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Functional Interaction of the v-Rel and c-Rel Oncoproteins with the TATA-Binding Protein and Association with Transcription Factor IIB

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Rel family proteins regulate the expression of genes linked to κB-binding motifs. Little is known, however, of the mechanism by which they enhance transcription. We have investigated the ability of the v-Rel and c-Rel oncoproteins to interact with components of the basal transcription machinery. Here we report that both the acidic transcription activation domain mapping to the unique C terminus of chicken c-Rel and the F9 cell-specific activation region common to both v-Rel and c-Rel interact with the TATA-binding protein (TBP) and transcription factor IIB (TFIIB) in vitro and in vivo. We also demonstrate that TBP interaction with Rel activation regions leads to synergistic activation of transcription of a κB-linked reporter gene. Combined with the observation that the mouse c-Rel and human RelA proteins also interact with TBP and TFIIB in vitro, these results suggest that association with basal transcription factors is important for the transcriptional activities of Rel family proteins.

The v-Rel, c-Rel, RelA, and RelB proteins belong to the Rel/NF-κB family of transcription factors (reviewed in references 2 and 3) and modulate expression of genes linked to κB DNA-binding motifs such as those present in the human immunodeficiency virus long terminal repeat and the interleukin 2 receptor α (IL-2Rα) promoters (1, 21, 32, 37). Despite extensive sequence similarity in the N-terminal Rel homology region (RHR), responsible for DNA binding and protein dimerization, sequences responsible for activation of transcription by Rel family members differ greatly (reviewed in references 2 and 3). Two distinct activation regions have been described for the chicken c-Rel protein. The first region identified maps to its acidic C terminus and is responsible for activation of transcription in a wide variety of cells (23, 24). A second activation domain was recently localized to a region common to both v-Rel and chicken c-Rel, 3′ to the RHR (23, 40, 47) and shown to selectively activate transcription in undifferentiated cells (22, 23, 47). Interestingly, this region is required for v-Rel-induced transformation of avian lymphoid cells (10, 12, 40, 48) as well as for Rel-induced differentiation of P19 embryonal carcinoma cells (22).

Initiation of eukaryotic transcription involves the cooperative binding of the TATA-binding protein (TBP) and transcription factor IIA (TFIIA) to the TATA element of the promoter and then the binding of TFIIB, that of RNA polymerase II, and those of other essential factors (reviewed in references 49 and 50). The binding of TFIIB is believed to be a rate-limiting step in the formation of the preinitiation complex that may be facilitated by sequence-specific transcriptional activators (30, 31; reviewed in reference 50). These proteins are believed to accelerate assembly of the complex by directly contacting TBP and/or TFIIB or by interacting with coactivators to bridge their interaction with basal initiation factors (30, 31; reviewed in reference 18). Activator proteins such as herpes simplex virus VP16 (31, 43), adenovirus E1A (20, 26), p53 (41, 45), transcription factor PU.1 (16), *Drosophila* Fushi tarazu (8), cytomegalovirus (CMV) IE2 (17), and the Epstein-Barr virus Zta protein (28) have indeed been shown to bind directly to TBP and/or TFIIB in vitro (reviewed in reference 14), whereas other activators such as Sp1 do not (16).

Given the central role that Rel and NF-κB proteins play in cell differentiation and transformation and the accumulating evidence for their transcriptional activity, it is important to understand the details of Rel-mediated activation. To gain an insight into the mechanism by which Rel activation regions enhance gene expression, we have investigated the ability of Rel proteins to interact with basal transcription factors. Here we report that both the acidic activation domain in the unique C terminus of chicken c-Rel and the F9 cell-specific activation region common to both v-Rel and c-Rel interact with TBP and TFIIB in vitro and in vivo. We also demonstrate that Rel and TBP can function in synergy to stimulate κB-dependent transcription. The observation that other Rel family members can also interact with TBP and TFIIB suggests that association of Rel proteins with general transcription factors is important for the transcriptional activity of Rel and NF-κB proteins.

MATERIALS AND METHODS

Plasmids. v-HincII lacks 172 amino acids from the C terminus of v-Rel because of the insertion of a stop codon at a unique *Hin*cII site (25). Likewise, c-HincII, c-AccI and c-RelΔXbaI, respectively, lack 276, 103, and 56 amino acids from the C terminus of chicken c-Rel. The control b10 gene contains a stop codon positioned two residues downstream of the initiating ATG of v-Rel (32). Rel/NF-κB-related proteins were derived from the chicken c-Rel (5a), mouse c-Rel

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(5), mouse p105 NF-κB precursor (11), and human RelA genes (38). pCG147 encodes the yeast GAL4 DNA-binding domain (39). GAL4-Rel fusion genes derived from the pCG vector (44) encode the yeast GAL4 DNA-binding domain (residues 1 to 147) fused in frame to residues 323 to 598 (pCG147/CCRHB), 323 to 495 (pCG147/CCRHA), 323 to 394 (pCG147/CCRHE), 323 to 542 (pCG147/CCRHX), or 514 to 598 (pCG147/CCRPB) of chicken c-Rel (23). Rel genes were expressed in vitro from the SP6 promoter of pGem-2 (v-Rel, v-HincII, and p105), the T7 promoter of pSelect-1 (chicken and mouse c-Rel and c-HincII), the SP6 promoter of pSelect-1 (GAL4-Rel fusions) or the T3 promoter of pBluescript (RelA [38]). Glutathione *S*-transferase (GST)-TBP, GST-TFIIB, and GST-TBPΔ166-335, deleted of the conserved C-terminal basic core of TBP, were described previously (15).

