein, and lanes 3–6 show the results of mixing $^{32}$P-labeled NF-κB-p50 protein and unlabeled DNA. The DNA probes used are indicated at the side of the panel. DNA M is the major histocompatibility complex class I gene promoter sequence, DNA m is a double-point mutant of the M sequence, and DNA κB is the NF-κB binding element of the immunoglobulin κ enhancer.

A second λgt11 library screen was performed by using 150 mM NaCl in the incubation buffer to identify eight additional recombinant phage. Four of these phage contained cDNAs that encode small molecular mass proteins that have a structural domain related to the high mobility group family proteins. Two of the other cDNAs were partially sequenced and were found to be overlapping fragments of the same cDNA. This cDNA sequence is not represented in any available data bases, and its nucleotide and predicted amino acid sequences also suggest that it is not a Rel protein family member.

The cDNA inserts of the two remaining recombinant phage isolates (B and C) were sequenced and shown to be fragments of the human NF-IL6 cDNA. The NF-IL6 transcription factor belongs to the C/EBP family of proteins that contain a region of basic amino acids adjacent to a leucine-zipper element (19). Fig. 3 shows the NF-IL6 amino acid sequence aligned with the deduced protein sequences of the cDNAs

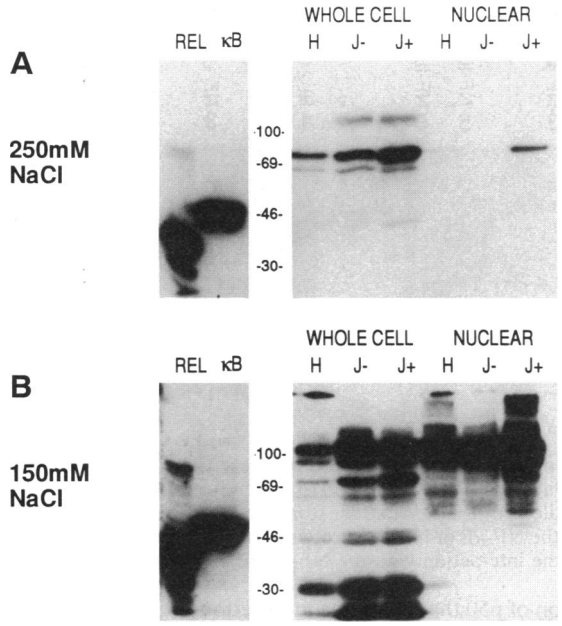


FIG. 2. Western blotting with $^{32}$P-labeled NF-κB-p50 protein. (*A* and *B*) Results of incubating nitrocellulose filters containing immobilized SDS/PAGE electrophoresed proteins with $^{32}$P-labeled NF-κB-p50 protein. The results of blotting with 250 mM (*A*) and with 150 mM (*B*) NaCl in the buffers are shown. Gel lanes "REL" and "κB" contain crude bacterial extracts containing human c-Rel and NF-κB-p50 protein, respectively. Whole-cell and nuclear extracts from HeLa cells (H), nonstimulated Jurkat cells (J−), and Jurkat cells after a 6-hr stimulation with phytohaemagglutinin and phorbol myristate acetate (J+) are loaded where indicated. The positions of $^{14}$C-labeled protein standards (in kDa) are indicated.

contained in recombinant phages B and C. The region of overlap immediately identified the last 159 amino acids of the NF-IL6 protein as adequate for association with the NF-κB-p50 protein.

**The NF-IL6 and NF-κB-p50 Proteins Interact on Western Blots.** A direct interaction between the NF-κB-p50 and NF-IL6 proteins was demonstrated by probing Western blots with $^{32}$P-labeled proteins. The NF-IL6-B and -C cDNA fragments were subcloned into the FLAG-HMK vector. These NF-IL6-B and NF-IL6-C proteins expressed in bacteria formed homodimers and heterodimers with each other and bound the NF-IL6 DNA element (data not shown). Western blots of the host bacterial cell extracts (control BL21) and of cells expressing NF-κB-p50, NF-IL6-B, and NF-IL6-C proteins were analyzed. Also included was an extract of a truncated NF-κB protein (NF-κB-Spe) that has only a partial Rel homology domain (4, 18). Purified NF-κB-p50 and NF-IL6-C proteins were $^{32}$P-labeled by incubation with HMK, and each was used to probe one blot (Fig. 4). The

