Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 28–32, January 1994
Biochemistry

Autoregulation of IκBα activity

PAUL J. CHIAO, SHIGEKI MIYAMOTO, AND INDER M. VERMA

Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Communicated by Leslie E. Orgel, September 20, 1993 (received for review August 13, 1993)

ABSTRACT **Transcription factor NF-κB regulates the expression of a plethora of genes. The activity of NF-κB proteins is regulated by IκB proteins. We report that induction of IκBα, a member of the IκB family of proteins, is preceded by activation of NF-κB complex. The promoter of the IκBα gene contains a κB site that is directly involved in its induction by the NF-κB complex. Degradation of IκBα protein precedes activation of NF-κB DNA binding activity, whereas newly synthesized IκBα protein inhibits NF-κB activity. If the degradation of IκBα is prevented, the induction of DNA binding activity of NF-κB complex is severely curtailed. These data suggest the existence of an autoregulatory loop whereby IκBα regulates the activity of transcription factor NF-κB, which in turn regulates the IκBα activity.**

Nuclear factor NF-κB was identified as a lymphoid-specific heterodimeric protein complex that binds to a decameric sequence motif located in the immunoglobulin κ light chain enhancer (1). Subsequently, it was shown that the NF-κB transcription factor participates in the regulation and transcriptional activities of many cellular and viral genes (2–4). Molecular cloning of the p50 and p65 subunits, the two components of the NF-κB complex, revealed a region in the N-terminal half of these proteins that shares extensive sequence homology with protooncogene *rel* and the *Drosophila* morphogen dorsal (5–9). The Rel homology region contains the DNA binding and the dimerization domain (4, 9). A number of other genes have now been identified as members of the Rel/NF-κB/dorsal gene family, which include genes for RelA (p65), RelB, p52, and p100 (4, 10–13). The NF-κB transcription factor is constitutively present and active only in the nucleus of a few subsets of cells, such as B cells and monocytes (1, 14). In general, NF-κB is found in the cytoplasm of many other cell types in an inactive form (4, 15). This inactive form contains an inhibitor protein, IκB, that is complexed with the RelA/p65 subunit of NF-κB and thought to retain the p50–p65 complex in the cytoplasm (4, 16). A number of IκB proteins have now been identified and molecularly cloned, including IκBα (MAD-3, pp40, and RL/IF-1), p105, IκBγ (C-terminal portion of p105), bcl3, and cactus in *Drosophila* (17–26).

Upon stimulation of cells with a variety of agents, including tumor necrosis factor α, phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), and interleukin 1, IκBα presumably dissociates from the complex and NF-κB translocates to the nucleus, where it can modulate the expression of a number of genes. Although there is evidence that IκBα is a phosphoprotein (16, 27–31) and that its activity is modulated by phosphorylation, *in vivo* the critical sites of phosphorylation of IκBα have not yet been identified. The model for activation of NF-κB assumes that upon induction, the nascently phosphorylated form of IκBα is released from NF-κB complex and rapidly degraded (32–34). Interestingly, IκBα mRNA is induced upon transfection of cells with RelA/p65 (32–34) or during differentiation of pre-B to B cells, where NF-κB activity is constitutively activated (S.M., unpublished data). Thus, it is paradoxical that IκBα gene product is both the inhibitor and the target of NF-κB complexes. We therefore undertook a study of the regulation of IκBα to specifically determine whether IκBα gene induction by κB proteins is mediated by cis elements containing κB sites. Recently, several investigators have reported a similar mechanism for regulation of IκBα gene expression (32–35).

MATERIALS AND METHODS

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay. 70Z/3 cells were grown in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum and 50 μM 2-mercaptoethanol to 10^6 cells per ml and treated with PMA (50 ng/ml) or LPS (10 μg/ml) and with or without cycloheximide (20 μg/ml) for the indicated times. L-1-Tosylamido-2-phenylethyl chloromethyl ketone (TPCK; 5–100 μM) was added 1 h prior to the PMA or LPS treatment. The nuclear extracts were prepared by the method of Andrews and Faller (36). The protein concentration of extracts was ≈5 mg/ml. For mobility shift assays, 10 μg of nuclear extract was incubated with 1 μg of poly(dI-dC) (Pharmacia) in 15 μl of binding buffer [75 mM NaCl/15 mM Tris·HCl, pH 7.5/1.5 mM EDTA/1.5 mM dithiothreitol/25% (vol/vol) glycerol/bovine serum albumin (20 μg/ml)] for 30 min at 4°C. ^{32}P-labeled double-stranded oligonucleotides containing the κB site (underlined) found in the Igκ gene (5′-CTCAACAGAGGGGACTTTCCGAGAGGCCAT-3′) or the κB site (underlined) found in IκBα gene (5′-CTGGCTTGGAAATTCCCCGCGCCTGAC-3′) were included as probes. The mutant κB site for Igκ (5′-CTCAACAGAGTTGACTTTTCGAGAGGCCAT-3′) or IκBα (CTGGCTTCGAAATTAATTGCGCCTGAC-3′) were used for competition studies (where underlined bases are the mutated sequences). The binding of the probe was performed for 20 min at room temperature. Reaction products were analyzed on 4% polyacrylamide gels containing 0.25× TBE (22.5 mM Tris/22.5 mM borate/0.5 mM EDTA, pH 8.0) buffer.