Rel proteins and TBP were expressed in vivo under the control of the CMV immediate-early promoter of pJDCMV19SV (9). pIL6CAT expresses the chloramphenicol acetyltransferase (CAT) reporter gene from the IL-6 promoter with three copies of the IL-6–κB DNA-binding motif (34). κB_2TKCAT contains two copies of an oligonucleotide with two NF-κB motifs derived from nucleotides −106 to −78 of the human immunodeficiency virus long terminal repeat inserted into the *Bam*HI site of pBLCAT2 (23). pG5BCAT contains five copies of the yeast GAL4 DNA-binding motif inserted upstream of the adenovirus E1B gene TATA box (29).

GST pull-down assays. GST fusion proteins were synthesized in *Escherichia coli* and immobilized on glutathione-agarose beads, as described by the manufacturer (Pharmacia). [^{35}S]methionine- or [^{35}S]methionine- and [^{35}S]cysteine-labeled Rel proteins were produced by in vitro transcription and translation in rabbit reticulocyte lysates, and equal amounts of labeled proteins were immunoprecipitated with antibodies raised against a 12-mer peptide (MDFLTNLRFTEC; Multiple Peptide Systems, San Diego, Calif.) corresponding to the unique N-terminal envelope-derived sequence of v-Rel (Ab 1967) or with antibodies that recognize the RHR (Ab 3 [12]). Parallel translation reaction mixtures were incubated with 2 μg of GST, GST-TBP, GST-TBPΔ166-335 (residues 1 to 165, containing the species-specific amino terminus of human TBP), or GST-TFIIB fusion proteins immobilized on glutathione-agarose beads for 2 h at 4°C in 0.2 ml of buffer containing 40 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES pH 7.5), 100 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 0.5% Nonidet P-40 (NP-40), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) (15). Complexes were washed four times with the same buffer containing 200 mM KCl and once with phosphate-buffered saline (PBS). Proteins were eluted with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer and resolved by electrophoresis.

Coimmunoprecipitation assays. v-Rel, v-HincII and human TBP (hTBP) RNAs were individually translated in rabbit reticulocyte lysates in the presence or absence of [^{35}S]methionine or [^{35}S]methionine and [^{35}S]cysteine. Equal amounts of unlabeled v-Rel and v-HincII proteins, on the basis of the quantitation of parallel radiolabeled translations, were incubated with ^{35}S-hTBP for 1 h at 30°C in 50 μl of buffer containing 20 mM HEPES (pH 7.3), 50 mM KCl, 1 mM DTT, 50 μg of bovine serum albumin, and 10% glycerol, prior to immunoprecipitation with Ab 1967 specific for the N terminus of v-Rel. Immune complexes were washed four times with 1 ml of buffer C (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.4% NP-40, 0.2% Triton X-100, 0.1 mM PMSF), eluted, and resolved by SDS-PAGE.

The in vivo association of Rel proteins with endogenous chicken TBP and TFIIB was assayed in whole-cell extracts from v-Rel-transformed (Rev-T) or c-Rel-transformed (CCR-1) chicken spleen cells, prepared as previously described (25). Briefly, cells were washed twice in PBS, lysed in ice-cold TNT buffer (20 mM Tris [pH 7.5], 200 mM NaCl, 1% Triton X-100, 1 mM PMSF, 200 kallikrein units of aprotinin [42]) and adjusted to 15% glycerol. To facilitate the detection of endogenous Rel-TBP and Rel-TFIIB complexes, extracts from the equivalent of 2×10^6 Rev-T or 4×10^6 CCR-1 cells were incubated with 50 fmol of ^{32}P- and bromodeoxyuridine-labeled double-stranded oligonucleotides containing one copy of the IL-2Rα κB-binding site, UV cross-linked, and resuspended in ELB buffer (50 mM HEPES [pH 7.0], 250 mM NaCl, 5 mM EDTA, 0.5 mM DTT, 0.1% NP-40, 1 mM PMSF). Lysates were precleared with protein A-Sepharose and incubated with affinity-purified anti-hTBP or anti-hTFIIB rabbit antisera (15). Immune complexes were precipitated with protein A-Sepharose and washed four times with ELB buffer and once with PBS prior to elution and electrophoresis. Control immunoprecipitations were performed in SDS buffer (20 mM Tris [pH 7.4], 100 mM NaCl, 5 mM $MgCl_2$, 0.5% SDS, 1% NP-40, 1% aprotinin).

Cell transfection and CAT assays. COS-7 simian virus 40-transformed African green monkey kidney cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transfections were performed by a modified calcium phosphate procedure, as previously described (6). Cells (3×10^5) were transfected with 0.5 μg of Rel plasmid DNA, 0.5 μg of b10 control DNA, 3 μg of pIL6CAT, 3 μg of calf thymus carrier DNA, and 5 μg of CMV-TBP or control CMV19SV DNA. The transcriptional activity of GAL4-Rel proteins was determined by transfection of 2 μg of GAL4-Rel plasmid DNA, 3 μg of pG5BCAT, 3 μg of calf thymus DNA, and 5 μg of CMV-TBP or control CMV19SV DNA. In all cases, the total amount of DNA transfected (15 μg) was kept constant by the addition of pGem-2 DNA. Transfections of undifferentiated mouse F9 teratocarcinoma stem cells were performed with 1.5 μg of Rel vectors, 3 μg of κB_2TKCAT, and 15 μg of CMV-TBP or CMV19SV DNA by the calcium phosphate procedure (6). Extracts were prepared 48 h posttransfection and normalized to protein concentration as determined by the method of Bradford (4). Assays were performed with 25 μg of total protein for 2 h, as described previously (13, 35). Although the extent of CAT activation varied somewhat between transfection assays, the absolute effect of TBP on Rel-mediated activation was consistent.