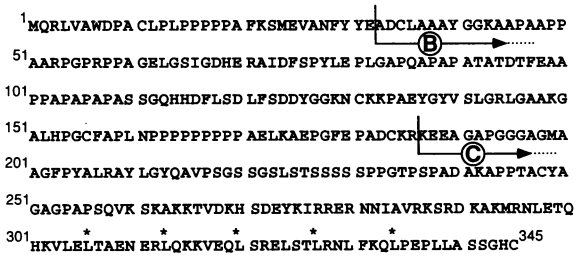


FIG. 3. Human NF-IL6 sequence. The amino acid sequence of the human NF-IL6 protein (19) is shown; the leucine residues of the leucine zipper are denoted by asterisks. The portions of the NF-IL6 protein found in bacteriophages B and C are indicated.

$^{32}$P-labeled NF-κB-p50 protein recognized itself and the NF-IL6-B and -C proteins, but not the truncated NF-κB-Spe protein or any other bacterial protein in the control extract. Similarly, the $^{32}$P-labeled NF-IL6-C protein bound only itself, the NF-IL6-B protein, and, to a lesser degree, the NF-κB-p50 protein. This strongly suggests that the two proteins, NF-κB-p50 and NF-IL6, directly interact.

**Immune Coprecipitation of NF-κB-p50 and NF-IL6.** Immunoprecipitations were performed to establish which domains of the NF-κB-p50 and NF-IL6 proteins are necessary for the association between these proteins. The antigenic FLAG epitope on the bacterially expressed NF-κB-p50 and NF-IL6-C proteins allowed use of the M2 anti-FLAG monoclonal antibody in the immunoprecipitations. The NF-κB-p50 and NF-IL6-C cDNAs were transcribed and translated *in vitro* to produce proteins labeled with [$^{35}$S]methionine, but lacking the FLAG determinant. Unlabeled, bacterially expressed NF-κB-p50 or NF-IL6 proteins (with FLAG elements) or control bacterial extracts (BL21) were separately combined with the [$^{35}$S]methionine-labeled NF-κB-p50 or NF-IL6-C proteins, and complexes were immunoprecipitated with the M2 anti-FLAG monoclonal antibody (Fig. 5). The $^{35}$S-labeled NF-κB-p50 protein was efficiently immune coprecipitated both through association with the bacterially expressed NF-κB-p50 protein (Fig. 5*A*, lane 3) as well as through association with the NF-IL6 protein (Fig. 5*A*, lane 5). Likewise, $^{35}$S-labeled NF-IL6-C protein was efficiently immune coprecipitated via an association with the bacterially expressed form of itself (Fig. 5*B*, lane 5) or with the NF-κB-p50 protein (Fig. 5*B*, lane 3).

To determine which portions of the NF-κB-p50 and NF-IL6 proteins were responsible for the observed interaction, shorter forms of these proteins (NF-κB-Spe and NF-IL6-Sac) were produced by *in vitro* translation of RNAs transcribed from truncated cDNA templates. The NF-κB-Spe protein was not immune coprecipitated by either the bacterially expressed NF-κB-p50 (Fig. 5*A*, lane 7) or NF-IL6-C proteins (Fig. 5*A*, lane 9). In a similar fashion, truncation of the NF-IL6 cDNA at the *Sac* I site generated a protein lacking the leucine-zipper motif contained in the last 41 amino acids (19). As expected, this truncated protein was not immune coprecipitated by the bacterially expressed NF-IL6-C protein (Fig. 5*B*, lane 9). More interestingly, this truncated form of the NF-IL6-C protein also was not immune coprecipitated by the bacterially expressed NF-κB-p50 (Fig. 5*B*, lane 7).