Northern Blot Analysis, Primer Extension, and DNA Sequencing. 70Z/3 cells stimulated by PMA or LPS in the presence or absence of TPCK or cycloheximide were harvested at the same time intervals as in the electrophoretic mobility shift assay and RNAs were isolated as described by Chomczynski and Sacchi (37). RNA (25 μg) was electrophoresed through a 1.2% agarose gel containing formaldehyde, transferred to a Hybond nylon filter (Amersham), UV-crosslinked, and hybridized with ^{32}P-labeled 1.1-kb mouse IκBα cDNA (*Eco*RI–*Eco*RI) probe.

Total RNA (25 μg) isolated from WEHI231 cells was hybridized with 0.1 ng of the ^{32}P-end-labeled primer (5′-CGTGCCCAGCTGGCTGGCTGAAACACCG) in 40 mM Pipes, pH 6.4/1 mM EDTA, pH 8.0/0.4 M NaCl/80%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PMA, phorbol 12-myristate 13-acetate; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; CAT, chloramphenicol acetyltransferase; LPS, lipopolysaccharide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

(vol/vol) formamide for 8 h at 37°C. After ethanol precipitation, primer extension was carried out at 42°C for 2 h in 50 mM Tris·HCl, pH 7.6/60 mM KCl/10 mM $MgCl_2$/all four dNTPs (each at 2.5 mM)/1 mM dithiothreitol/RNase inhibitor (1 unit/μl)/actinomycin D (50 μg/ml)/50 units of murine leukemia virus reverse transcriptase. The reaction products were treated with 5 μg of RNase or 150 units of S1 nuclease at 37°C for 30 min, extracted with phenol/chloroform, precipitated with ethanol, and analyzed in 12% denaturing polyacrylamide gels along with DNA sequencing reaction products of the IκBα genomic clone using the same primer and DNA size marker (^{32}P-end-labeled MspI-digested pBR322 DNA fragments). IκBα cDNA clones and one genomic clone containing the 1.6-kb upstream region of the IκBα gene were analyzed by DNA sequencing using a Sequenase kit (United States Biochemical).

Transient Transfections and Chloramphenicol Acetyltransferase (CAT) Assays. The 1.6-kb *Hin*dIII–*Nar* I and 0.2-kb *Eco*0109I–*Nar* I genomic fragments of IκBα gene promoter were cloned into the *Hin*dIII and *Xba* I sites in pBLCAT3 vector (see Fig. 3*A*). By using PCR-mediated site-directed mutagenesis, a mutant κB site (5′-CGAAATTAATT-3′, where underlined bases are the mutated sequences) was generated in the 0.2-kb (*Eco*0109I–*Nar* I) IκBα gene promoter. For construction of the expression plasmid for p65, p50, and c-Rel, the 1.8-kb *Bam*HI–*Eco*RI c-Rel cDNA was inserted into the *Bam*HI–*Eco*RI sites of the expression plasmid pCMX (38), the 2.5-kb *Sac* II (blunted)–*Xho* I p65 cDNA was ligated into *Eco*RV–*Xho* I site in the pCMX vector, and the 1.3-kb *Nco* I p50 cDNA was ligated to *Bam*HI–*Nhe* I site of pCMX plasmid. The IκBα promoter–CAT plasmid (5 μg) and pCMXp65, pCMXp50, and pCMX c-Rel expression plasmids (5–10 μg) were used in each cotransfection. Forty-eight hours after calcium phosphate transfection, cells were collected, the relative transfection efficiency was determined by cotransfected LacZ expression plasmid (1 μg, Rous sarcoma virus LacZ), and subsequent β-galactosidase activities in cell extracts were used to normalize the transfection efficiencies.

CAT activity (percent conversion to acetylated chloramphenicol) was determined by phosphor image analysis.

Western Blot Analysis. 70Z/3 cells were grown to 10^6 cells per ml, treated with PMA (50 ng/ml) or LPS (10 μg/ml) in the presence or absence of cycloheximide (20 μg/ml), lysed, and subjected to immunoprecipitation with anti-p65 sera raised against the N-terminal 19-aa peptide (MDDLFPLIFPSEPAQASGP). The precipitates were resolved by SDS/PAGE, transferred to nylon membranes (Immobilon-P; Millipore), and detected with IκBα antibody specific for the N terminus (aa 1–56) of IκBα protein. The subsequent Western blot analysis was carried out with ECL Western blotting kit (Amersham) by the manufacturer's recommendations.