RESULTS

In vitro association of TBP and TFIIB with the transcription activation regions of v-Rel and c-Rel. Recently reported interactions between acidic activators of transcription and basal transcription factors led us to investigate whether Rel proteins could interact with human TBP and TFIIB. ^{35}S-labeled in vitro-translated v-Rel and c-Rel proteins (Fig. 1A) were incubated with TBP or TFIIB proteins produced as bacterial fusions to GST and tested for association in GST pull-down assays. Complexes of v-Rel or chicken c-Rel with GST-TBP (Fig. 1B, lanes 4 and 16) or GST-TFIIB (Fig. 1B, lanes 5 and 12) were easily detected over the background association of Rel proteins with the control GST protein

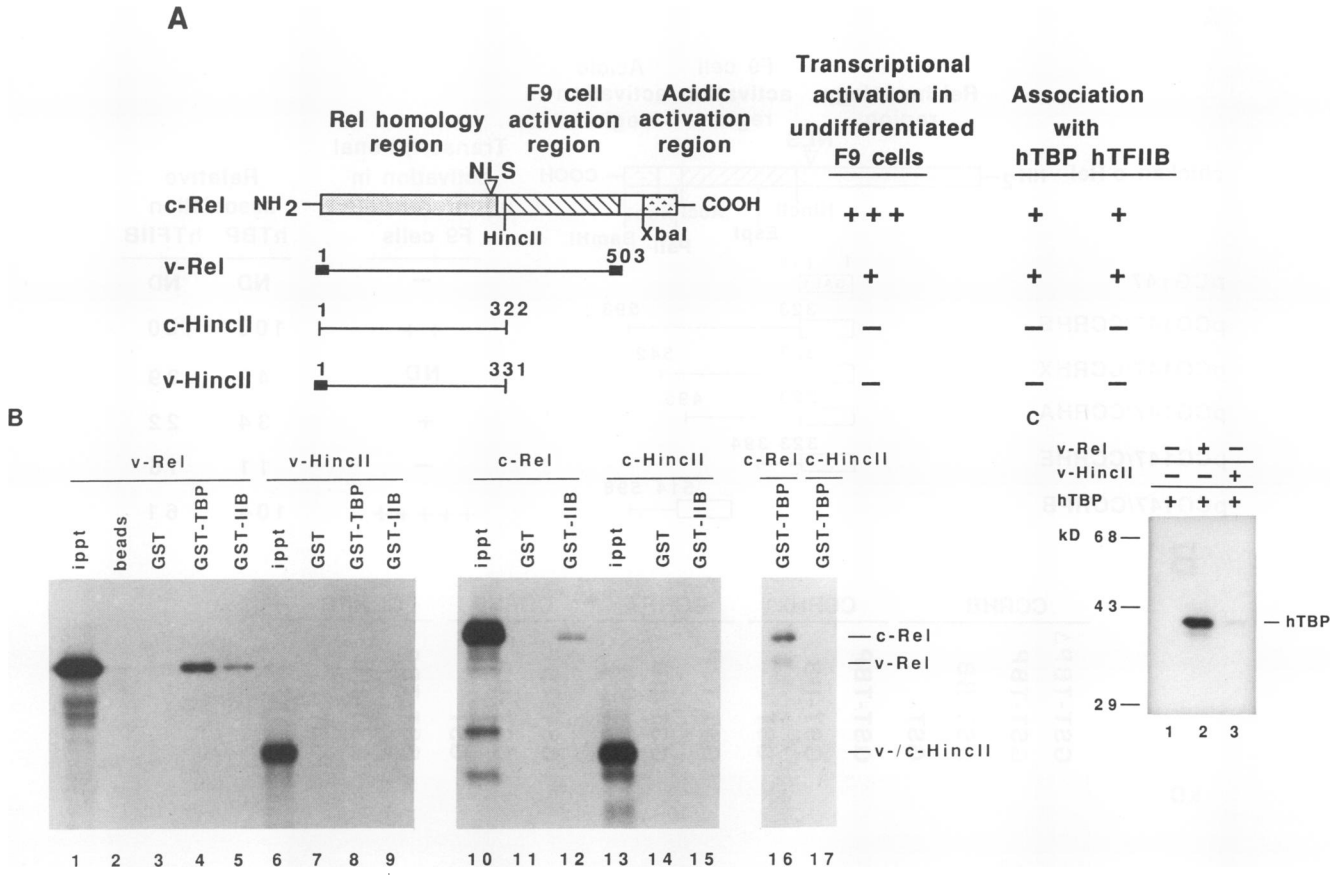

FIG. 1. (A) Structure, transcriptional activity, and in vitro association of v-Rel and c-Rel proteins with hTBP and TFIIB. (B) In vitro interaction of v-Rel and c-Rel with GST-TBP and GST-TFIIB. Equal amounts of in vitro-translated [^{35}S]methionine (v-Rel and c-Rel)- or [^{35}S]methionine- and [^{35}S]cysteine (v-HincII and c-HincII)-labeled Rel proteins were incubated with glutathione-agarose beads (lane 2) or with GST (lanes 3, 7, 11, and 14), GST-TBP (lanes 4, 8, 16, and 17), or GST-TFIIB (lanes 5, 9, 12, and 15) bound to glutathione-agarose, washed extensively, and resolved by SDS–15% PAGE. As a control, Rel proteins were immunoprecipitated with Ab 1967 specific for the N terminus of v-Rel (lanes 1 and 6) or Ab 3 that recognizes the RHR (lanes 10 and 13). (C) Coimmunoprecipitation of in vitro-translated TBP with v-Rel. ^{35}S-labeled hTBP was incubated with equal amounts of in vitro-translated unlabeled v-Rel (lane 2) or v-HincII (lane 3) prior to immunoprecipitation with Ab 1967 specific for the N terminus of v-Rel. ^{35}S-TBP was immunoprecipitated with Ab 1967 as a control (lane 1). Proteins were resolved by SDS–12.5% PAGE. The position of hTBP is indicated.