## DISCUSSION

Observations of specific protein–protein interactions are increasingly common in the study of the activities of transcription factors. In addition to interactions between individual proteins within a given family of factors, recent reports suggest that interactions between members of disparate classes of proteins are possible. For example, proteins containing a helix–loop–helix element have been shown to interact with the AP-1 family of leucine-zipper proteins (11, 23). By applying a generally applicable method in which a bacterially expressed fusion protein is specifically $^{32}$P-labeled *in vitro* and used as a molecular probe, we have demonstrated that the p50 subunit of the NF-κB complex interacts with the NF-IL6 protein, a member of the C/EBP class of leucine-zipper transcription factors.

Use of *in vitro* phosphorylated NF-κB-p50 protein in a modified Western blotting technique revealed that, whereas the $^{32}$P-labeled NF-κB-p50 interacted with itself or with the c-Rel protein when incubated at either 150 mM or 250 mM NaCl, interactions with other proteins were sensitive to the ionic strength of the buffer. For example, at 250 mM NaCl, the $^{32}$P-labeled NF-κB-p50 interacted predominantly with proteins with relative mobilities of ≈75 kDa and 115 kDa.

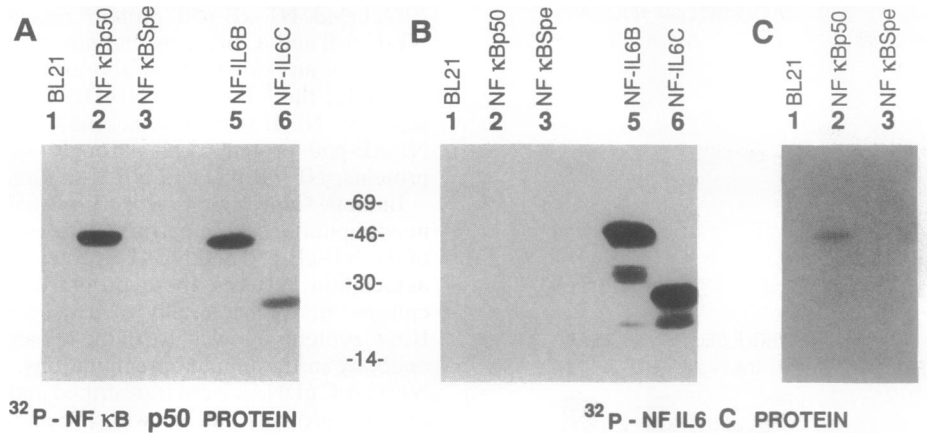


FIG. 4. Western blotting of bacterial extracts. Replicate nitrocellulose membranes containing electrophoresed and transferred bacterial extract proteins were probed with $^{32}$P-labeled NF-κB-p50 protein (*A*) or with $^{32}$P-labeled NF-IL6-C protein (*B* and *C*) in phosphate-buffered saline (150 mM NaCl). The positions of $^{14}$C-labeled protein standards (in kDa) are indicated. Lane 1, extract from the BL21 pLysS bacteria (BL21) as a control. Lanes 2, 3, 5, and 6, extracts from BL21 pLysS bacteria expressing the NF-κB or NF-IL6 protein form indicated. A longer exposure of lanes 1–3 of the $^{32}$P-NF-IL6-probed membranes is shown in *C* to illustrate the interaction with the NF-κB-p50 protein.

Other less intense bands were observed, including ones at ≈50 kDa and 65 kDa, which would correspond to the cellular NF-κB-p50 and -p65 proteins. The ≈75-kDa protein that interacts with the NF-κB-p50 protein is present in both HeLa and Jurkat whole-cell extracts, but is found only in the nuclear extract of stimulated Jurkat T cells. Such nuclear translocation is a hallmark of the NF-κB-p65 protein, but the size of this protein on this gel is not consistent with it being NF-κB-p65 (22, 24).

When the Western blot was developed at 150 mM NaCl, additional interactions with several low molecular mass proteins of ≈10 kDa, 35 kDa, and 45 kDa and with larger proteins of ≈90 kDa and 105 kDa were detected. The molecular mass of the 105-kDa protein suggests that it might be the cellular NF-κB-p105 protein that is the NF-κB-p50 precursor (4, 7). If this protein is the p105 precursor, it is present at a surprisingly high level compared to that of the mature p50 protein. From the nature of cDNAs identified by screening the λgt11 library at the 150 mM NaCl condition, we suggest that the 10-kDa protein could be the small molecular mass high mobility group protein. The NF-IL6 protein whose cDNA was isolated twice during the screen at 150 mM NaCl has a molecular mass of 38 kDa and could therefore correspond to the ≈35-kDa band observed to interact with the $^{32}$P-labeled NF-κB-p50 protein. Any one of the other bands that interact with the $^{32}$P-labeled NF-κB-p50 protein could correspond to the protein encoded by the cDNA that remains to be characterized.