RESULTS

Induction of IκBα Gene. Electrophoretic mobility shift assays and Northern blot analyses were performed using pre-B (70Z/3) cells stimulated by either PMA or LPS (Fig. 1). The NF-κB DNA binding activities can be detected within 15 min after LPS or PMA stimulation (Fig. 1*A*, lanes 2–7), whereas the expression levels of IκBα mRNA lagged and increased to the maximum by 60 min (Fig. 1*B*, lanes 4 and 9). These results suggest that IκBα is likely to be one of the target genes regulated by NF-κB proteins. It is not clear from our data whether the IκBα gene is regulated by complex I (p50–p65 heterodimer) or complex 2 (p50–p50 homodimer) (Fig. 1*A*; S.M., unpublished data).

IκBα Promoter Has a κB Binding Site. We next cloned the promoter and other upstream sequences of murine IκBα gene. The sequence of 651 nt upstream of the IκBα mRNA

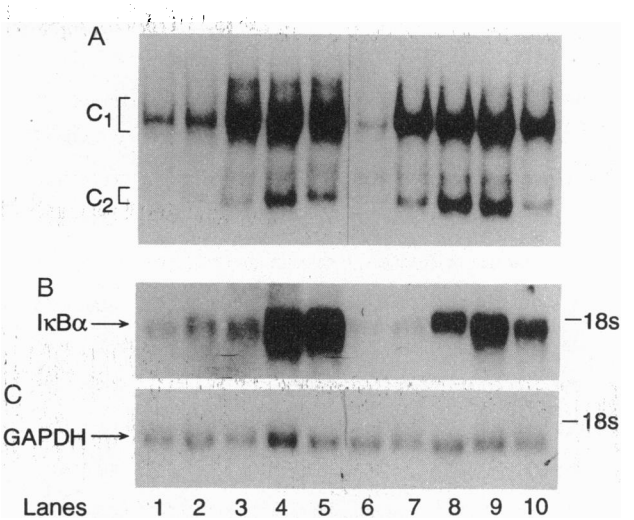

FIG. 1. Induction of NF-κB DNA binding activities and IκBα mRNA in 70Z/3 cells treated with PMA or LPS. Lanes: 1–5, 70Z/3 cells plus LPS; 6–10, 70Z/3 cells plus PMA; 1 and 6, 0 min; 2 and 7, 15 min; 3 and 8, 30 min; 4 and 9, 1 h; 5 and 10, 2 h. (*A*) Electrophoretic mobility shift assays of NF-κB DNA binding activities in the nuclear extracts of PMA- or LPS-treated 70Z/3 cells at the indicated times. C1 is likely to be p50–p65 heterodimer and C2 is a p50–p50 homodimer (S.M., unpublished data). (*B* and *C*) Northern blot analysis of IκBα mRNA induction by PMA or LPS in 70Z/3 cells at the same times used in the electrophoretic mobility shift assay. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were analyzed as an internal control for loading.

transcription start site is shown in Fig. 2*A*. Sequence analysis reveals the presence of a TATA box, a κB binding site, and many other previously identified cis-regulatory elements, including SP-1, a possible AP-1 site, AP-2, AP-3, CREB, interferon γ response element, etc. (Fig. 2*A*). The κB site (GGGAATTTCC) in the IκBα promoter is identical to the κB site in the promoter region of tumor necrosis factor α (4). To define the mouse IκBα gene transcription initiation site, primer-extension reactions were performed (Fig. 2*B*), which revealed a 96-bp DNA fragment with or without S1 nuclease digestion (Fig. 2*B*, lanes 2 and 3). The same primer was also used in the nucleotide sequence reactions and the products were analyzed on the same gel carrying the primer-extension reaction products (Fig. 2*B*, lanes 4–7), thus allowing direct gel comparison of the size of primer-extension products and the sequencing products. The results show that the initiation of transcription started 75 bp upstream of ATG (marked by a thick arrow). The TATA element is located 26 bp upstream of the transcription initiation site (Fig. 2*A*). We conclude that IκBα gene contains a bona fide NF-κB binding site.

IκBα Gene Promoter Is Inducible with p65. To determine whether the κB site identified in the IκBα promoter is functional, 1.6-kb *Hin*dIII–*Nar* I and 0.2-kb *Eco*01091–*Nar* I genomic fragments of IκBα gene promoter were cloned into pBLCAT3 vector (Fig. 3*A*). By using PCR-mediated site-directed mutagenesis, a mutant κB site was generated in the 0.2-kb (*Eco*01091–*Nar* I) fragment of IκBα gene promoter (Fig. 3*A*). High levels of CAT activities were detected in COS cells cotransfected with the 1.6-kb or 0.2-kb IκBα gene promoter–CAT construct and the expression vector encoding the p65 subunit of NF-κB (Fig. 3*B*, lanes 5 and 6). Little or no CAT activity was detected in the cells cotransfected with IκBα gene promoter–CAT construct with mutant κB site (plasmid C; Fig. 3*B*, lane 4). Increased transactivation was observed when p65 was cotransfected with a construct generating p50 (lanes 8 and 9) but c-Rel was unable to transactivate either construct A or B (data not shown).