(Fig. 1B, lanes 3 and 11). In sharp contrast, v-Rel and c-Rel mutants deleted of sequences 3′ to the RHR failed to associate with either TBP or TFIIB (v-HincII and c-HincII [Fig. 1A and B, lanes 8, 9, 15, and 17]). Similar results were obtained in coimmunoprecipitation assays of ^{35}S-labeled hTBP with unlabeled in vitro-translated v-Rel and v-HincII proteins (Fig. 1C, lanes 2 and 3), as well as in GST pull-down assays with proteins produced in wheat germ extracts (data not shown). These experiments indicate that both v-Rel and c-Rel can interact with TBP and TFIIB in vitro and show that RHR sequences are not sufficient for this interaction, as deletion of sequences mapping 3′ to the RHR and comprising both Rel transcription activation regions abolished Rel association with TBP and TFIIB.

To identify Rel sequences important for these interactions and to investigate the possible participation of Rel activation regions in these contacts, we analyzed the association of GST-TBP and GST-TFIIB with hybrid proteins containing the yeast GAL4 DNA-binding domain fused to chicken c-Rel sequences mapping 3′ to the RHR. The structure of the hybrids analyzed and their transcriptional activities in undifferentiated F9 cells are shown in Fig. 2A. ^{35}S-labeled GAL4-Rel fusion proteins were produced by in vitro translation, resolved by SDS-PAGE, and quantitated by phosphorimage analysis. Equivalent amounts of labeled proteins were assayed for association with GST-TBP and GST-TFIIB in GST pull-down assays. A GAL4-Rel fusion containing all of c-Rel sequences mapping 3′ to the RHR associated efficiently with both GST-TBP and with GST-TFIIB (Fig. 2A, pCG147/CCRHB; Fig. 2B, lanes 2 and 3) but showed no significant association with the GST control or with GST fused to the N terminus of hTBP (GST-TBPΔ166-335) (Fig. 2B, lanes 1 and 4).

GAL4-Rel fusion proteins CCRHX, -HA, -HE, and -PB, containing various subfragments derived from the C-terminal half of c-Rel, were used to further delineate the interacting domains of c-Rel. Deletion of the acidic transcription activation region mapping to the unique C terminus of c-Rel led to a twofold decrease in association with GST-TBP in comparison to that seen with the intact C terminus of c-Rel (Fig. 2B, lanes 2 and 5, compare CCRHB with CCRHX). Likewise, association of the CCRHX protein with GST-TFIIB was decreased 2.5-fold by this deletion (Fig. 2B, lane 6). Conversely, a GAL4-Rel fusion protein containing the