The human NF-IL6 cDNA was originally cloned (19) by screening λgt11 expression libraries with $^{32}$P-labeled DNA-binding site probes (16, 17). The NF-IL6 protein is a member of the C/EBP family of transcription factors, which have several basic amino acids adjacent to a leucine-zipper structural motif composed of a heptad repeat of leucine residues (25). The leucine-zipper structure facilitates the formation of homodimers and heterodimers with certain other leucine-zipper protein family members (reviewed in ref. 26). Surprisingly, immune coprecipitation studies indicate that the leucine-zipper motif is also essential for the ability of the NF-IL6 protein to interact with the NF-κB-p50 protein. The NF-κB-p50 protein does not interact with all zipper structures since the c-jun protein is not immune coprecipitated when incubated with NF-κB-p50 protein (data not shown). The basis of the specificity of NF-κB for interactions with these leucine-zipper tracts has not been determined. The interaction between the NF-IL6 and NF-κB-p50 proteins is dependent on an intact Rel homology domain in NF-κB-p50, the same region of p50 that mediates interactions with other Rel family members. It is not known whether the precise determinants in NF-κB-p50 that control interactions with c-Rel-related proteins and with NF-IL6 are similar.

A recent report indicates that the rat NF-IL6 protein undergoes a cAMP-dependent nuclear translocation and contributes to the activation of c-*fos* transcription by binding to the serum response DNA element (27). This study also showed that anti-NF-IL6 antibody coprecipitated two cellular proteins of 43 kDa and 65 kDa, which are antigenically unrelated to NF-IL6. With the finding that a direct physical interaction occurs between NF-κB and NF-IL6, the possibility that these associated proteins might be related to the proteins of the NF-κB-p50/p65 heterodimer complex warrants investigation.

Although NF-κB-p50 and NF-IL6 proteins interact in solution, it has proven difficult to demonstrate a similar interaction between these two proteins when bound to DNA. Probe DNAs with binding sites for both proteins have been used unsuccessfully to test for cooperative interactions. However, it has been reproducibly observed that the presence of the NF-IL6 protein specifically augments the amount of NF-κB-p50 dimer bound to the NF-κB DNA probe on gel shifts when compared to the same amount of NF-κB-p50 without NF-IL6 protein present (data not shown). The molecular basis for this observation remains to be elucidated.

NF-IL6 gene expression is induced by stimulation of cells with interleukin (IL) 1, IL-6, and tumor necrosis factor α (19). The NF-IL6 protein was defined as a transcription factor that binds to the IL-1-responsive element in the IL-6 gene promoter to control its expression (28). The NF-IL6 factor is also induced during the acute-phase response of cells to inflammation and acts to control the expression of acute-phase protein and several cytokine genes, including the tumor necrosis factor α, IL-8, and granulocyte–colony-stimulating factor genes (29). Studies have also indicated that in addition to the NF-IL6 factor, NF-κB factor induction is required for maximal IL-6 gene expression in response to inflammatory cytokines such as tumor necrosis factor α or IL-1 (30, 31). The IL-6 gene promoter has an NF-IL6 binding site located ≈70 base pairs 5′ of the NF-κB DNA element (28). Analysis of the promoters of other immune response or acute-phase response genes reveals that binding sites for both NF-κB and NF-IL6 are frequently present. For example, the IL-8 gene promoter contains binding site elements for these two factors that are directly adjacent, yet not overlapping (32). In contrast, the promoter of the gene encoding the acute-phase