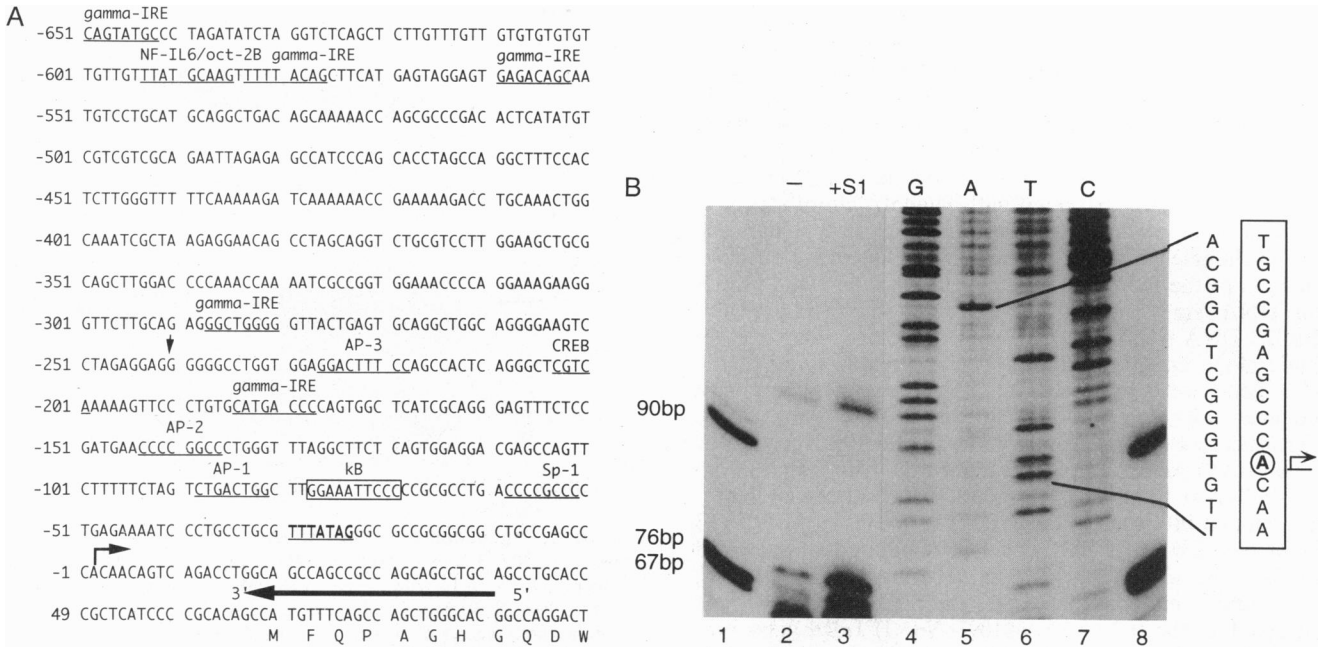

FIG. 2. (*A*) Analysis of IκBα promoter. Genomic DNA containing IκBα promoter was molecularly cloned from a mouse 3T3 library (Stratagene). The nucleotide sequence of the IκBα promoter region is shown. The thin arrow marks the start site of transcription determined by primer-extension analysis. IκBα cDNA coding sequence is shown with the amino acid sequence, and the primer used for primer-extension and DNA sequencing analyses is underlined with a thick arrow; TATA box and other known nuclear protein binding motifs are indicated. The κB site is indicated by a box. The nucleotide sequence is numbered with respect to the nucleotide start site (position +1, the start site) and the upstream sequence from the mRNA start point is indicated with negative numbers. The DNA sequence of IκBα promoter region was analyzed by the computer program, SIGNAL SCAN (39). Gamma-IRE, interferon γ response element. (*B*) The primer-extension analysis for determining the start site of transcription. The primer sequence is indicated in *A*; molecular size markers are in lanes 1 and 8. The primer-extension products and S1 nuclease-treated primer-extension products are shown in lanes 2 and 3, respectively. By using the same primer, IκBα genomic clone was subjected to DNA sequence analysis and the reaction products were electrophoresed in lanes 4–7. The DNA sequence in the vicinity of the primer-extension product is indicated. The start site is indicated with an arrow.