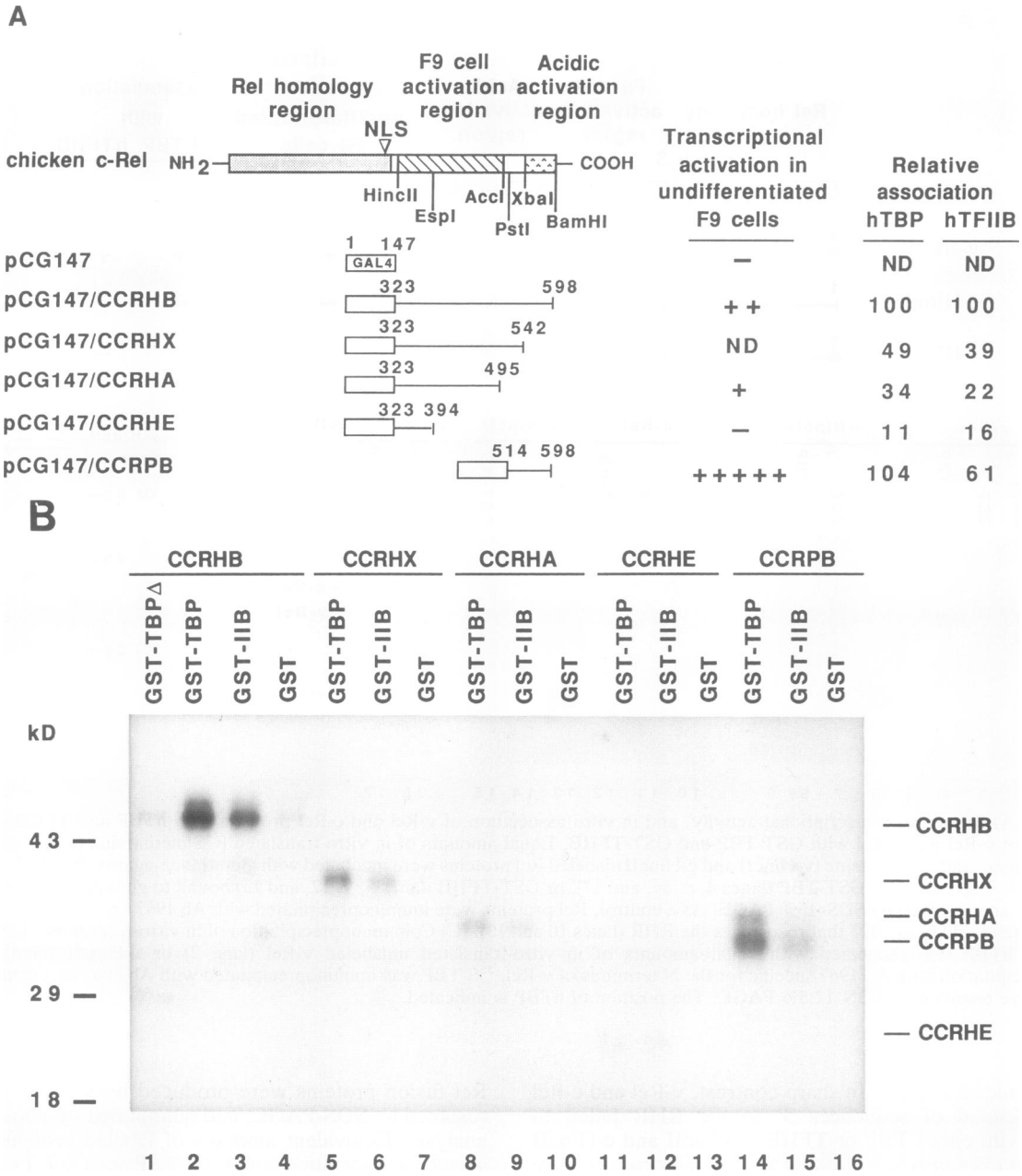

FIG. 2. (A) Structure, transcriptional activity, and in vitro association of GAL4-Rel fusion proteins with hTBP and TFIIB. (B) Mapping of TBP- and TFIIB-binding sites in the C-terminal half of chicken c-Rel by GST pull-down assays. Equal amounts of in vitro-translated [^{35}S]cysteine-labeled GAL4-Rel fusion proteins pCG147/CCRHB (lanes 1 to 4), pCG147/CCRHX (lanes 5 to 7), pCG147/CCRHA (lanes 8 to 10), pCG147/CCRHE (lanes 11 to 13), or pCG147/CCRPB (lanes 14 to 16) were incubated with GST-TBPΔ166-335 (lane 1), GST (lanes 4, 7, 10, 13, and 16), GST-TBP (lanes 2, 5, 8, 11, and 14), or GST-TFIIB (lanes 3, 6, 9, 12, and 15) fusion proteins bound to glutathione-agarose, washed extensively, and resolved by SDS–15% PAGE.

unique C-terminal acidic activation region of c-Rel showed efficient association with GST-TBP and GST-TFIIB (Fig. 2B, lanes 14 and 15, CCRPB).

Sequences mapping between the *Hin*cII and *Acc*I sites of chicken c-Rel (residues 323 to 495) also associated with GST-TBP and GST-TFIIB, although these interactions were respectively 3- and 4.5-fold-less efficient than those seen with the intact C terminus of c-Rel (Fig. 2B, lanes 8 and 9, CCRHA). This region is well conserved between v-Rel and c-Rel and includes sequences capable of activating transcription in undifferentiated cells (23, 40, 47) and essential for promoting the differentiation of P19 embryonal carcinoma cells (22) as well as v-Rel-induced transformation (10, 12, 40, 48). C-terminal deletion of c-Rel sequences in the CCRHA mutant virtually abolished Rel association with both TBP and TFIIB (Fig. 2B, lanes 11 and 12, CCRHE). Combined, these results point to the interaction of v-Rel and c-Rel transcription activation regions with TBP and TFIIB.

Several members of the Rel family associate with TBP and TFIIB. The significant contribution of Rel and NF-κB pro-

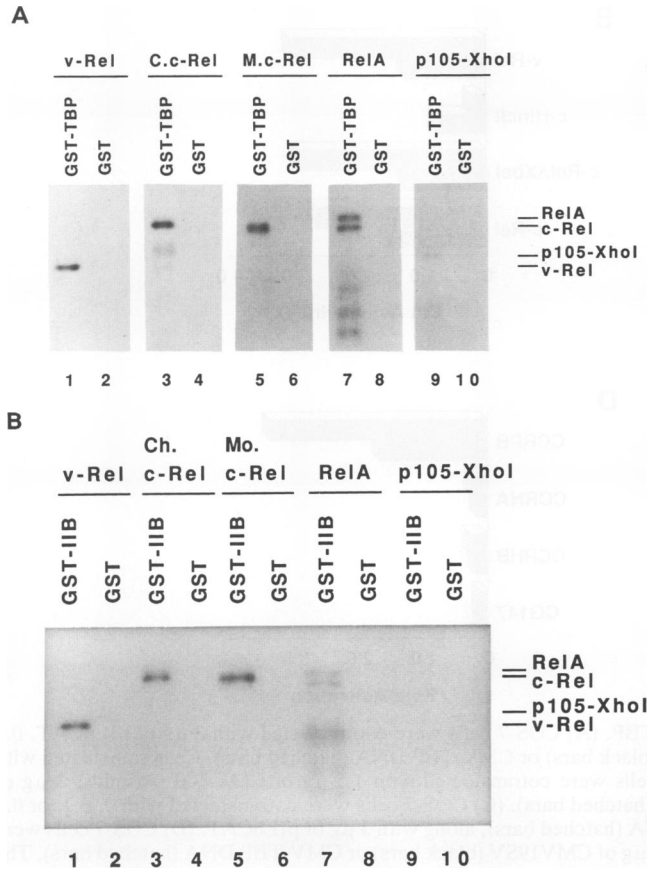

FIG. 3. (A) In vitro interaction of hTBP with members of the Rel/NF-κB family. [^{35}S]methionine-labeled v-Rel (lanes 1 and 2), chicken c-Rel (lanes 3 and 4), mouse c-Rel (lanes 5 and 6), mouse p105-XhoI (lanes 9 and 10), or [^{35}S]methionine- and [^{35}S]cysteine-labeled human RelA (lanes 7 and 8) were produced in rabbit reticulocyte lysates. Equal amounts of labeled proteins were incubated with GST or GST-hTBP immobilized on agarose beads, washed extensively, and analyzed by SDS–12.5% PAGE. (B) In vitro interaction of TFIIB with members of the Rel/NF-κB family. Equal amounts of [^{35}S]methionine-labeled v-Rel (lanes 1 and 2), chicken c-Rel (lanes 3 and 4), mouse c-Rel (lanes 5 and 6), mouse p105-XhoI (lanes 9 and 10), or [^{35}S]methionine- and [^{35}S]cysteine-labeled human RelA (lanes 7 and 8) were incubated with GST or GST-TFIIB immobilized on agarose beads, washed, and analyzed as described for panel A.