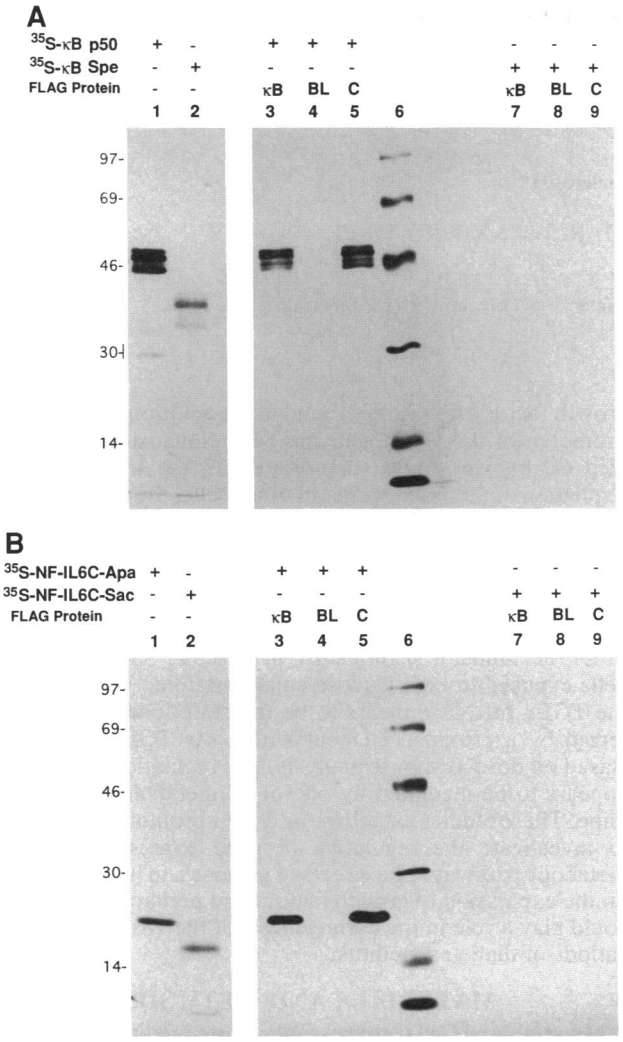


FIG. 5. Immune coprecipitations. (*A*) *In vitro*-translated, $^{35}$S-labeled NF-κB-p50 protein (shown in lane 1) or the shorter NF-κB-Spe form (lane 2) was combined with bacterial extract, precipitated with the M2 anti-FLAG monoclonal antibody, and run on SDS/polyacrylamide gels. Lanes 3–5 show the material immunoprecipitated by mixing equal amounts of the *in vitro*-translated NF-κB-p50 protein with bacterial extract containing NF-κB-p50 protein (κB; lane 3), with control bacterial extract (BL; lane 4), or with the NF-IL6-C protein (C; lane 5). Lanes 7–9 show the immunoprecipitation of the smaller NF-κB-Spe protein after incubation with bacterial extracts containing NF-κB-p50 protein (κB; lane 7), with control bacterial extract (BL; lane 8), or with the NF-IL6-C protein (C; lane 9). (*B*) Lane 1 shows the *in vitro* translated, $^{35}$S-labeled NF-IL6-C protein (NF-IL6C-Apa) and lane 2 shows the form of the NF-IL-C protein lacking the leucine-zipper portion (NF-IL6C-Sac). Lanes 3–5 show the material immunoprecipitated by mixing equal amounts of $^{35}$S-radiolabeled, *in vitro*-translated NF-IL6-C protein with bacterial extract containing NF-κB-p50 protein (κB; lane 3), with control bacterial extract (BL; lane 4), or with the NF-IL6-C protein (C; lane 5). Lanes 7–9 show the immunoprecipitation of the smaller NF-IL-C protein after incubation with bacterial extract containing NF-κB-p50 protein (κB; lane 7), with control bacterial extract (BL; lane 8), or with the NF-IL6-C protein (C; lane 9). The positions of $^{14}$C-labeled protein standards (in kDa) are shown in lane 6.

response protein angiotensinogen has overlapping binding sites for NF-κB and NF-IL6 (33, 34). In view of the overlapping signals that control NF-κB and NF-IL6 activities and the relative positioning of their binding sites in these promoters, the interaction of subunits of these two transcription factors could be highly important in regulating cellular physiology.

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