Regulation of IκBα Gene. Since IκBα regulates the activity of NF-κB complexes in the cytoplasm, it was of interest to study whether IκBα provides an autoregulatory loop for regulation of κB proteins. We therefore studied the kinetics of induction of IκBα mRNA and protein and its correlation to NF-κB DNA binding activity. Fig. 4*A* shows that using an oligonucleotide probe corresponding to the κB site present in the IκBα promoter (κB–IκBα), the κB binding activity is detected within 15 min of the addition of PMA to 70Z/3 cells, reaches a maximum by 60 min, and rapidly declines to basal levels by 4 h (Fig. 4*A*, lanes 2–6). Interestingly, the expression of IκBα mRNA is induced maximally by 60 min (Fig. 4*B*, lane 4), then declines, and remains essentially unchanged for the next 24–48 h (Fig. 4*B*). This result correlates well with the synthesis of IκBα protein, which is hardly detectable by 30–60 min after induction, and then the level increases and essentially remains unchanged (Fig. 4*D*). A simple interpretation of this data will be that upon induction with PMA, the IκB protein is dissociated from NF-κB complex and is rapidly degraded, allowing the NF-κB complex to traverse to the nucleus and activate genes containing κB sites, one of which is the IκBα gene. Once IκBα protein is synthesized, it binds to NF-κB complex and keeps it sequestered in the cytoplasm, thus providing the autoregulatory loop.

NF-κB Complex Induces IκBα Gene Expression. To test whether activated NF-κB complex, upon stimulation with PMA or LPS, is directly involved in the induction of IκBα gene expression, we blocked the activation of NF-κB complex by a chymotrypsin protease inhibitor, TPCK. Fig. 5*A* shows that TPCK (50 μM) prevents LPS-induced NF-κB activity in 70Z/3 cells. Little or no IκBα mRNA was detected upon stimulation in the presence of TPCK (Fig. 5*B*). TPCK was not a general inhibitor of transcription because the levels of GAPDH mRNA were essentially unchanged (Fig. 5*B*). Similar results were obtained with c-fos mRNA, which has short half-life (30 min) (data not shown). Although mecha-

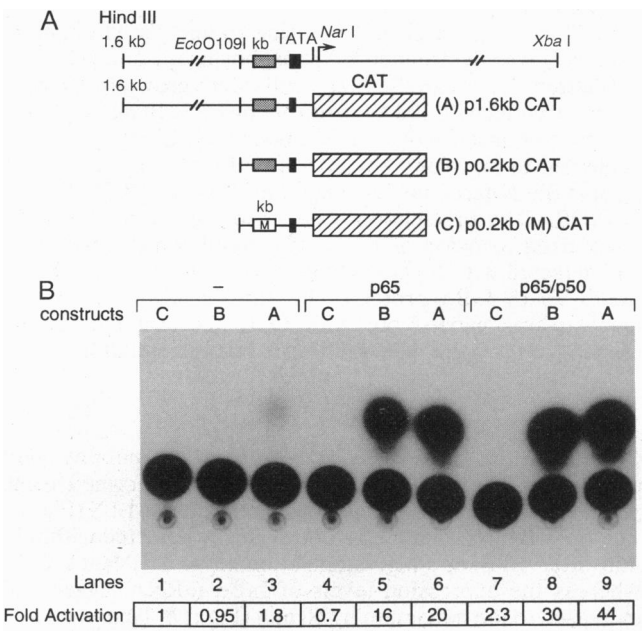

FIG. 3. Analysis of the IκBα promoter. (*A*) Schematic diagram of the IκBα genomic clone and the reporter constructs. Plasmids A and B represent the 1.6-kb and 0.2-kb upstream fragments linked to CAT gene whereas plasmid C contains the 0.2-kb fragment but the κB site is mutated. (*B*) CAT assays for analysis of IκBα promoter. The reporter plasmids were cotransfected in COS cells with p65 and p50 expression plasmids. The results shown here are representative of five CAT assays.