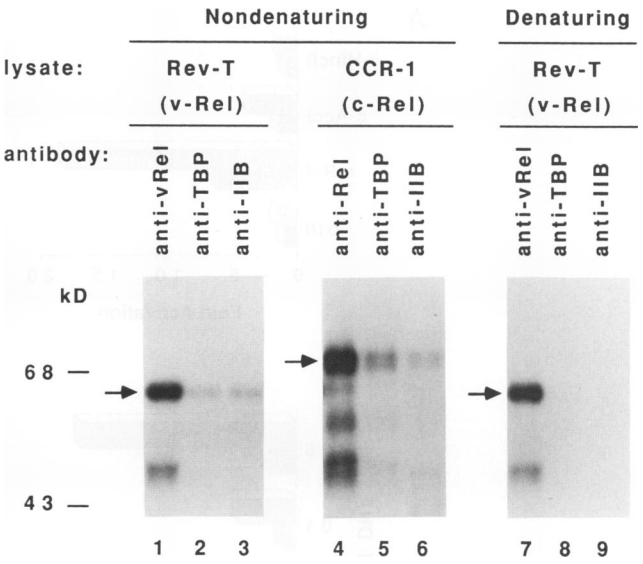

FIG. 4. In vivo association of v-Rel and c-Rel with endogenous TBP and TFIIB in transformed chicken spleen cells. Whole-cell lysates from chicken spleen cells transformed by either v-Rel (Rev-T) or c-Rel (CCR-1) were incubated with ^{32}P- and bromodeoxyuridine-labeled IL-2Rα κB oligonucleotides and subjected to UV cross-linking. Precleared lysates were then immunoprecipitated with anti-hTBP (lanes 2 and 5) or anti-hTFIIB (lanes 3 and 6) antisera and resolved by SDS–9% PAGE; 25-fold-diluted cell extracts were immunoprecipitated with anti-Rel antibodies Ab 1967 (lane 1) or Ab 3 (lane 4). As a control, Rev-T cell extracts were immunoprecipitated under denaturing conditions with anti-TBP (lane 8) and anti-TFIIB antibodies (lane 9) or were diluted 25-fold prior to immunoprecipitation with Ab 1967 (lane 7).

teins to transcriptional regulation led us to investigate whether other Rel family members could also interact with TBP and TFIIB. ^{35}S-labeled v-Rel, chicken c-Rel, mouse c-Rel, human RelA, and a C-terminally truncated derivative of the mouse p105 precursor for the p50 subunit of the NF-κB transcription factor (p105-XhoI) were produced in vitro and tested for association with GST-TBP and GST-TFIIB in GST pull-down assays. v-Rel, chicken c-Rel, mouse c-Rel, and RelA, all of which contain sequences capable of activating transcription, showed significant association with hTBP (Fig. 3A, lanes 1, 3, 5, and 7) and TFIIB (Fig. 3B, lanes 1, 3, 5, and 7). In contrast, the p105-XhoI protein that lacks an activation domain showed little TBP or TFIIB association above background association with GST (Fig. 3A, lanes 9 and 10; Fig. 3B, lanes 9 and 10), despite the fact that equivalent amounts of ^{35}S-labeled proteins were used in all cases (data not shown). Combined with the results described above, these findings indicate that several NF-κB-related proteins can interact with TBP and TFIIB and suggest that the transcription activation regions of Rel family members use a common mechanism to enhance transcription.

The v-Rel and c-Rel proteins associate with endogenous TBP and TFIIB in transformed spleen cells. The results described above suggest that the in vitro interaction of Rel proteins with TBP and TFIIB reflects functional interactions occurring in vivo. We addressed the biological significance of these associations by determining whether the v-Rel or c-Rel proteins from transformed chicken spleen cells could be found complexed with endogenous chicken TBP and/or TFIIB. It was anticipated that if such complexes did exist in vivo, their low abundance might limit their detection. To improve our ability to detect Rel proteins complexed with endogenous chicken TBP and TFIIB, we sought to reveal endogenous TBP-Rel and TFIIB-Rel complexes with ^{32}P-labeled κB oligonucleotides.

Extracts from v-Rel (Rev-T)- or chicken c-Rel (CCR-1)-transformed chicken spleen cells were incubated with ^{32}P- and bromodeoxyuridine-labeled oligonucleotides containing an NF-κB-binding motif (κB-pd [1]). After UV cross-linking, DNA-protein complexes were immunoprecipitated with affinity-purified anti-hTBP antibodies (Fig. 4, lanes 2 and 5) or anti-hTFIIB antibodies (Fig. 4, lanes 3 and 6). In parallel reactions, 25-fold-diluted cell extracts were immunoprecipitated with anti-peptide antibodies specific for the unique envelope-derived N terminus of v-Rel (Fig. 4, lane 1) or anti-Rel antibodies directed against the RHR (Fig. 4, lane 4). Both anti-TBP and anti-TFIIB antibodies successfully im-

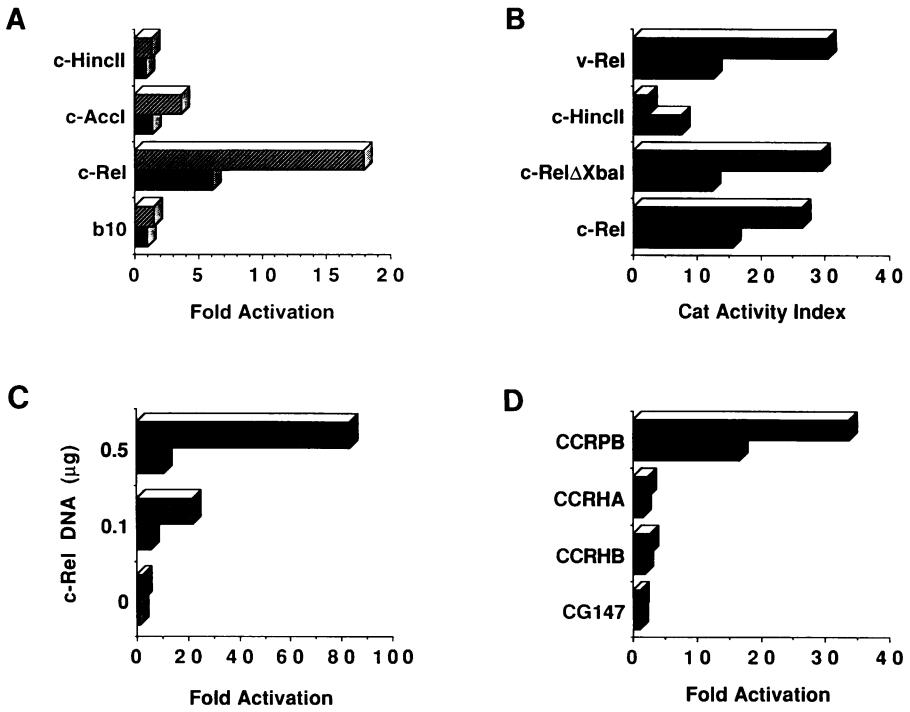

FIG. 5. Synergistic activation of transcription by Rel proteins and TBP. (A) COS-7 cells were cotransfected with 3 μg of pIL6CAT, 0.5 μg of CMV-b10, 0.5 μg of CMV-Rel plasmids, and 5 μg of CMV19SV (black bars) or CMV-TBP DNA (hatched bars). Cells transfected with 1.0 μg of b10 DNA were used as controls. (B) Undifferentiated F9 cells were cotransfected with 1.5 μg of CMV-Rel plasmids, 3 μg of κB_2TKCAT, and 15 μg of CMV19SV (black bars) or CMV-TBP DNA (hatched bars). (C) COS-7 cells were cotransfected with 0, 0.1, or 0.5 μg of CMV–c-Rel and 5 μg of CMV19SV (black bars) or CMV-TBP DNA (hatched bars), along with 3 μg of pIL6CAT. (D) COS-7 cells were cotransfected with 3 μg of pG5BCAT, 2 μg of GAL4-Rel vectors, and 5 μg of CMV19SV (black bars) or CMV-TBP DNA (hatched bars). The pCG147 vector was used as a control. In all cases, assays were performed with 25 μg of total cellular proteins for 2 h. The average fold activation over that of control plasmids from three independent experiments (A, C, and D) or the CAT activity index (35) (B) is plotted. Assays whose results are shown in panels A, C, and D were performed with different batches of cells.

munoprecipitated v-Rel from Rev-T cells and c-Rel from CCR-1 cells bound to ^{32}P-labeled κB oligonucleotides. In contrast, anti-TBP and anti-TFIIB antibodies failed to efficiently precipitate v-Rel in assays performed under denaturing conditions (Fig. 4, lanes 8 and 9), indicating that its immunoprecipitation with anti-TBP and anti-TFIIB antibodies under nondenaturing conditions resulted from its association with endogenous TBP and TFIIB. The combined observations that anti-hTBP antibodies can supershift endogenous chicken TBP from spleen cell extracts bound to TATA-containing oligonucleotides and that anti-TBP and anti-TFIIB antibodies showed no cross-reactivity for the v-Rel and c-Rel proteins in Western blot (immunoblot) analyses further suggest that these interactions are specific (data not shown).

Together, these results demonstrate that a fraction of the v-Rel and c-Rel protein population from transformed chicken spleen cells exists in complexes with endogenous chicken TBP and chicken TFIIB. Similar coprecipitation assays with antibodies raised against the Jun protein failed to reveal any complexes with v-Rel or c-Rel (data not shown), further suggesting that interactions between endogenous Rel proteins and TBP or TFIIB are specific. These results support the hypothesis that the association of Rel proteins with TBP and TFIIB is biologically significant.

c-Rel and TBP synergize to activate transcription. The in vivo association of v-Rel and c-Rel with chicken TBP in transformed spleen cells raises the possibility that this interaction is functional. This possibility was tested by characterizing the effect of TBP on the transcriptional activity of v-Rel and c-Rel in cotransfection assays. Wild-type and mutant c-*rel* genes expressed from a CMV immediate-early promoter were transfected into COS-7 cells along with a CMV expression vector for hTBP and a CAT reporter gene expressed from the IL-6 enhancer/promoter. As anticipated, chicken c-Rel led to a sixfold activation of transcription in COS-7 cells over the control b10 vector, and its progressive C-terminal truncation rapidly decreased its activity (Fig. 5A, compare c-Rel with c-AccI and c-HincII). This is in agreement with our previous findings indicating that the bulk of c-Rel activation in differentiated cells is mediated through its unique C-terminal acidic activation region (23).

Transfection of the TBP expression vector with the b10 control plasmid had little or no effect on the basal level of CAT expression. In sharp contrast, cotransfection of TBP with Rel expression vectors led to a threefold synergistic activation of transcription by both the c-Rel and c-AccI proteins over that seen in the absence of TBP (Fig. 5A). However, TBP was unable to enhance transcription by the c-HincII deletion mutant that contains only RHR sequences and therefore lacks both c-Rel activation regions (23). Modest synergy (1.5-fold) of v-Rel with TBP was also observed, although activation by v-Rel in COS-7 cells was much lower than seen with c-Rel (data not shown). This was anticipated, since the v-Rel activation region functions preferentially in undifferentiated cells (23, 47). In agreement with the results

described above, TBP also increased the activity of c-Rel, c-RelΔXbaI, and v-Rel but not that of c-HincII in undifferentiated F9 cells (Fig. 5B). Cooperative activation of transcription by Rel and TBP was dose dependent, as titration of increasing amounts of c-Rel with a constant amount of TBP and vice versa resulted in increased synergy (Fig. 5C, and data not shown). Combined, these results provide compelling evidence that the interaction of Rel with TBP promotes Rel-mediated activation of transcription.