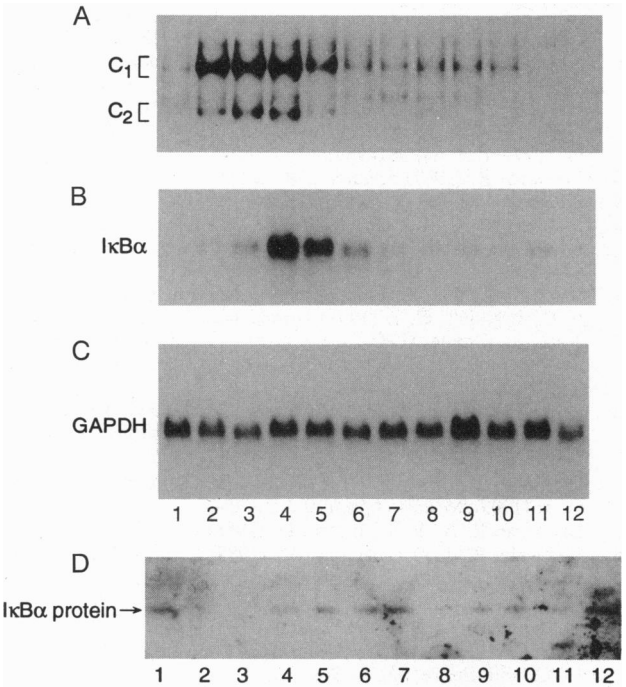

FIG. 4. Kinetics of activation of NF-κB and IκB gene expression. Lanes: 1, 0 min; 2, 15 min; 3, 30 min; 4–12, 1, 2, 4, 6, 8, 12, 24, 32, 48 h, respectively. (*A*) Time course of activation of NF-κB activity after addition of PMA to 70Z/3 cells. (*B*) Induction of IκBα mRNA. (*C*) Control GAPDH mRNA. (*D*) Western blot using IκBα antibodies.

nism of TPCK inhibition of IκBα mRNA or NF-κB activity is not understood, it is likely that it prevents degradation of IκBα protein and, thereby, does not allow activation of NF-κB complex. In agreement with this proposal, degradation of IκBα protein is inhibited in a B-cell line (WEHI 231) upon addition of TPCK (S.M., unpublished data).

If the newly synthesized IκBα is required to inhibit the NF-κB activity, then its inhibition should allow constitutive activity of the NF-κB complex, as there will be no blocking protein to sequester it in the cytoplasm. In Fig. 6, we show that as expected, NF-κB activity is transiently induced for ≈2 h upon stimulation of 70Z/3 cells with PMA; however, the NF-κB activity can be detected for nearly 12 h if induction is carried out in the presence of cycloheximide, an inhibitor of protein synthesis. The IκBα mRNA is, however, induced and the levels remain high because of the sustained amount of activated NF-κB complex or because no IκBα protein is being translated in the presence of cycloheximide (Fig. 6). The lack of newly synthesized IκBα protein may in part be responsible for sustained activation of NF-κB activity.

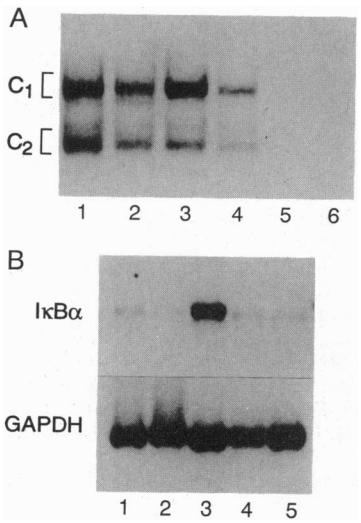

FIG. 5. Inhibition of NF-κB activity with TPCK. (*A*) NF-κB activity in LPS-stimulated 70Z/3 cells (30 min) in the absence and presence of increasing concentrations of TPCK. Lanes: 1–6, 0, 5, 10, 25, 50, and 100 μM TPCK, respectively. (*B*) Induction of IκBα mRNA in 70Z/3 cells stimulated with LPS for 15 or 30 min, in the absence or presence of 50 μM TPCK. Lanes: 1, no addition; 2, LPS for 15 min; 3, LPS for 30 min; 4, TPCK plus LPS for 15 min; 5, TPCK plus LPS for 30 min.

DISCUSSION

We have demonstrated that transcription of IκBα, the inhibitor of activity of NF-κB complex, is rapidly induced by NF-κB complex in pre-B-cell line 70Z/3 upon treatment with PMA or LPS. The induction of IκBα mRNA is likely mediated directly by the κB site present in the upstream promoter element of IκBα gene. IκBα is further regulated posttranscriptionally, since the stability of IκBα protein is greatly reduced upon activation of NF-κB activity (Fig. 4*E*; refs. 32–34).

Several investigators have recently shown that activation of cytoplasmic NF-κB complex is accompanied by modification of IκBα protein leading to its rapid degradation (32–34). The release of IκBα from the dormant cytoplasmic NF-κB complex in response to external signals leads transport of NF-κB complex to the nucleus, resulting in κB-site DNA binding activity. The release and degradation of IκBα protein are prerequisites for activation of NF-κB DNA binding activity. Furthermore, IκBα gene expression is induced after activation of NF-κB DNA binding activity. It thus