To confirm that the interaction of TBP with either of the c-Rel activation regions could lead to cooperative activation, we tested the effect of TBP on transcription by GAL4-Rel fusion proteins derived from the C-terminal half of c-Rel. Coexpression of TBP with CCRPB, which encodes the C-terminal acidic activation region of c-Rel, led to a twofold cooperative activation of CAT expression from a vector containing five copies of the yeast GAL4 DNA-binding motif in COS-7 cells (Fig. 5D). Modest activation was observed with the CCRHA vector, which contains the F9 cell-specific activation region of Rel, a region that functions preferentially in undifferentiated cells and that is weakly activating in differentiated cells such as the COS-7 cells used here (23, 47). As expected, limited synergy was observed between TBP and CCRHA (Fig. 5D). Combined, these results demonstrate that c-Rel sequences responsible for activation of transcription can functionally interact with TBP to bring about efficient promoter activity. These findings also suggest that endogenous complexes of Rel proteins with basal transcription factors are critical for Rel protein function.

DISCUSSION

In this report, we demonstrate that Rel proteins interact with two components of the basal transcription machinery, namely, TBP and TFIIB. Using GAL4-Rel fusion proteins, we show that Rel sequences involved in these interactions overlap with those responsible for activation of transcription. Coimmunoprecipitation assays revealed that Rel-TBP and Rel-TFIIB complexes also exist in vivo, as v-Rel and c-Rel proteins from transformed chicken spleen cells could be found associated with endogenous chicken TBP and TFIIB. The presence of these complexes in transformed cells, combined with the participation of Rel activation subdomains in TBP and TFIIB interactions, suggested that these complexes are important for Rel-mediated activation of transcription. Consistent with this hypothesis is the fact that cotransfection of TBP with v-Rel, c-Rel, or GAL4-Rel fusion proteins led to synergistic activation of κB-directed transcription. Our identification of TBP as a target for Rel is an important step in understanding the mechanism of transcriptional activation by Rel and NF-κB proteins and suggests a physiological role for Rel complexes with basal transcription factors.

The exact composition of Rel-TBP and Rel-TFIIB complexes remains to be determined. Our experiments clearly show that Rel proteins associate with TBP or TFIIB; however, it is premature to conclude that these interactions are direct, as intermediate proteins such as coactivators or TBP-associated factors (TAFs) present in chicken spleen cell extracts or translation lysates could be involved in these complexes. If Rel association with basal transcription factors was indeed affecting the assembly of active transcription complexes, a direct interaction of Rel with TBP could influence TBP binding to the TATA element, promoting the assembly of the initiation complex and RNA polymerase II (49, 50) as proposed for the Zta protein of Epstein-Barr virus (28). Alternatively, Rel-TFIIB interactions may mediate Rel-activating effects on TBP. This model is supported by studies with acidic activators that function to recruit and/or retain TFIIB during complex assembly (30) and by a report that the interaction of the glutamine-rich activation region of the *Drosophila* Fushi tarazu protein with TFIIB is responsible for efficient promoter activation (8). A third possibility is that Rel proteins interact with intermediate factors (TAFs or coactivators) that tether Rel activation domains to the promoter region, bridging its interaction with the transcription machinery (29, 36). The requirement for TAFs or coactivators in Rel-mediated activation will need to be addressed with purified proteins in in vitro transcription assays.

Of equal importance will be the characterization of the sequences involved in Rel protein interactions with general transcription factors. The unique C terminus of c-Rel, rich in acidic residues, is likely to interact with TBP in a way that is similar to that of other activators (20, 26, 31, 43), that is, with the conserved basic region in the C terminus of TBP. The inability of the TBPΔ166-335 mutant protein, deleted in this region, to interact with Rel activation sequences supports this hypothesis. Recent mutagenesis and structural studies have indicated that the acidic activation domains of the yeast GAL4 and GCN4 proteins are not α-helical but rather form β sheets and that the conformation of these regions may be more important to their function than the acidic residues themselves (27, 46). It is thus interesting to note that the acidic activation region in the C terminus of chicken c-Rel is predicted by Chou-Fasman analysis (7) to adopt a β-sheet conformation (data not shown).

Little is known about the F9-specific activation region common to v-Rel and c-Rel. In contrast to the C-terminal activation domain of c-Rel, this region does not show any significant homology to the glutamine-rich, acidic, or proline-rich activators. Furthermore, despite its interaction with TBP and TFIIB, its activating function is primarily confined to undifferentiated cells (23, 47). This suggests that, unlike the acidic activation region in the C terminus of c-Rel, its ability to interact with the transcription machinery must be tightly regulated. The high serine and threonine content (23%) of this region is reminiscent of the C-terminal activation region of the *Drosophila* homeodomain protein Zerknüllt (25% [8, 19]) and raises the intriguing possibility that phosphorylation of critical residues mimics the charge and/or conformation of an acidic activation domain. It is thus tempting to speculate that controlled posttranslational modification converts this region into a potent activator and could involve developmentally regulated kinase and phosphatase activities. It will be of interest to identify the residues critical for the interaction of Rel activation regions with TBP and TFIIB.

The biological significance of Rel-mediated effects on transcription has yet to be clarified, but accumulating evidence suggests an important role in cell differentiation and transformation. This is supported by observations that DNA binding is critical for Rel transacting and transforming functions (25, 33, 47) and by the extensive overlap between Rel activation domains and sequences important for Rel-induced cell differentiation (22) and transformation (10, 12, 40, 48). One model for v-Rel-induced oncogenesis was derived from its ability to modulate expression of genes linked to κB enhancer sequences. The ability of the RHR of v-Rel to competitively inhibit c-Rel and NF-κB-mediated transcription was postulated to block expression of genes important for lymphoid cell differentiation. The observation that v-Rel can selectively activate gene expression in undifferentiated

cells has provided an additional model for v-Rel-induced transformation, via activated expression of developmentally regulated cellular genes in immature lymphoid cells. Although the results described herein do not directly address the mechanisms by which Rel proteins function in cell differentiation and transformation, they suggest that cell-specific and developmental stage-specific activation of transcription by Rel proteins result from specific interactions with basal transcription factors. Experiments aimed at further mapping Rel activation sequences important for association with TBP and TFIIB and at establishing their role in the biological function of Rel family members will help to resolve these questions.

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