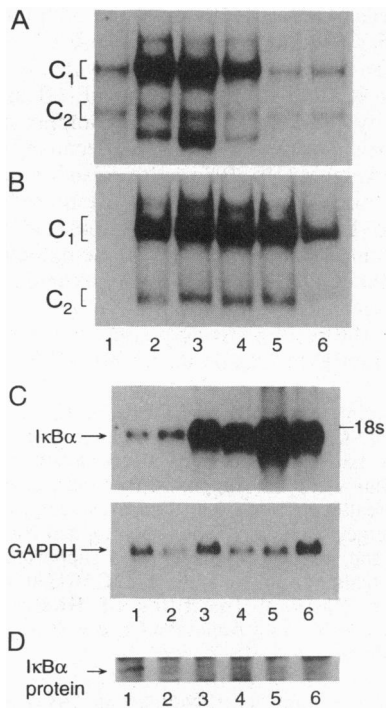

FIG. 6. Effects of cycloheximide on induction of NF-κB activity. (*A*) Induction of NF-κB activity with PMA in 70Z/3 cells using κB site from the IκBα promoter as probe. Lanes: 1, 0 min; 2, 15 min; 3, 1 h; 4, 2 h; 5, 6 h; 6, 12 h. (*B*) Same as in *A* except cycloheximide (20 μg/ml) was added at the time of induction. (*C*) Induction of IκBα mRNA in the presence of cycloheximide. (*D*) The levels of IκBα protein during the time course of induction in the presence of cycloheximide.

appears that in the uninduced cell—for instance, the pre-B cell line 70Z/3—the NF-κB complex is present in an inactive form in association with IκBα protein. After addition of external signals in the form of PMA or LPS, the IκBα protein, known to be associated with the p65 subunit of the p50–p65 NF-κB complex, is modified, presumably by phosphorylation, and rapidly degraded. It is not clear at present whether phosphorylation is a signal for release or degradation of IκBα proteins. However, it is now well established that free IκBα protein is unstable and can be stabilized by association with p65 (33, 34). Once the p50–p65 complex is released from the complex with IκBα, it traverses to the nucleus and binds to cognate κB DNA binding sites. The promoter of IκBα gene contains κB sites and, hence, is a target of NF-κB complex. The newly synthesized IκBα protein can then bind to NF-κB proteins in the cytoplasm and prevent their translocation to the nucleus and, thereby, regulates the transcription of genes containing κB sites, including that of IκBα gene. The product of IκBα gene is a negative regulator of the NF-κB complex, but its synthesis is dependent on the presence of activated NF-κB complex. Therefore, the regulator of IκBα gene is regulated by the IκBα gene product, thereby providing the autoregulatory loop in the regulation of expression of genes containing a κB site. In this manuscript we provide crucial data supporting this model. These data are as follows. (*i*) DNA binding activity of NF-κB complex precedes the transcription of IκBα gene (Figs. 1 and 4). (*ii*) The IκBα gene promoter contains a κB site that when linked to a reporter gene is induced by p65, a member of the κB DNA binding proteins (Fig. 3). Interestingly, no transcription from IκBα gene promoter could be detected when transfected with protooncogene *rel* (data not shown), in agreement with results reported (35), suggesting that members of the κB family may have different functions. (*iii*) The level of IκBα protein decreases immediately upon the addition of PMA or LPS, which parallels the increase in NF-κB DNA binding and transactivation activity (Fig. 4). The levels of IκBα protein then increase leading to decrease in NF-κB activity or no NF-κB activity (Fig. 4). (*iv*) NF-κB complex directly activates the transcription of IκBα mRNA because TPCK, which blocks the activity of NF-κB complex, also inhibits transcription of IκBα mRNA (Fig. 5). (*v*) In the presence of cycloheximide, no IκBα protein is resynthesized and, consequently, sustained NF-κB activity can be detected for >12 h (15, 33). Although TPCK and cycloheximide are general inhibitors of proteases and protein synthesis, respectively, nevertheless, the results strongly support the proposed autoregulatory model for regulation of NF-κB/IκB proteins.

We are grateful to Dr. U. Siebenlist for sharing unpublished information, Dr. L. Kerr for providing phages containing MAD-3 genomic DNA, and members of the Verma laboratory for helpful discussions. This work was supported by funds from the National Institutes of Health, the American Cancer Society, the H. N. and Frances C. Berger Foundation, and the Council for Tobacco Research–USA, Inc. (to I.M.V.). P.C. was supported by National Institutes of Health Training Grant 2T32CA09370 and S.M. was supported by National Institutes of Health Fellowship 1F32CA09115. I.M.V. is an American Cancer Society Professor of Molecular Biology.

1. Sen, R. & Baltimore, D. (1986) *Cell* **46**, 705–716.
2. Lenardo, M. J. & Baltimore, D. (1989) *Cell* **58**, 227–229.
3. Baeuerle, P. A. (1991) *Biochim. Biophys. Acta* **1072**, 63–80.
4. Grilli, M., Chiu, J. J.-S. & Lenardo, M. J. (1993) *Int. Rev. Cytol.* **143**, 1–62.
5. Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Noland, G. P. & Baltimore, D. (1990) *Cell* **62**, 1019–1029.
6. Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., Le Bail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. A. & Israël, A. (1990) *Cell* **62**, 1007–1018.
7. Nolan, G. P., Ghosh, S., Liou, H.-C., Tempst, P. & Baltimore, D. (1991) *Cell* **64**, 961–969.
8. Steward, R. (1987) *Science* **238**, 692–694.
9. Gilmore, T. (1991) *Cell* **62**, 841–843.
10. Ryseck, R.-P., Bull, P., Takamiya, M., Bours, V., Siebenlist, U., Dobrzanski, P. & Bravo, R. (1992) *Mol. Cell. Biol.* **12**, 674–684.
11. Schmid, R. M., Perkins, N. D., Duchett, C. C., Andrews, P. C. & Nabel, G. J. (1991) *Nature (London)* **352**, 733–736.
12. Neri, A., Chang, C.-C., Lombardi, L., Salina, M., Corradini, P., Maiolo, A. T., Chagauti, R. S. K. & Dalla-Favera, R. (1991) *Cell* **67**, 1075–1087.
13. Bours, V., Burd, P. P., Brown, K., Villalobos, J., Park, S., Ryseck, R.-P., Bravo, R., Kelly, K. & Siebenlist, U. (1992) *Mol. Cell. Biol.* **12**, 685–695.
14. Griffin, G. E., Leung, K., Folks, T. M., Kunkel, S. & Nabel, G. J. (1989) *Nature (London)* **339**, 70–73.
15. Baeuerle, P. A. & Baltimore, D. (1988) *Cell* **53**, 211–217.
16. Baeuerle, P. A. & Baltimore, D. (1988) *Science* **242**, 540–546.
17. Haskill, S., Beg, A. A., Tompkins, S. M., Morris, J. S., Yurochko, A. D., Sampson-Johannes, A., Mondal, K., Ralph, P. & Baldwin, A. S., Jr. (1991) *Cell* **65**, 1281–1289.
18. Davis, N., Ghosh, S., Simmons, D. L., Tempst, P., Liou, H. C., Baltimore, D. & Bose, H. R., Jr. (1991) *Science* **253**, 1268–1271.
19. Tewari, M., Mohn, K. L., Yue, F. E. & Taub, R. (1992) *Nucleic Acids Res.* **20**, 607.
20. Inoue, J.-I., Kerr, L. D., Kakizuka, A. & Verma, I. M. (1992) *Cell* **68**, 1281–1289.
21. Rice, N. R., Mackichan, M. L. & Israël, A. (1992) *Cell* **71**, 243–253.
22. Kidd, S. (1992) *Cell* **71**, 623–635.
23. Geisler, R., Bergmann, A., Hiromi, Y. & Nusslein-Volhard, C. (1992) *Cell* **71**, 613–621.
24. Hatada, E. N., Nieters, A., Wulczyn, F. G., Naumann, M., Myer, R., Giuseppina, N., McKeithan, T. W. & Scheidereit, C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2489–2493.
25. Inoue, J.-I., Kerr, L. D., Rashid, D., Davis, N., Bose, H. R., Jr., & Verma, I. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4333–4337.
26. Leveillard, T. & Verma, I. M. (1993) *Gene Exp.* **3**, 135–150.
27. Ghosh, S. & Baltimore, D. (1990) *Nature (London)* **344**, 678–682.
28. Tung, H. Y. L., Bargmann, W. J., Lim, M. Y. & Bose, H. R., Jr. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2479–2483.
29. Kerr, L. D., Inoue, J.-I., Davis, N., Link, E., Baeuerle, P. A., Bose, H. R., Jr., & Verma, I. M. (1991) *Genes Dev.* **5**, 1464–1476.
30. Link, E., Kerr, L. D., Schreck, R., Zabel, U., Verma, I. M. & Baeuerle, P. A. (1992) *J. Biol. Chem.* **267**, 239–246.
31. Beg, A. A., Finco, T. S., Nantermet, P. V. & Baldwin, A. S., Jr. (1993) *Mol. Cell. Biol.* **13**, 3301–3310.
32. Brown, K., Park, S., Kanno, T., Franzoso, G. & Siebenlist, U. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2532–2536.
33. Sun, S.-C., Ganchi, P. A., Ballard, D. W. & Green, W. C. (1993) *Science* **259**, 1912–1915.
34. Scott, M. L., Fujita, T., Liou, H.-C., Nolan, G. P. & Baltimore, D. (1993) *Genes Dev.* **7**, 1266–1276.
35. de Martin, R., Vanhove, B., Cheng, Q., Hofer, E., Csizmadia, V., Winkler, H. & Bach, F. H. (1993) *EMBO J.* **12**, 2773–2779.
36. Andrews, N. C. & Faller, D. V. (1991) *Nucleic Acids Res.* **19**, 2499.
37. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
38. Inoue, J.-I., Kerr, L. D., Ransone, L. J., Bengal, E., Hunter, T. & Verma, I. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3715–3719.
39. Prestridge, D. S. (1992) *CABIOS* **7**, 203–206.